The Biological Control of Spotted Knapweed in the Southeastern United States

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
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By

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ABSTRACT

Spotted knapweed is an invasive, short-term-perennial plant that is native to Eurasia. It was accidentally introduced into North America in the early 1890's and has since spread across The United States and Canada. Spotted knapweed degrades rangelands and pastures by negatively impacting native plants, increasing soil surface runoff and stream sediment yields, and reducing soil infiltration. A biological control program for spotted knapweed using *Larinus minutus* (Coleoptera: Curculionidae), was initiated in Arkansas in 2008. In this dissertation I described the releases of *L. minutus* and investigated the adult activity in the southeastern United States (Chapter 1), investigated the effects of timed mowings on spotted knapweed and the effect of these mowings on *L. minutus* (Chapter 2), investigated the efficacy of *L. minutus* in reducing spotted knapweed infestations (Chapter 3), determined if there were any interactions between *L. minutus* and *Urophora quadrifasciata* (Chapter 4), and determined if it was feasible to use multispectral remote sensing to detect and monitor spotted knapweed populations. Releases of *L. minutus* were made at 39 sites in 7 different counties between 2008 and 2012. Thorough monitoring of the sites indicated establishment of the weevil. In Arkansas, *L. minutus* emerges earlier in the year than in the Pacific Northwest, but is still univoltine. It was determined that the most effective time for mowing spotted knapweed when *L. minutus* is not present is May, but if weevils are present in high numbers the most opportune time is in July. *L. minutus* reduced spotted knapweed seed production and rosette densities, but monitoring of the release sites needs to continue for several more years to document the impact of the release program on spotted knapweed in the region. The occurrences of *Larinus minutus* and *Urophora quadrifasciata* in the capitula of spotted knapweed are not independent of each other, although, this interaction had no effect on the number of seeds found in a capitulum. Finally it was determined that it is feasible to detect spotted knapweed with multispectral remote sensing throughout the growing

season and it is feasible to monitor the change in spotted knapweed populations due to control efforts.

This dissertation is approved for recommendation
to the Graduate Council.

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Special thanks Dagne Duguma whose research led to the start of this project. Thanks for paving the road I have traveled for the past four years. To June Shen for the field assistance, the countless hours of dissecting capitula and collecting, releasing and sweeping of weevils with me. I positively could not have done this without you. To Bevin McWilliams and Adam Alford for the assistance in the field and for the intellectual and emotional support as we travelled the road of higher education together. To my fellow graduate students in the Department of Entomology, thank you for the seemingly endless number of study groups, the laughs, and the stories. I hope that out paths cross again in the future. To my committee members thank you for the knowledge and the guidance as I traveled this path. I am sure it wasn't easy, but we made it through. To Tim Kring, my major advisor, you once told me that a PhD program should be fun and I have to admit that you were right. It wasn't always fun (but what is?), but I will remember my time as a PhD student fondly. Thank you for nudging me in the right direction all while letting me learn from my own mistakes. I think that you have earned the right to take the asterisk off of your "World's Best Advisor" coffee cup. To my friends and family who have always been so supportive of my dreams and aspirations and for being there to pick me up when I have fallen along the way.

DEDICATION

This dissertation is dedicated to my loving husband Levon Killian and my two wonderful children, Bishop and Draven Killian. Without your constant love and support this would have not been possible.

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I. Introduction

An invasive plant species is any non-native species (or propagules of that species), whose introduction does, or is likely to, cause harm to the environment, the economy, or human health (National Invasive Species Council, 2006). Invasive species may compete so well in new environments that virtual monocultures are sometimes formed. Invasion ecologists have formulated many hypotheses in attempt to explain the improved success of some invaders in new regions. One of the most noteworthy of these is the *Enemy Release Hypothesis* (Keane and Crawley 2002, Liu and Stiling 2006). The *Enemy Release Hypothesis* states that species are successful in new areas due to the lack of control by natural enemies. Classical (importation) biological control theory and practice is based on this assumption. Simplistically, the goal of classical biological control endeavors is to establish a natural enemy population so that pest densities are regulated without further intervention. Classical biological control is considered a safe, sustainable, and cost-effective method to control invasive species. While classical biological control is normally safe, there are risks involved, particularly to non-target species that may be directly or indirectly affected by the releases of the natural enemy. Many biological control programs rely on the introduction of multiple natural enemies. The introduction of multiple natural enemies could increase the potential for pest control, but it could also increase the risk of impacts on non-target species, thus increasing the probability of indirect effects on the ecosystem. Several studies suggest that release of a single natural enemy species represents a better solution (Myers 1985, Myers et al. 1989, Denoth et al. 2002, Liu and Stiling 2006).

In this dissertation I present research designed to contribute to an effective control and monitoring program of spotted knapweed (*Centaurea stoebe* ssp. *micranthos*) in the southeastern United States. Spotted knapweed is a pink-flowered perennial in the Asteraceae. In the United

States spotted knapweed is a tetraploid, short-lived perennial that typically survives for 3-5 years, but may live up to 9 years (Boggs and Story 1987). Spotted knapweed spends its first year of life as a basal rosette of leaves. During the second and subsequent years of life, the plant produces 1-20 upright stems originating from the basal rosette. The stems are up to 100 cm tall and branched in the upper half. Each branch is topped with a single, egg-shaped capitulum. The capitula have distinctive, black tipped phyllaries, which give spotted knapweed its common name (Tutin et al. 1976, Mauer et al. 2001).

Spotted knapweed is native to Europe, with its distribution ranging from central and southeast Europe to central Russia. Achenes (referred to as seed for the remainder of the dissertation) of spotted knapweed were accidentally introduced into North America in the 1890's in contaminated alfalfa seed as well as contaminated ship ballast (Tutin et al. 1976, Mauer et al. 2001). Spotted knapweed is now present in 46 of the 50 United States and is listed as a noxious or prohibited weed in 17 of them (United States Department of Agriculture 2007). Spotted knapweed was first reported in Arkansas in 1941 (UARK herbarium records) and a survey conducted in 2007 found that spotted knapweed is present in 20 Arkansas counties (Minteer 2007).

Spotted knapweed usually dominates dry, disturbed sites and does not compete well with grass species in moist areas (Harris and Cranston 1979). Spotted knapweed has a large taproot and can access deeper resources than the fibrous root systems of grasses, and can therefore compete more effectively with grasses at dry sites (Watson and Renney 1974). Spotted knapweed prefers high sun exposure and is most often found on disturbed sites, such as roadsides; however, in the Pacific Northwest it is known to invade pastures where it causes the overgrazing of native grasses (Tyser and Key 1988, Lacey et al. 1990, Ochsmann 2001). Spotted

knapweed is also thought to be allelopathic by secreting (−)-catechin from its roots. This chemical has been shown *in vitro* to negatively impact the germination of several native plant species (Weir et al. 2003). However, other studies show that (−)-catechin levels found in the soil at spotted knapweed infestation sites are three-fold lower than that shown to negatively impact native plant species (Blair et al. 2006).

Spotted knapweed reproduces mostly by seed but may spread by some lateral root sprouting that produces new rosettes (Watson and Renney 1974). Plants produce an average of 1,000 seeds per plant or 5,000 to 40,000 seeds/m^2 (Sheley et al. 1998). The seeds are dispersed by wind, animals, vehicles and hay transported by farm equipment. Upon maturation the seeds can remain viable for up to eight years (Davis et al. 1993).

Objectives of this research were to release and establish *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) in Arkansas and to describe the seasonal dynamics of the adult weevils. Other objectives were to investigate the impact of *L. minutus* on spotted knapweed infestations and the impact of mowing intervals on knapweed seed production and *L. minutus* survival. The final objectives were to examine the feasibility of using multispectral remote sensing techniques to map spotted knapweed infestations, and to provide the foundation for long-term monitoring of the statewide distribution of spotted knapweed using remote sensing techniques.

Chapter 1

Releases, Establishment, and Adult Activity of *Larinus minutus* (Coleoptera: Curculionidae), a Biological Control Agent of Spotted Knapweed in Arkansas

Abstract

Spotted knapweed is an invasive plant from Eurasia that causes degradation of pastures and rangelands in the western United States and Canada. A biological control program for this invasive weed was initiated in the 1960s. Thirteen exotic insect species have been established in the United States and Canada for control of spotted and diffuse knapweeds and the program has largely been considered a success. *Larinus minutus* Gyllenhal is thought to be one of the key agents responsible for the reduction of spotted and diffuse knapweeds in the western United States and Canada. Previous to start of this program there was no targeted control program for spotted knapweed in the Southeastern United States. *Larinus minutus* was released into Arkansas from 2008 through 2012. Releases were monitored for establishment. Weekly sweep netting of select release sites was conducted to monitor activity of adult weevils. *Larinus minutus* was released into six Arkansas counties and established in five. *Larinus minutus* in the southeastern United States was still univoltine, as seen in the west. Sex ratios were approximately 50:50, except during peak flowering, where the proportion of females captured increased. Eggs were not present inside females until one week after flowering of spotted knapweed.

Introduction

Spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek) is an invasive perennial that is native to Eurasia. It was accidentally introduced into North America in the late 1800s in contaminated ship ballast and alfalfa seed and has now spread throughout much of the United States and Canada (Mauer et al. 2001). Spotted knapweed invades disturbed fields and pastures, roadways, and other dry, open, disturbed habitats (Harris and Cranston 1979, Tyser and Key 1988, Lacey et al. 1990).

A biological control program for diffuse (*Centaurea diffusa* Lamarck) and spotted knapweeds was initiated in the 1960s and 13 insect species have been released and established in the United States to control these invaders (Story 2002). Although there has been decline in both diffuse and spotted knapweeds, it is not completely clear which of the 13 insect species is responsible. *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) is suspected in being the agent (or one of the agents) responsible for the decline (Myers 2004, Myers et al. 2009).

Larinus minutus is native to Europe and was first released into the United States in 1991 (Story 2002). Larvae feed on the seeds of spotted and diffuse knapweeds and can destroy up to 100% of the seed in the capitula (Kashefi and Sobhian 1998). In the western United States, *L. minutus* is a univoltine species that first appears on knapweed plants in June, typically four weeks previous to knapweed buds appearing. Weevils copulate throughout the growing season, but repeated mating does not seem to be required. Adult females must feed on knapweed flowers to complete ovarian development. Oviposition occurs in newly opened flower heads. During laboratory studies, oviposition occurred for 11 weeks (Groppe 1990). Weevils show some sensitivity to heat and during the hottest portion of the day tend to retreat to areas under branches and flower heads. Under laboratory conditions (25° C) eggs hatch in three to four days.

Larval development takes approximately four weeks and larvae go through three instars (Groppe 1990). Larvae feed on the seeds and pupate in the capitula, making a cocoon out of the seed head material (Kashefi and Sobhian 1998). The adults emerge in late September, in the Western United States, and feed on plants until winter, when they overwinter in the soil and emerge in June (Jordan 1995). In their native range the lifespan of males is 48 to 97 days and females from 17 to 58 days (Kashefi and Sobhian 1998).

Nothing has been reported about the seasonal dynamics of *L. minutus* in the southern United States. In Arkansas spotted knapweed flowers in May and can sometimes still be in flower in October. In the western United States, *L. minutus* emerge from overwintering sites in June and are active until September (Jordan 1995). Because of the longer growing season in Arkansas, I hypothesize that an additional generation of *L. minutus* may occur, and therefore cause a larger decrease in spotted knapweed than that seen in the West. The objective of this study is to describe the release and establishment of *L. minutus* in Arkansas and compare adult *L. minutus* activity in the southern United States to that reported for the Northwest.

Materials and Methods

Larinus minutus adults were collected in Colorado Springs, Colorado, USA during June of 2008 through 2011 and released into the Ozark Plateau and the Gulf Coastal Plain Regions of Arkansas. Releases in 2008 (2) were completed during the day. All subsequent releases were conducted at night. As weevils readily fly during the heat of the day, night releases were chosen so that weevils would remain near the release site. Releases were monitored visually and via sweep net for the presence of *L. minutus* a few months after release and in June of the years following release until establishment was confirmed, or until no *L. minutus* was found for two years.

From 2010 through 2012 three release sites were surveyed weekly for adult activity via sweep net (four sets of 25 sweeps at each site) starting in April of each year and continuing until two weeks after no *L. minutus* were found at any site. As weevils are reported to hide during the hottest portion of the day (late afternoon), weevil sweeps were conducted between the hours of 10 am to 12 pm (Groppe 1990, Kashefi and Sobhian 1998). *Larinus minutus* collected during the sweeps were preserved in alcohol and held in a refrigerator until sex ratio could be determined using the method described in (Kashefi 1993). Females were then dissected to determine the presence of eggs. Ten mature capitula (flowers senesced, but capitulum still closed) per site were also collected. These capitula were placed individually in plastic diet cups with cardboard lids and placed in a screen house**.** *Larinus minutus* emergence was monitored weekly for the remainder of the season.

Results and Discussion

Releases

Over 29,000 *L. minutus* adults were released at 40 sites in six Arkansas counties (Washington, Madison, Benton, Howard, Carroll, and Boone). Releases ranged from 300 to 1500 weevils per site, depending on the size of the knapweed infestation. An average of 750 weevils was released per site. Of the 40 releases, 38 were successful and *L. minutus* was established or present during sweeps and/or visual assessment in 2011 and/or 2012 (for releases made in 2010 and 2011) (Table 1). Several release sites were augmented with additional weevils if the number of weevils collected via sweep net was abnormally low (as compared to other sites) following establishment. This was typical at earlier release sites, where the number of *L. minutus* released was low (~300 individuals). Releases failed at two sites due to herbicide

destruction of the knapweed infestation (Washington County) and at an extremely small, isolated knapweed infestation at the Howard county release site.

Adult Activity

As expected, the numbers of weevils across release sites increased across the years of monitoring (Figure 1). I was expecting a possible second generation per year, because of the longer growing season in the southeastern United States. Spotted knapweed flowers earlier and *L. minutus* is active earlier in the year (late-April to early-May) in the southeastern United States than it is in its native range (late-May to early-June) (Groppe 1990). Knapweed flowers also persist much longer, as they are readily evident into mid-October or later. Adult *L. minutus* activity (monitored via sweep nets) indicates that only one generation per year occurs in Arkansas (Figure 1). After the initial increase in the number of adult weevils seen (mid to late-April), there is a slight dip in adult activity followed by another increase during all three years of monitoring. I believe that both of these peaks in adult activity are due to the adults emerging from their overwintering sites and becoming active. I do not believe that the second peak is the F1 generation emerging from the seedheads of spotted knapweed, because there are no spotted knapweed flowers until mid-May to early-June. Although there are flowers available during this second peak, there has not been enough time for egg laying, hatch, and full larval development, which takes approximately 4 weeks (Groppe 1990). Data from the capitula collection in 2011 (data from 2010 are not shown due to low numbers of emergence) show that emergence from the capitula starts in mid- to late-July, so this second peak seen from the sweep net sampling must be due to the emergence of the overwintering adults (Figure 2). I believe that two peaks were seen because of the sampling method used. Sweeping collected the active adult weevils from the early bolted portions of the plants, but was not appropriate for monitoring the weevils that are

active at the base of the plants. *Larinus minutus* feed on the leaves of the plant, but prefer to feed on flowers (Groppe 1990). When flowers are present more weevils move to the top of the plants and are more easily collected via sweep net. The F1 generation emerged in early to mid-July, as seen by the third peak of adult activity and by the presence of emergence holes on plants. Weevils were active until mid-September (Figure 1).

Male and female weevils became active at the same time of the year, based on our sweeps. Females became active in larger numbers at the start of emergence from overwintering sites in 2010 (Figure 3). This trend in 2010 may have been a product of the sampling method used combined with the lower weevil density. Females may be active at the tops of the plants earlier in the year than the males and therefore showed an early activity based on sweeps. This was not seen in 2011 or 2012 when the weevil numbers were higher, therefore increasing the proportion of males captured earlier in the growing season (Figure 3).

Sex ratio

Sex ratios over the study hovered around 50:50 (Figure 4). Exceptions to this were directly before peak flowering (May), where there were more males than females ($P<0.0001$) and during peak flowering time (June and July) ($P<0.0001$ and $P<0.0001$, respectfully) where there were more females than males. This skew in sex ratio during the beginning of the season and peak flowering is consistent with previous studies (Kashefi and Sobhian 1998). This may also have been a product of the sampling method used, as females were found ovipositing at the tops of plants during peak flowering.

Egg Production

Female *L. minutus* were dissected to determine the presence of eggs. Eggs were found in females starting the week after the first flowers were seen during all years of the study. This

result agrees with the published literature, which states that feeding on flowers is required for ovary development (Groppe 1990, Kashefi and Sobhian 1998). However, no studies have been conducted to verify this correlation.

Larinus minutus was successfully released and established in Arkansas. There was one generation/year in the southeastern United States. Sex ratios were skewed towards more males prior to flowering of spotted knapweed, but during peak flowering, the ratio shifts to a higher proportion of females. Long-term monitoring studies are currently being conducted to determine the effectiveness of *L. minutus* in the region.

Table 1. *Larinus minutus* releases in Arkansas from 2008 through 2011 by county.

Date	Latitude	Longitude	# of *L. minutus*	County
7/23/2008	36.091384	-94.19132	300	Washington
7/26/2008	36.048849	-94.19142	400	Washington
6/13/2009	36.102207	-94.17495	800	Washington
6/13/2009	36.07678	-94.19767	600	Washington
6/13/2009	36.03448	-94.18479	700	Washington
6/13/2009	35.92496	-94.19784	700	Washington
6/13/2009	35.9845	-94.19894	700	Washington
6/14/2009	36.10053	-94.18552	700	Washington
6/25/2010	36.2985	-94.16965	1000	Benton
6/25/2010	36.17105	-93.91383	700	Madison
6/25/2010	36.354611	-94.17525	700	Benton
6/28/2010	36.1022	-94.17489	750	Washington
6/29/2010	36.21117	-93.67454	1200	Madison
6/29/2010	36.31833	-94.18419	600	Benton
6/29/2010	36.322325	-94.18543	600	Benton
6/29/2010	36.32331	-94.18563	600	Benton
6/29/2010	36.05995	-94.12745	900	Washington
6/30/2010	33.94957	-93.91564	300	Howard
7/1/2010	36.17577	-93.53704	300	Carroll
7/1/2010	36.17681	-93.53625	300	Carroll
7/1/2010	36.23018	-93.5303	600	Carroll

7/1/2010	36.04124	-93.48668	840	Madison
7/1/2010	36.10334	-94.0061	800	Washington
7/1/2010	36.10069	-94.05145	400	Washington
7/1/2010	36.25772	-93.63195	1200	Madison
7/1/2010	36.25634	-93.63939	1200	Madison
7/2/2010	36.03682	-93.96311	400	Washington
7/2/2010	36.120473	-94.153211	400	Washington
7/2/2010	36.26712	-93.85767	400	Madison
7/2/2010	36.2985	-94.16965	800	Benton
7/6/2010	36.20656	-92.99887	400	Boone
6/24/2011	36.294535	-94.14799	700	Benton
6/24/2011	36.265286	-94.147888	1500	Benton
6/24/2011	36.334011	-94.162925	1500	Benton
6/25/2011	35.90324,	-93.9313	600	Madison
6/25/2011	35.91032	-93.9362	600	Madison
6/25/2011	35.96508	-93.99424	800	Washington
6/25/2011	36.08855	-94.22562	500	Washington
6/25/2011	36.1022	-94.17489	400	Washington
6/26/2011	36.111396	-94.162396	700	Washington

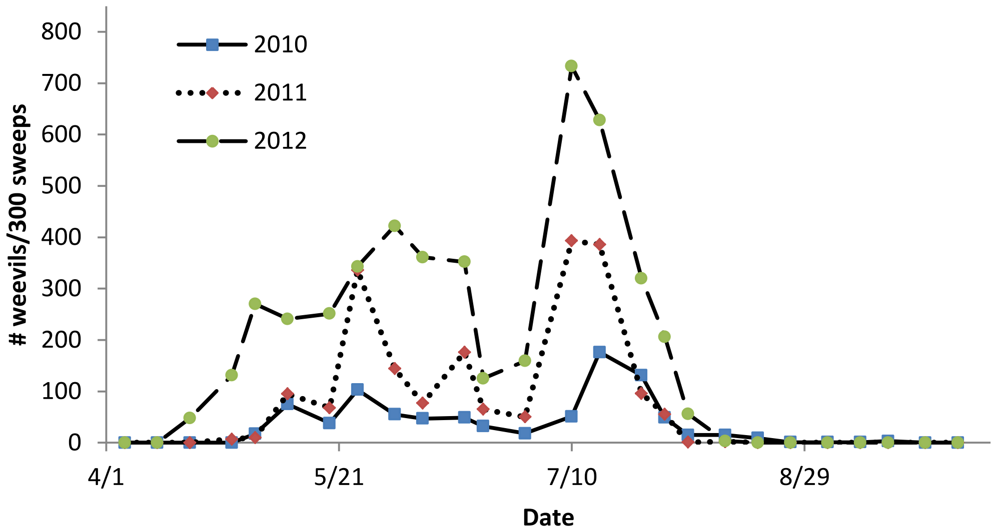


Figure 1. Adult *Larinus minutus* activity during 2010 through 2012 at three release sites in Arkansas. Weevils were monitored weekly with sweep nets (Four sets of 25 sweeps at each of three release sites).

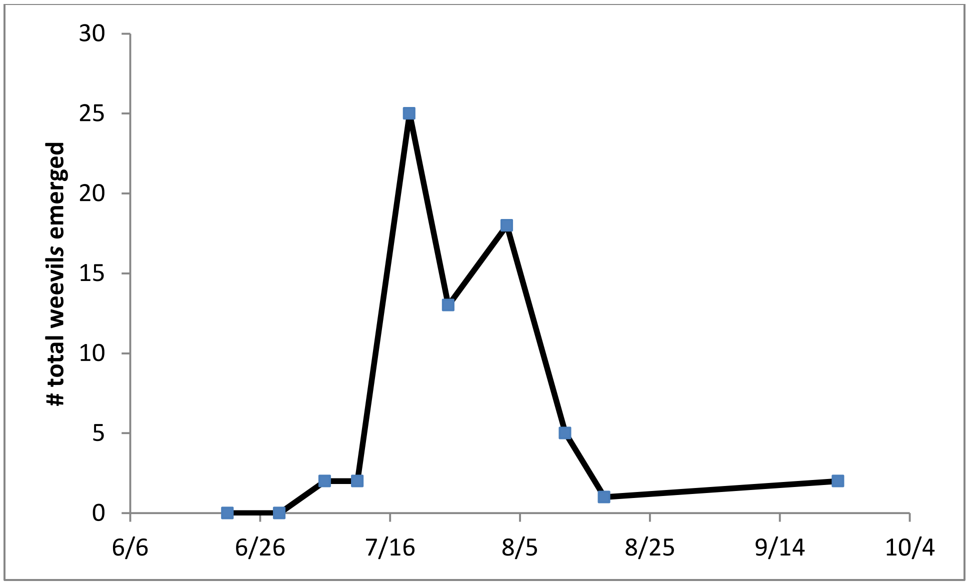
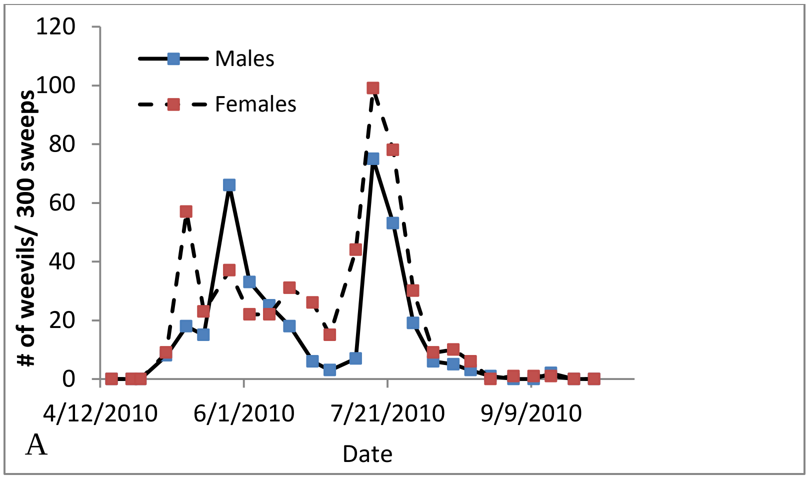


Figure 2: *Larinus minutus* emergence from capitula collected weekly from three release sites in the Ozark Plateau region of Arkansas in 2011. Capitula were collected weekly and held in a screen house. Numbers here indicate the sum total of *L. minutus* (per week) that emerged from all capitula collected.



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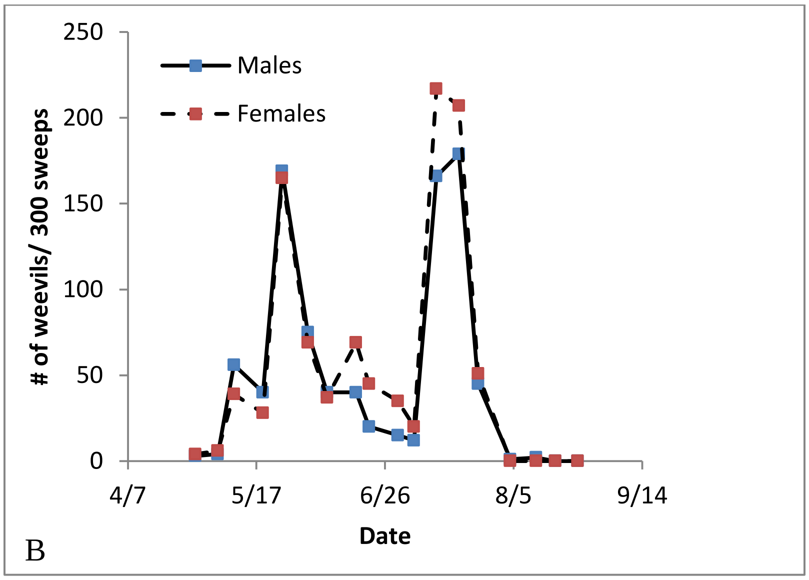

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Figure 3: Adult *L. minutus* activity by sex, as determined by weekly sweep net samples in 2010 (A) and 2011 (B) (Four sets of 25 sweeps at each of three release sites).

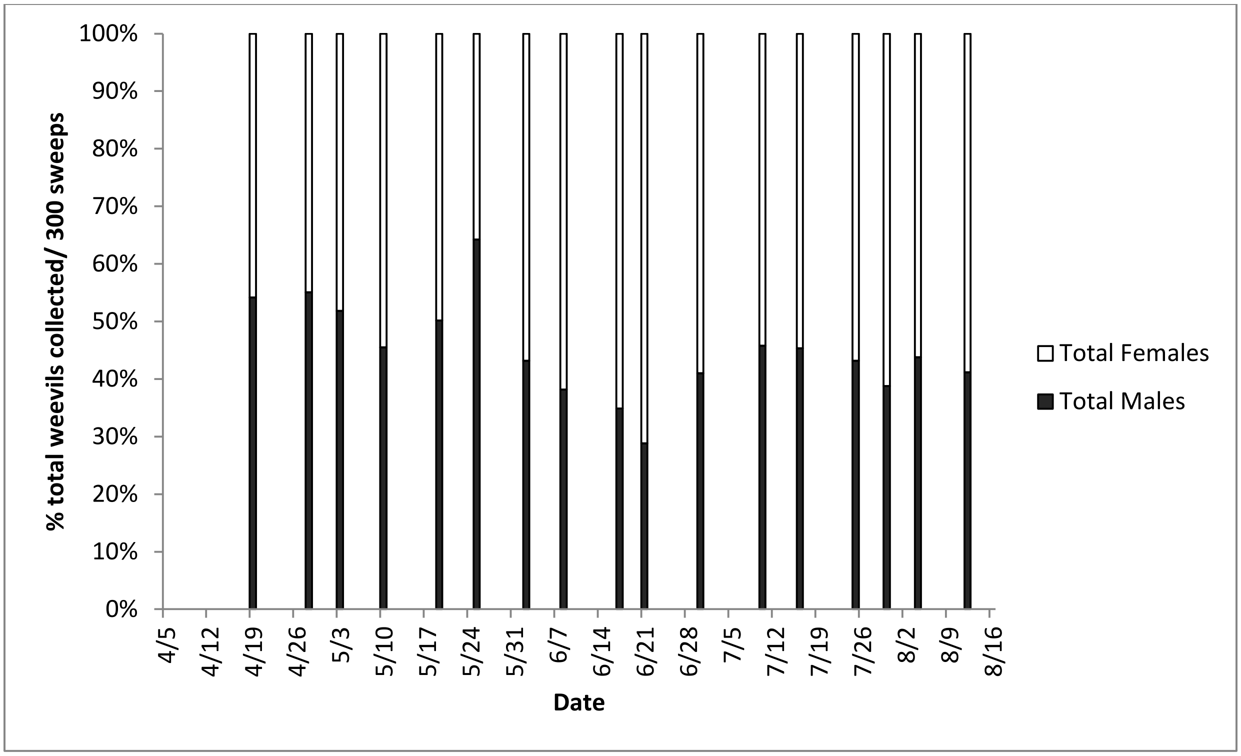


Figure 4. Sex ratios of *L. minutus* in Arkansas collected during weekly sweep net sampling during 2010 through 2012 (Four sets of 25 sweeps at each of three release sites). Sex ratios are only shown if weevil counts were > 10 weevils.

Chapter 2

Impact of Selected Mowing Timing on Spotted Knapweed and *Larinus minutus* Survival in the Southeastern United States

Abstract

Spotted knapweed in the southeastern United States often occurs as a roadside weed and populations have been increasing in size in recent years. Seeds of this invasive plant are spread by wind, animals, and anthropogenic means (e.g., mowing equipment). Mowed spotted knapweed will re-sprout and produce another set of flowers. It is unknown if this second set of flowers produces comparable numbers of seeds as un-mowed plants. Objectives of this study were to investigate if the timing of a single mowing can reduce the number of spotted knapweed seeds entering the seed bank and to investigate if timing of mowing has an impact on biological control agents that are present. Four mowing treatments (May, June, July, and August) and an un-mowed control were arranged at random with 4 replicates each at 2 sites in northwest Arkansas in 2011 and 2012. Drought conditions in 2011 led to a failure of any mowed plants to re-sprout, so plots were irrigated in 2012. In 2012 there were no differences in the number of rosettes/ m^2 or size of rosettes between post-mow experimental plots and the "peak" growth control plots. Number of bolted plants/m^2 differed for June, July, and August, but not for plots mowed in May. Number of stems/ m^2 was lower for all mowed plots than the controls.

Plant height was significantly lower in plots mowed in May when compared to the controls, but not for June or July. There was no difference in total seeds, immature seeds, or mature seeds/ capitulum for plots mowed in May, June, or July when compared to controls. The proportion of capitula with emerged *Larinus minutus* post-mow was significantly lower in May and June than in unmowed controls. Based on the results of this study May is the best time to mow infestations of spotted knapweed without *L. minutus* present and mid-July is the best time to mow infestations with *L. minutus*.

Introduction

Spotted knapweed (*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek) is an invasive perennial from Eurasia that was accidently introduced into North America in the 1890s (Tutin et al. 1976, Mauer et al. 2001). It is primarily a problem in rangelands and pastures in the western United States where it occupies > 2,000,000 ha and spreads at an annual rate of 10-24% (Duncan et al. 2004). Spotted knapweed increases surface runoff and sediment yield, results in the overgrazing of native grasses, reduces available forage for livestock and replaces indigenous plants (Tyser and Key 1988, Lacey et al. 1989, Lacey et al. 1990, Kedzie-Webb et al. 2001, Ochsmann 2001). Plants produce an average of 1,000 seeds per plant, with the seeds remaining viable for 8 or more years. Conservative estimates of seed production estimate that plants produce between 5,000 and 29,600 seeds/m^2 (Davis et al. 1993, Sheley et al. 1998).

In the Southeastern United States spotted knapweed is primarily found along roadsides and in unmanaged fields and pastures (pers. obs.). Weeds in these and other ruderal areas are largely ignored when it comes to targeted control. Herbicides and mowing tactics are commonly used to control weeds along transportation corridors.

Dicamba + 2,4-D and picloram have been shown to be effective at reducing spotted knapweed and increasing perennial grass biomass (Rice et al. 2000, Sheley et al. 2000). Resistance to these herbicides is not common but has been seen in yellow star-thistle (*Centaurea solstitialis L*.), as well as other plant species (Sabba et al. 2003, Jugulam et al. 2005). Overuse of herbicides along transportation corridors increases the risk of weeds developing resistance to these herbicides. Potential water contamination is also sometimes associated with using herbicides to control roadside weeds (Wood 2001).

Mid-spring burning has been shown to reduce spotted knapweed adult and rosette densities (Emery and Gross 2005, MacDonald et al. 2007). While burning may reduce spotted knapweed density and/or growth rate, there are obvious challenges for burning along roadsides and it is not a common practice in most areas of the United States.

Spotted knapweed has also been a target of biological control, and natural enemies recently have provided suppression of knapweed populations in the Western United States and Canada (Myers 2004, Smith 2004). Myers et al. (2009) found that knapweed declined at sites where *Larinus minutus* had become established in high numbers, but not at other sites where other natural enemy species were present, but *L. minutus* was not present in high numbers.

Two biological control agents for spotted knapweed are currently found in northwest Arkansas, where this study was conducted, *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) (Duguma 2008, Minteer et al. 2011). *Urophora quadrifasciata* spread naturally into Arkansas and *L. minutus* was released. *U. quadrifasciata* only reduced spotted knapweed seed production late in the growing season, when seed production is presumed to be at its lowest (Duguma 2008). *Larinus minutus* significantly reduces knapweed seed production over the entire growing season (Minteer, unpublished data). Because it is not economical to control weeds in ruderal areas such as roadsides, importation biological control programs are the tactic of choice for amenable exotic invasive weeds. Biological control (once establishment is achieved) is sustainable and long-lasting with little to no input (monetary or otherwise) from humans, so it is cost-effective for long term control.

Mowing is often used by departments of transportation to reduce vegetation along roadsides. In Arkansas mowing is used almost exclusively for this purpose. Watson and Renny

(1974) found that in British Columbia mowing spotted knapweed led to a reduction in the number of plants that produced seed, when compared to un-mowed controls when plants were mowed during the bud or flowering stage, or plants that were mowed during both the bud and flowering stage. They also found that seed germination was reduced in areas that were mowed during flowering and areas that were mowed twice (bud and flowering), but they did not find a decrease in germination of seeds produced by plants that were mowed only during the bud stage (Watson and Renney 1974). Rinella et al. (2001) found that mowing had variable effects on spotted knapweed adult density, but they did find that in Montana, mowing one time in the fall, when spotted knapweed is at the flowering or seed-producing stage, reduced spotted knapweed cover and adult plant density as much as any mowing regimen in which the plants were mowed multiple times. They also found that a single fall mowing that is repeated for a period of 3 years reduced adult plant density (Rinella et al. 2001).

A single fall mowing may not lead to the same result in the southeastern United States, because spotted knapweed in these areas blooms in May-June and has already started to set seed by July, whereas plants in the Pacific Northwest do not set seed until mid-August (Watson and Renney 1974). A single fall mowing (in the Southeast), therefore, would spread seed, and potentially increase spotted knapweed density instead of decreasing it. Mowing times in the Southeastern United States may not follow the same results as mowing in Montana due to the longer growing season and the shift in phenological timing between two regions.

Roadsides in Arkansas, where this study was conducted, are maintained by mowing. The Arkansas Department of Transportation contracts out most mowing duties to private companies or individuals, and routes that have mowing contracts do not receive any herbicide treatments. Guidelines require that roadsides are mowed three times per year, once prior to Memorial Day,

once in July, and once between October 1 and Thanksgiving. The sides of divided highways are mowed in a 9.1 m (30 ft) swath on each side of the road. A 3 m (10 ft) swath on each side of an undivided roadway is mowed on the first and second mow of the year and a 9.1 m (30 ft) swath is mowed on the third mow (or up to the fence line or well-established vegetation lines. These set mowing widths leave a refuge of plants for the biological control agents present at these sites (if natural enemies are present), but mowing timings could possibly reduce the populations of agents.

If mowing is used to manage spotted knapweed there are three possible outcomes for spotted knapweed: (1) mowing too early, thus providing time for spotted knapweed to re-sprout and produce viable seeds (little or no impact), (2) a reduction in the amount of seeds entering the seed bank and an eventual reduction in adult plant density, or (3) the spread of seeds to new areas through contaminated mowing equipment.

The mow prior to Memorial Day will cut spotted knapweed plants that have already bolted, but have not produced flowers. Mowed plants will have sufficient time to re-sprout, but are significantly shorter and will potentially not be affected by subsequent mows (Rinella et al. 2001). This combination of factors could result in spotted knapweed seed load and adult plant density not being reduced by the pre-Memorial Day mow, but it may allow for the survival of seed-feeding biological control agents. The July mow could provide for some reduction in seed load if the plants have not set seed. It is currently unknown if plants (in the Southeastern United States) mowed during this time will produce viable seed upon re-sprouting and what effect this has on *L. minutus*. The late mow (October 1-Thankgiving) occurs after spotted knapweed has already produced mature seed. Seed dispersal in spotted knapweed is achieved by movement of the stems after the bracts have become dehydrated and have opened (Watson and Renney 1974).

The action of the mowing equipment would, therefore, spread more spotted knapweed seed and carry it even further on the decks of the equipment than it would spread naturally. My objectives for this project were to investigate (1) the effects on spotted knapweed and *L. minutus* when plants are mowed are different times, (2) to investigate if there is an optimal time to mow spotted knapweed populations that would reduce the input of seed into the system, and (3) to investigate if there is an optimal time to mow, with respect to the conservation of established biological control agents.

Materials and Methods

This study was conducted at two sites in the Ozark Plateaus in 2011 and 2012. The first site was at the University of Arkansas Experiment Station Farm in Washington County, Arkansas in an experimental plot, where spotted knapweed was planted in 2006. Soils are silty, cherty, and moderately well drained (as defined by the United States Department of Agriculture Soil Conservation Service), and while spotted knapweed dominates at this site, other vegetation (*Conyza canadensis* (L.) Cronquist, *Tridens flavus* (L.) Hitchc., *Trifolium arvense* L., and *Setaria* P. Beauv. spp.) is present. Site two was a disturbed field in Benton County, Arkansas with moderately well drained, loamy, cherty soils (as defined by the United States Department of Agriculture Soil Conservation Service) with spotted knapweed, sumac, johnsongrass, and purpletop as the dominant vegetation.

I investigated four treatments and a control. Treatments were a May, June, July, or August mow and an un-mowed control. Four reps for each of the 5 treatments were set up at each site (total of 8 reps for each treatment). Multiple mows were not investigated, as Rinella et al. (2001) found that in Montana the season of mowing was more important than frequency of mowing because spotted knapweed re-sprouts after a mow, but plants are significantly shorter

and, therefore, not affected by subsequent mowings. All plots were selected in late April of each year and had a spotted knapweed adult plant density between 15-25 plants/m^2 when chosen. Treatments were then assigned randomly among the selected plots.

Mowing was scheduled in relation to key phenological time frames pre-flower (May), start of flower (June), peak flower and beginning seed set (July), and seed set (August). Before a plot was mowed the plot was evaluated to determine the number of rosettes, adult plants, stems, and capitula and rosette diameter. Rosettes were defined as unbolted plants that had at least one dissected leaf. Unbolted plants with un-dissected leaves were considered seedlings. Plant height was determined by measuring the distance between the root crown and the tip of the tallest stem of each plant in the subplot and averaged. Twenty capitula (if present) were collected from each plot prior to mowing and dissected to determine the number of mature and immature seeds. A 56-cm Tanaka dual sided hedge trimmer was used to mow each of the mowed plots to a height of 10-13 cm. After the mow, the plots were monitored for re-bolting and flower development. The date of re-flowering was recorded and the same data that were collected pre-mow were recollected in October. Plant height was also measured after post-mow bolting occurred. After the post-mow when flowers had started to mature (petals starting to wilt) white tulle bags were placed over 20 capitula per plot and left on the plants until September, so that the seeds could mature on the plant.

The control plots were surveyed each month (May through August). Fabric bags were placed around 20 capitula in each of the un-mowed plots in July (when the seeds start to mature) in order to ensure that seed number would not be reduced because of natural seed dispersal. Bags were collected from un-mowed plots in September and mowed plots in October for counting of mature and immature seed. Seed bags were collected later for mowed plots, so that

seed would have a chance to mature. Upon dissection of the capitula, the number of *L. minutus* adults (both emerged and un-emerged) and un-emerged *L. minutus* larvae were recorded. The presence of an emerged *L. minutus* was determined either by observing an emergence hole in the capitulum or by the presence of an adult weevil in the fabric bag containing the capitulum.

The Tukey-Kramer HSD test was used to analyze all data to lower the chance of a Type I error from multiple comparisons. Analyses were performed on pre-mow data (among control plots at each of the set mow times and the experimental month pre-mow data) to determine any differences among the "paired" plots. Post-mow experimental plot data were compared to the control plots surveyed in August. These control plots were chosen for comparison because plants had started to senesce in August and growth had ceased, giving measurements for the peak size, flower number, and seed numbers for the un-mowed plants. Sites were analyzed separately.

Results

The Benton County site was mowed by the landowner prior to the final post-mow data collection, so data for this site could not be analyzed. The area experienced a severe drought in 2011 and an extreme drought in 2012 (based on the Palmer Hydrological Drought Index). Plants did not re-sprout over any mowing treatment in 2011, so no analysis was performed and irrigation was added to the protocol in 2012. Irrigation of 2.5 cm/wk in 2012 provided the study plots with near normal "rainfall" for the season.

Pre-mow comparisons

Total number of rosettes, rosette size, number of bolted plants, and number of stems did not differ between any of the pre-mow experimental plots and the control plots for that same time frame (e.g., no difference between the May experimental plots pre-mow and the control plots measured in May) (Figures 1 and 2). To prevent an impact on final measurements, only

non-destructive data collection was done in control plots previous to the final data collection in August, so seed counts were not conducted on the control plots until September.

Post-mow comparisons

There were no differences in the number of rosettes/ m^2 (May P=0.9; June P=0.9; July P=0.8; August P=0.9) or size of rosettes (May P=0.5; June P=0.6; July P=0.9; August P=0.7) between post-mow experiment plots and the “peak” growth control plots (surveyed in August) (Figure 3). Numbers of bolted plants/m^2 differed for June, July, and August (P=0.039, 0.039, and 0.035, respectively), when compared to the controls, but not for May (Figure 4). Number of stems/ m^2 was lower for all mowed plots than the control plots (May P=0.0008; June P=0.0003; July P<0.001; August P<0.001) (Figure 4). Plant height was significantly shorter in plots mowed in May when compared to the controls (P=0.031), but no difference was observed during June or July (Figure 4). There were no bolted plants in the plots mowed in August, so no analysis on plant height could be conducted.

There were no differences in total seeds, immature seeds, or mature seeds/ capitulum for plots mowed in May, June, or July when compared to control plots (Figure 6). No analysis was conducted for the August mow, because no plants had re-bolted by the time final data were collected (October). The proportion of capitula with emerged *L. minutus* post-mow was significantly lower in May (0.17) (P<0.0001) and June (0) (P<0.001) than in unmowed controls (0.45). There was no difference in the proportion of capitula with emerged *L. minutus* emergence between plots mowed in July (0.5) and control plots (P=0.9). No analysis could be conducted on the August experimental plots, as no plants re-sprouted following mowing.

Discussion

2011 season

The absence of re-sprouting in 2011 is contrary to what I have observed in previous years, and I believe that it was related to the drought experienced in the area (based on the Palmer Hydrological Drought Index). This lack of re-sprouting led to the decision to irrigate the study plots in 2012. The lack of re-sprouting of plants during the drought suggests that water plays an important role in the re-sprouting of mowed plants and that timing of mowing during dry years may not be something that needs to be considered.

Pre-mow comparisons

The absence of differences between experimental plots (pre-mow) and the control plots surveyed at the same time shows the consistency of plot choices and lends confidence that any differences seen are due to experimental differences and not natural variation. When the plots were chosen in late April all plots had 15 to 25 spotted knapweed plants in each plot. However, when data collection began in May more plants had bolted in many of the plots, so several plots had >25 bolted plants. Pre-mow comparisons were done to ensure plot consistency.

Post-mow comparisons

There were no differences in the number of rosettes/ m^2 or size of rosettes between post-mow experiment plots and control plots surveyed at the end of the growing season. The lack of effect here was expected, as rosettes are below the area cut by the blade, and thus are not directly affected by mowing.

May

There was no difference in the number of bolted plants/m^2 in May compared to the control plots, but the plants were significantly shorter. Mowing in May allowed enough time in the growing season for mowed plants to re-sprout and complete development. In May there were no mature seeds in any of the capitula collected pre-mow, yet post-mow the number of mature

seeds/ capitulum was the same as the control plots. While the ability of spotted knapweed plants mowed in May to produce a similar number of bolted plants and mature seeds post-mow as the un-mowed controls was interesting, it will most likely have little effect changing the spread of spotted knapweed in the area. Even considering the mature seeds produced post-mow, mowing in May will not increase the number of mature seeds that are released into the system because of the absence of mature seeds pre-mow. It should be determined if the reduction in plant height will lead to a decrease in the number of capitula/ plant, thereby reducing the number of seeds produced/m^2. Studies investigating the effects of timed mowings on the above ground biomass of spotted knapweed, instead of measuring individual plant characteristics, may be more diagnostic.

June

The number of bolted plants/m^2 was significantly lower for plots mowed in June when compared to control plots, but there was no difference in the plant height. There was only one bolted plant in the June treatment group, so the average plant height was not a very robust average. I believe that, if there were more bolted plants in this group, the average plant height would have been much shorter. There was no difference in the number of mature seeds/ capitulum. This indicates that even after mowing in June spotted knapweed plants have the ability to re-sprout and produce mature seed. This gives the opportunity for mature seed to be released into the system twice if plants are mowed in June, because mature seed is already present in the capitula pre-mow. There will be a greater number of seeds pre-mow seeds than post-mow because of the lower number of bolted plants post-mow. Even with the decrease in the number of plants post-mow there is an increase in the number of seeds being released into the system, with the presence of mature seed post-mow. More research is needed to determine if

there is a difference in the total number of seeds per m^2 between mowed and un-mowed plots, as opposed to number of mature seeds/capitulum.

July

The number of bolted plants/m^2 was significantly less for plots mowed in July when compared to control plots, but there was no difference in plant height. There was only one bolted plant in the July treatment group, so the average plant height did not represent a strong average. I believe that if there were more bolted plants in both the June and July groups that the average plant height for these groups would have been much shorter. The number of mature seeds/ capitulum post-mow was the same as control plots. This indicates that even after mowing in July spotted knapweed plants have the ability to re-sprout and produce mature seed. However, in July there were so few capitula present post-mow that more research is needed to determine if this result will be supported further.

August

No plants re-sprouted after being mowed in August. Mature seed has already been released into the system by August, so this mow does little to inhibit seed production. It does not, however, allow the plants to produce additional seed post-mow, thereby not allowing additional seed to be spread like in plots mowed in June or July.

***Larinus minutus* emergence**

The proportion of capitula with emerged *L. minutus* post-mow was significantly smaller in May and June than in unmowed controls. Although the proportion of emerged *L. minutus* in plots mowed in July was no different from that of the control group, this was based only on a single plant that re-sprouted in the July plots. *Larinus minutus* in Arkansas emerges from spotted knapweed capitula in mid-July. If plants are mowed prior to this time *L. minutus* larvae within

the cut capitula may perish, but this needs further research. Mowing in May or June, before *L. minutus* emergence, could detrimentally affect the *L. minutus* numbers in an area. Mowing in July would allow for successful emergence of *L. minutus* pre-mow, but also spreads seed before mowing and still provides an opportunity for new seeds to be produced post-mow.

With the ability of plants to re-sprout and produce mature seed in plots mowed during May, June and July, mowing in May or early June before seeds develop may minimize the numbers of seed released in mowed areas without *L. minutus*. In areas where *L. minutus* is present the best time to mow is more complicated. *Larinus minutus*, in Arkansas, emerges from spotted knapweed capitula in mid-July and mowing before this time may likely increase the mortality of developing *L. minutus* in the capitula. However, mowing in July spreads mature seeds and still allows the plants to re-sprout and produce more seed in the years with adequate summer moisture. This spreading of seed more than once a year could increase the spread of spotted knapweed. I believe that because *L. minutus* significantly reduces spotted knapweed seed production, it is more beneficial to preserve the levels of *L. minutus* with mowing (when required) in July, even with the potential increase in seed dispersal. These findings suggest that roadside mowing times should be re-visited, in order to preserve the natural enemies present in areas with spotted knapweed. It is unknown what the long-term effects of timed mowings on spotted knapweed infestations with or without natural enemies present are. It is also unknown how timed mowings affect generalist seed predators. Generalist seed predators may have a significant effect on both the numbers of spotted knapweed seed entering the system, as well as the dispersal of seed. These unknowns should be investigated.

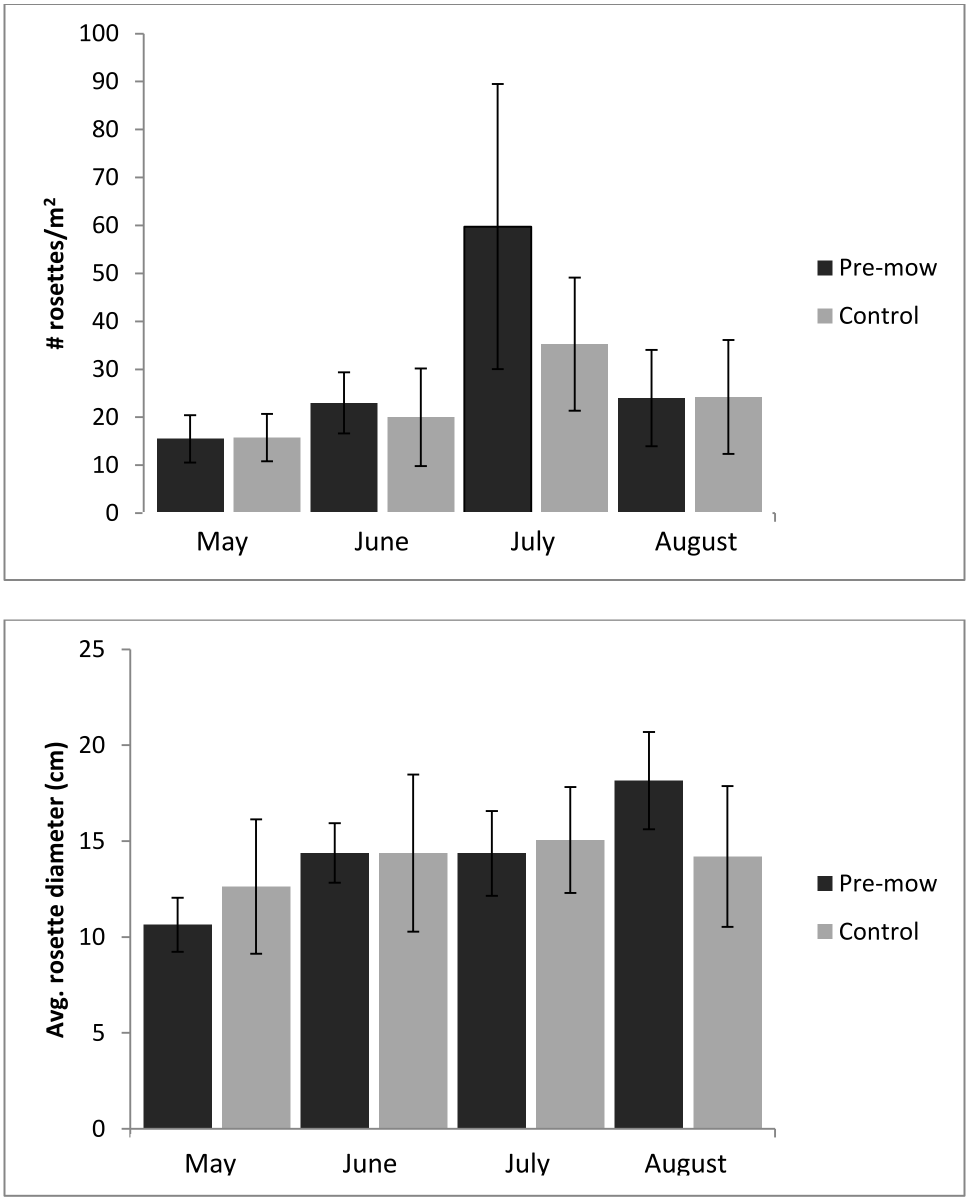
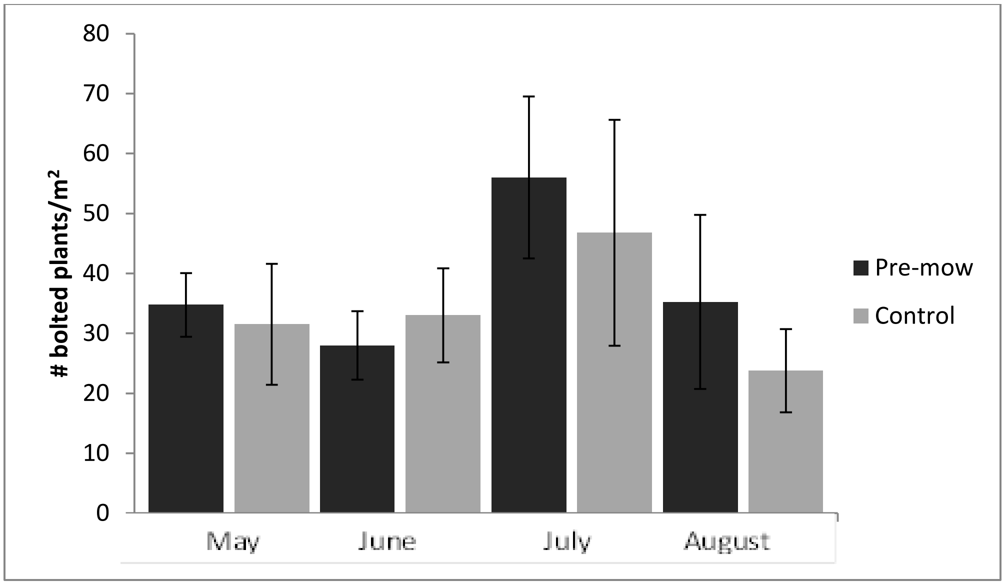


Figure 1: Numbers of rosettes (top) and average rosette diameter (bottom) of pre-mowed plots and control plots measured during the same time frame (with standard error bars).



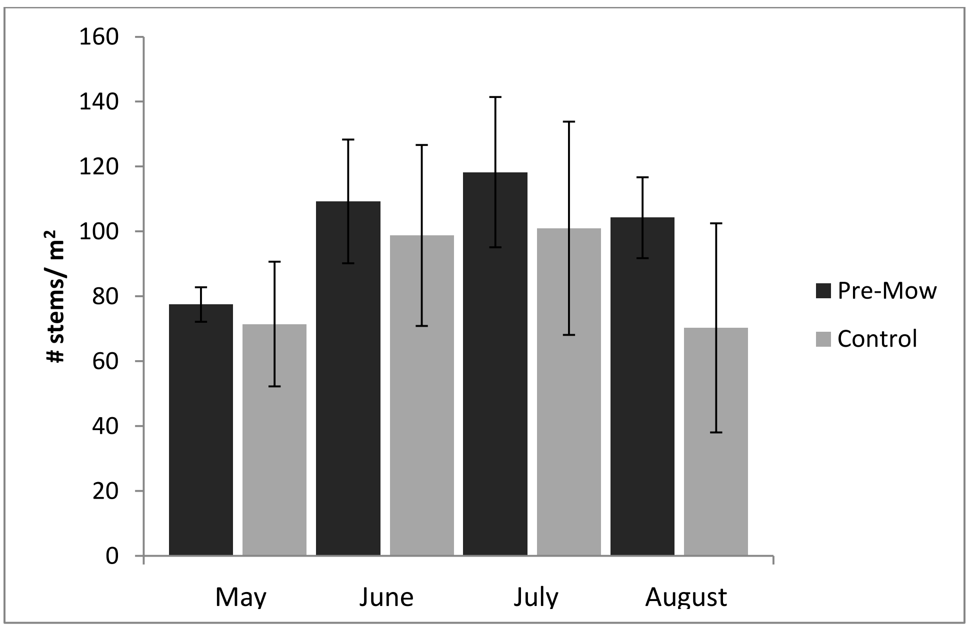


Figure 2: Numbers of bolted plants (top) and stems (bottom) of pre-mowed plots and control plots measured during the same time frame (with standard error bars).

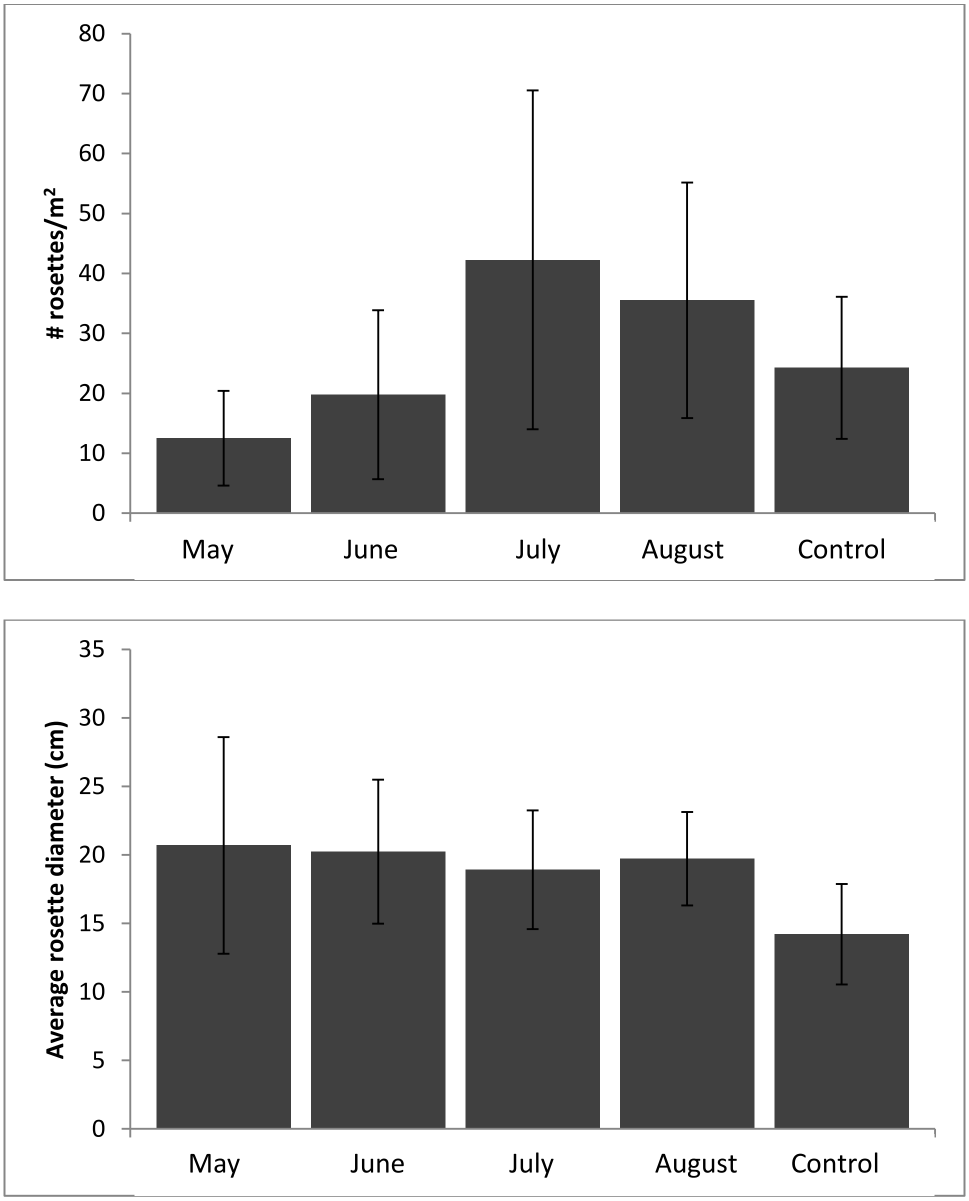
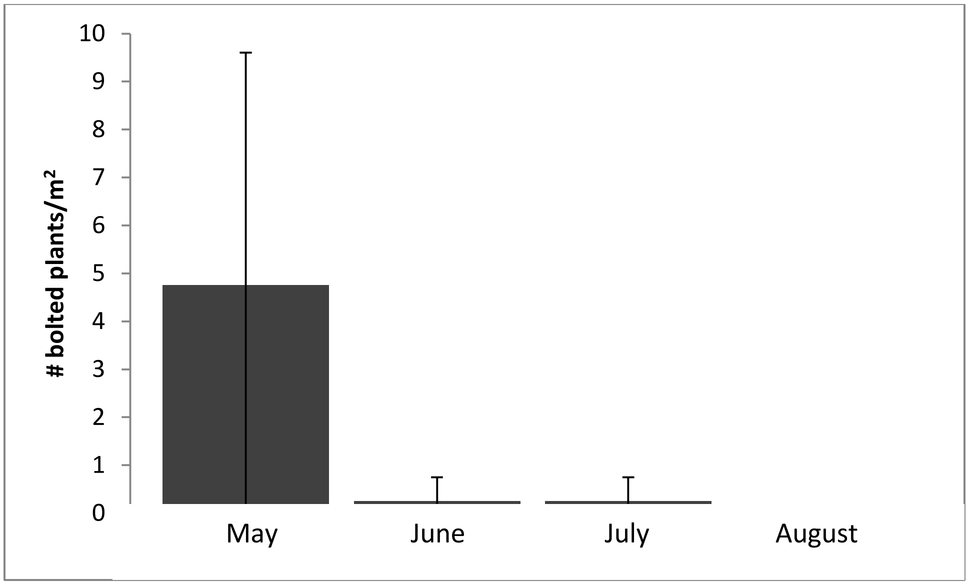


Figure 3: Number of rosettes (top) and average rosette diameter (bottom) of plots post-mowing in May, June, July, or August (with standard error bars).



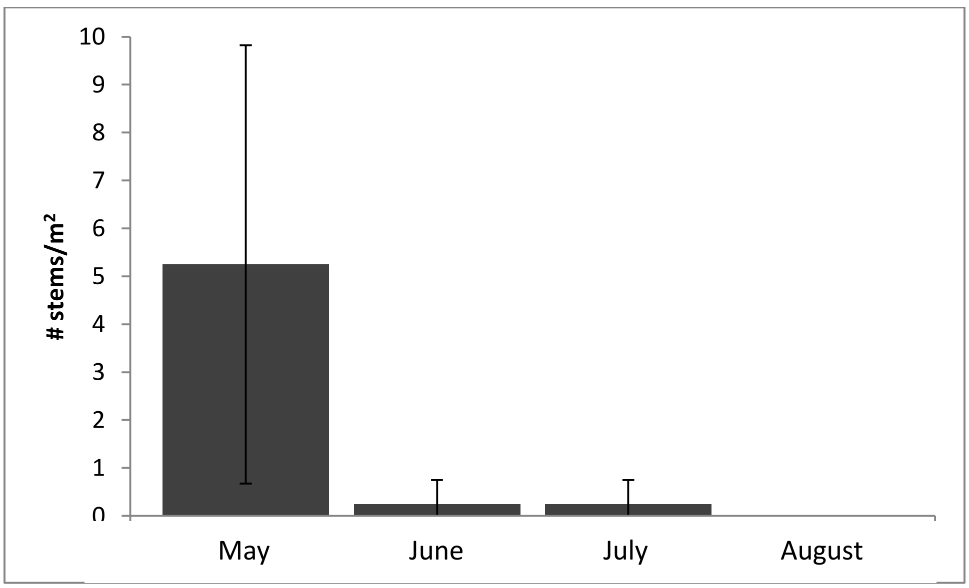


Figure 4: Number of bolted plants (top) and number of stems (bottom) of plots post-mowing in May, June, July, or August (with standard error bars). (Control (not shown): # plants =23.75 and # stems = 70.25)

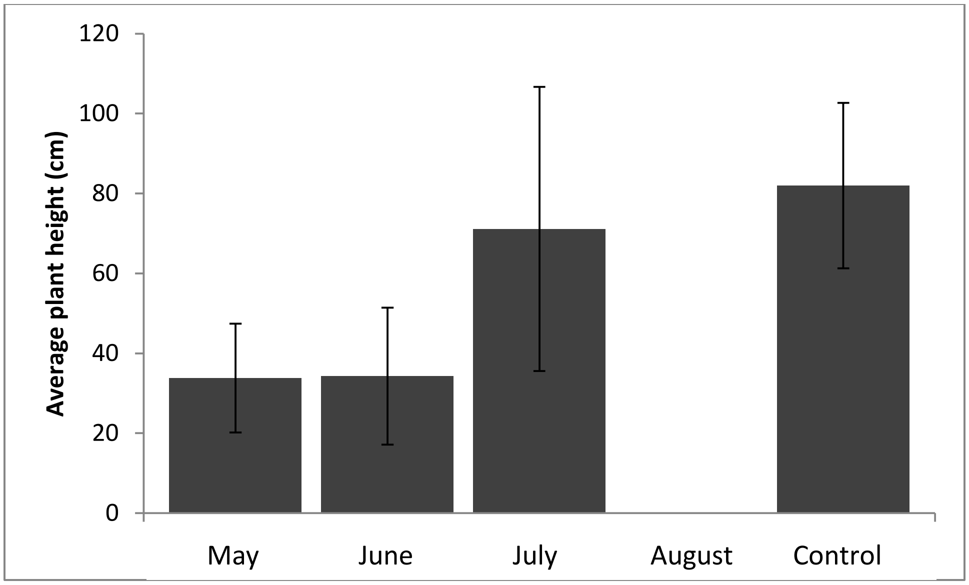


Figure 5: Average spotted knapweed plant height in plots post-mowing in May, June, July, and August of 2011 and un-mowed controls (with standard error bars).

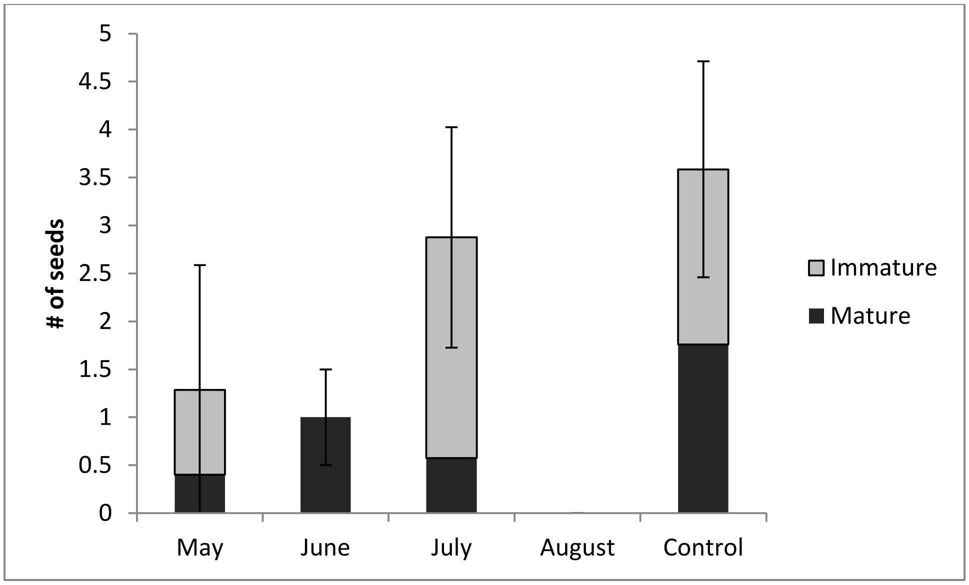


Figure 6: Total seeds/ capitulum (seeds collected in September) for plots mowed in May, June, July, and August 2011, along with un-mowed controls (with standard error bars).

Chapter 3

Investigation into the Cumulative Stress Hypothesis: A Case Study with Spotted Knapweed (*Centaurea stoebe* ssp. *micranthos*) and *Larinus minutus* (Coleoptera: Curculionidae)

Abstract

The Cumulative Stress Hypothesis suggests that successful projects for biological control of weeds are largely due to a combination of stresses when multiple natural enemy species are released. Another hypothesis, the Lottery Model, states that the likelihood of releasing the one (or few) natural enemies that are effective in controlling the weed is increased by the number of species released. The successful knapweed biological control program in the northwestern United States and southwestern Canada has been used to support both the Cumulative Stress Hypothesis and the Lottery Model in areas where multiple knapweed biological control agents are present. I investigated whether *Larinus minutus* will cause a change in spotted knapweed infestations in an area where only one other biological control agent is present (*Urophora quadrifasciata*) and has been shown to be ineffective. Ten sites in the Ozark Plateau region of Arkansas that were infested with spotted knapweed were monitored from 2010 through 2012 using ten static subplots at each site. Changes between years at each site where *L. minutus* was present were compared with plots where *L. minutus* was absent. *Larinus minutus* reduced rosette density during 2011 through 2012 and seed production from 2010 through 2011, but did not show a significant change over the entire study (2010 through 2012). I believe that the lack of

response over the entire study could be a result of the extreme drought conditions over the study area in 2012 and a loss of replicates in the experimental group due to mowing activities.

Introduction

The "Cumulative Stress Hypothesis" (CSH) in the field of biological control of weeds suggests that successful control of a weed is provided by the cumulative stress of multiple natural enemy species that have been released to control it, as opposed to the idea that a single species could provide the control (Harris 1985). Several successful biological control of weeds projects support this hypothesis: *Hypericum perforatum* L. (Australia), *Lantana camara* L. (United States), *Opuntia stricta* (Haworth) var. *stricta* (Haworth) (Australia), and *Rubus argutus* Link (United States) (Denoth et al. 2002).

Other theories have been offered as alternatives to the CSH. "The Lottery Model" (LM) (Myers 1985) suggests that although control of an invasive weed may not occur until many natural enemies have been released, the reduction in weed density is not a function of cumulative stress of the entire suite of natural enemies released, but that one or a few of the natural enemies is responsible. In this model, the likelihood of releasing the one (or few) natural enemies that are effective in controlling the weed (e.g., the "silver bullet") increases with the number released, like a lottery. Examples of single agent successes include *Rubus constrictus* Lefevre and P.J. Miller (Chile), *Salvia molesta* D.S. Mitchell (Zambia), *Sesbania punicea* (Cavanille) Bentham (South Africa), *Sonchus arvensis* L. (Canada), and *Pistia stratiotes* L. (Australia) (Denoth et al. 2002).

There is debate on which of these hypotheses best explains successes in weed biological control programs as well as debate on how many biological control agents are necessary to achieve control. This is a long-standing debate, with evidence for both sides coming sometimes from within the same weed system (Huffaker 1959). This is often the case because a species able to provide successful control in one region may be unable to provide control in another due

to climatic or other factors (Crawley 1989). Here I explore which hypothesis may explain the success of the biological control of spotted knapweed in the United States and Canada.

Spotted knapweed is an invasive herbaceous weed in the Asteraceae. It is a short-term perennial that lives up to 9 years. Seeds germinate in the spring or fall and produce a basal rosette of dissected leaves. If plants germinate in the fall they will typically overwinter as a rosette and bolt (produce a flowering stalk) the following spring. If plants germinate in the spring, they will typically spend one year as a rosette and then bolt the following year (Boggs and Story 1987, Tyser and Key 1988). Spotted knapweed is native to Europe and Asia and seeds were accidentally introduced into North America in the late 1800s. This invasive plant is now present in 46 of 50 states and is listed as a noxious or prohibited weed in 17 of them (United States Department of Agriculture 2007). Spotted knapweed usually dominates dry, disturbed sites and does not compete well with grass species in moist areas (Harris and Cranston 1979). It is most often found on disturbed sites such as roadsides; however, in the Pacific Northwest it is known to invade pastures where it causes the overgrazing of native grasses (Tyser and Key 1988, Lacey et al. 1990). Spotted knapweed infests over 3 million ha in the US (DiTomaso 2000) and increases soil surface runoff and stream sediment yields (Westbrooks 1998), reduces soil infiltration, inhibits canopy undergrowth due to allelopathic effects (Bais et al. 2003), and reduces native plant diversity (Sheley and Barko 1999). Infestations of spotted knapweed cause significant economic damage as well as ecological damage. Spotted knapweed contributes to a 50-90% loss of available forage (Sheley and Barko 1999). The weed displaces desirable forage and quickly dominates habitats, making invaded rangeland less valuable, as cattle avoid feeding on the weed (MacDonald et al. 2003). In one of the only estimates, $42 million in direct and

indirect economic damages are estimated to occur annually due to three species of knapweeds in Montana (Hirsch and Leitch 1996).

The knapweed biological control program is one of the oldest successful terrestrial classical biological control programs in North America. Several biological control agents (13), eight seed head feeders and five root borers, have been released and established in Canada and/or the western United States to reduce the spread and control these invaders (Müller and Schroeder 1989, Story 2002). The first releases of knapweed natural enemies started in the 1970s and continued through the 1990s (Story 2002).

There is debate on whether the observed success of the knapweed biological control program is due to a combination of multiple agents (CSH) or to a single agent (LM). Denoth et al. (2002) lists the diffuse knapweed program in Canada a success due to *Agapeta zoegana* L. (Lepidoptera: Tortricidae), *Sphenoptera jugoslavica* Obenberger (Coleoptera: Buprestidae), and *Urophora affinis* (Frauenfeld) (Diptera: Tephritidae). Seastedt et al. (2007) agree with CSH explaining the success, but they cite different natural enemies as responsible (*S. jugoslavica*, *U. affinis*, *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae), and *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae)). Knochel et al. (2010) found a reduction in spotted knapweed biomass and flower production over varying soil nutrient levels with the presence of *L. minutus* and *C. achates*.

Myers et al. (2009) investigated the control of diffuse knapweed (*Centaurea diffusa* Lam.) infestations in areas where four natural enemy species (*Larinus minutus, Urophora affinis*, *Urophora quadrifasciata*, and *Sphenoptera jugoslavica*) are established in British Columbia Canada. Diffuse knapweed declined at sites where *Larinus minutus* became established and

where numbers of the weevil were high, but a reduction of diffuse knapweed is not seen at other sites where all 4 natural enemy species are present, but *L. minutus* was not present in high numbers. Story et al. (2006) found that densities of spotted knapweed (*Centaurea stoebe* L. ssp. *micranthos* (Gugler) Hayek) were reduced in areas where the root-weevil *C. achates* and six other agents (*Agapeta zoegana, U. affinis, U. quadrifasciata, Metzneria paucipunctella* (Lepidoptera: Gelechiidae), *Larinus obtusus* (Coleoptera: Curculionidae), and *L. minutus*) were found, and spotted knapweed densities remained high in areas with the same natural enemies without *C. achates.* Both of these cases (Story et al. 2006, Myers et al. 2009) appear to support the lottery model and the effectiveness of a single natural enemy, but neither study can show if the reduction in knapweed density is due to a single natural enemy or if they actually support the cumulative stress hypothesis because of the presence of the other natural enemies.

A 2006 survey for knapweed natural enemies in Arkansas yielded only one species, *Urophora quadrifasciata*. Duguma et al. (2008) found that *U. quadrifasciata* significantly reduces the number of seeds produced by spotted knapweed by 44% late in the season (August), when the plants are more environmentally stressed. The fly does not, however, significantly reduce the number of seeds produced earlier in the season, when knapweed is most robust (Duguma 2008). Powell (1990) showed that even a 95% decrease in seed production, by three biological control agents, does not significantly reduce knapweed density. Thus, it may be assumed that *U. quadrifasciata* alone will not significantly suppress knapweed populations in Arkansas, or stop its spread further into the southern United States.

Larinus minutus was first released into the United States in 1991 (Story 2002)**.** Knapweed levels started to decline after the release of *L. minutus* (Smith 2004, Myers et al. 2009)**.** This reduction in knapweed could support the CSH if the reduction is a result of the

cumulative stress of all 13 arthropod species released. However, it may also support the LM, where knapweed was reduced, not because of the cumulative stress of all agents released, but because as the number of agents released increased, so did the chance that the "right" agent would be among them (Myers 1985). In this case, *L. minutus* could be the *"*silver bullet*"* and be responsible for the decline of knapweed in Western North America. An investigation in Western North America into which hypothesis explains the decline of knapweed is impossible, due to the widespread establishment of many (if not all) of the 13 natural enemies species released. The presence of only one spotted knapweed biological control agent in Arkansas, combined with the evidence that the agent is ineffective in reducing knapweed seed when the plant is actively growing, gave us a unique opportunity to be able to contribute to the discussion of the LM/CSH as it pertains to spotted knapweed biological control. *Larinus minutus* has been released an established in Arkansas in order to test the CSH hypothesis in areas with only one other biological control agent (*U. quadrifasciata*) (Minteer et al. 2011).

Larinus minutus is a univoltine species that first appears on knapweed plants in the Western United States in June, typically four weeks previous to knapweed flower buds appearing. In Arkansas, where this study was conducted, weevils become active in early May, approximately four weeks before flowers are seen (Chapter 1). Females typically feed on new foliage and on knapweed flowers. Flower feeding results in ovarian development. Weevils copulate throughout the growing season and oviposition occurs in newly opened flower heads. Larvae pass through three instars and development takes approximately four weeks (Groppe 1990). Larvae feed on the seeds and pupate in the capitula, making a cocoon out of the seed head material (Kashefi and Sobhian 1998). In Arkansas, adults emerge in July and feed on plants until winter, when they will overwinter in the plant litter and emerge in May (unpublished

data). One of the benefits of investigating this system in Arkansas is the ability to evaluate the impact of *L. minutus* in the absence of a full suite of competing natural enemies. Spotted knapweed is currently spreading further in the southeastern United States, where no comprehensive control program exists.

There are risks with any biological control program and these risks increase as the number of agents released increases. Risks include damage caused to non-target plants and insects, (McAvoy et al. 1987, Diehl and McEvoy 1990, Louda et al. 1997), damage to structures (Huelsman et al. 2002, Koch and Galvan 2008), higher trophic level effects of predators consuming biological control agents (Pearson and Callaway 2008), and sometimes human-health issues (Albright et al. 2006). With the risk associated with any release of a biological control agent, it is intuitively safer to release the fewest number of species possible that will still provide effective control. Negative effects from other natural enemies released during the knapweed biological control program in western North America have been reported. These effects include risks to human health and the reduction of native plants by second-order apparent competition (Pearson 1999, Pearson and Callaway 2006, Pearson and Callaway 2008). In order to reduce the risk associated with the spotted knapweed biological control program in Southeastern United States, the fewest number of agents needed to provide adequate control should be released. Objectives of this project were to investigate the effect of *L. minutus* on spotted knapweed in areas where the only other biological control agent present is *U. quadrifasciata.* I hypothesized that seed production will be reduced in areas with *L. minutus* soon after establishment of the weevils, but that will not lead to a reduction in plant density until the seed bank is weakened (7-10 years).

Materials and Methods

Non-release sites

Spotted knapweed populations fluctuate over time naturally, so it was essential to conduct baseline studies throughout the course of the study. Ten sites were selected in the northern portion of Arkansas within the Ozark Plateau Region. Sites were of varying size due to the difference in the sizes of spotted knapweed patches, but all occurred along roadsides. *Larinus minutus* has the ability to fly and can spread once released, so non-release sites were geographically separate from insect release sites and were checked twice annually to monitor for presence of *L. minutus*. Populations of *U. quadrifasciata* were expected to be found at every site. Both *L. minutus* and *Cyphocleonus achates* were released by the Missouri Department of Transportation (MODOT) during the summers of 2008 and 2009 (Swanigan, *pers. comm*.). The closest MODOT release to any one of my surveyed sites was 29 km. In order to determine if the weevils had spread into Arkansas from these releases, non-release sites and surrounding areas were sampled using a sweep net in June/July and September of 2010 through 2012.

Stratified random sampling was used to survey each site, beginning in June 2010. Stratified random sampling was used to insure that the sites' variability was accurately measured. Sites were divided into 10 equal-sized segments. A single 2 m X 0.5 m quadrat was randomly positioned within each of the segments, making sure that each quadrat was placed beyond the cut line (limit of mowing). A coin toss determined the orientation of the rectangular quadrat, unless there was a hill or other density-affecting gradient. If a gradient exists the quadrat was orientated to capture the potential variation in plant population dynamics due to the gradient. The quadrats were permanently marked and re-visited once per year from 2010 through 2012. Quadrats were surveyed for number of spotted knapweed plants, stems, capitula, seedlings, rosettes, and bolted plants. Rosettes were defined as non-bolted spotted knapweed plants that had dissected leaves. If no dissected leaves were present on a rosette, it was considered a seedling. Diameter of

rosettes and height of bolted plants were also measured. Five capitula were randomly selected and were bagged in each of the quadrats. Capitula selected were mature, but had not opened to spread seed. Bags were constructed of nylon tulle and were cinched around the branch of the plant with thick string. The bags were collected from the field in September, placed in paper sacks and held in the lab. The capitula were dissected to record the number and weight of mature and immature seeds.

Release sites

Releases of *L. minutus* were made at 9 sites in Washington County, Arkansas during the summers of 2008 and 2009. Because many of the spotted knapweed infestations are in disturbed, government-managed areas (such as roadsides), sites were carefully selected to reduce the probability of plants being mowed until establishment occurred. Release sites were surveyed in the same manner as non-release sites and were also monitored regularly to confirm presence of the weevils. *Larinus minutus* must have been established at a site for the site to be retained as a release site in this study. Four release plots were chosen for evaluation; however, only three plots were successfully surveyed all three years of the study, due to one of the release plots being completely mowed on several occasions.

Statistical analysis

The change in plant density variables (number of stems, bolted plants, seedlings, capitula, rosettes) as well as plant height and rosette diameter for each subplot at each site were calculated. Average change for each of these plant variables was calculated for each site, in each year (2010-2011 and 2011-2012), as well as for the entire period of study (2010-2012). T-tests were performed on the change data for the sites using JMP 10 (SAS Institute 2012). Change data were analyzed instead of directly analyzing plant density parameters to reduce year to year

variability by site and to determine if the presence of *L. minutus* was causing an overall reduction in plant density over time at the release plots. T-tests were also performed on the average # of seeds/capitulum. Sites that were compared statistically from year to year were subject to disruption by extrinsic forces. Mowing activities in Arkansas are sub-contracted by the Arkansas Department of Transportation to private companies or individuals. Guidelines require that roadsides are mowed three times per year with the sides of undivided roadways being mowed 3 m (10 ft) on each side of the roadway on the first and second mow of the year and a 9.1 m (30 ft) swath is mowed on the third mow (or up to the fence line or well-established vegetation lines). These guidelines are the minimum requirements. While all sites were completely mowed at the end of each season (after surveys have been completed) there were a few instances when a site was mowed completely before the plot was surveyed. If a plot was mowed prior to survey, it was not included in the analysis of change for that year. For example, if plot A was mowed pre-survey in 2012, but not in 2010 or 2011, it was included in the change analysis for the period of 2010-2011, but not 2011 through 2012 OR 2010 through 2012 and data for seed production was only used from 2010 and 2011.

Results

Plant density

There were no differences in the change in any spotted knapweed "life-stage" densities (seedling, rosette, or bolted) between release and control sites during the first year (2010-2011) or over the length of the study (2010-2012), but there was a difference in change in rosette density over the period from 2011-2012 (P=0.0147). There was a greater change in the number of rosettes in the release plots (-8.1 rosettes) when compared to the control plots (+0.5 rosettes), but no other differences in plant "life-stage" densities were found during that same time frame.

Changes in spotted knapweed "life-stage" densities were variable over the term of the study in both the release and non-release plots (Figure 1).

Plant size

There were no differences in change in plant size variables (rosette diameter, plant height, # of stems/plant) during the first year of study or the last year of study. The change in plant height in the release plots showed a trend to be numerically reduced (-8.53 cm) and the change in plant height in the control plots a trend toward numerical increase (+12.34 cm) on average (P=0.059), but these differences were not statistically significant. This trend was not seen in 2011-2012 or over length of the study (Figure 2).

Seed production

There was a difference in the average number of mature seeds/capitulum between release and control plots (7.2 and 13.5 seeds, respectively) in 2010 (P=0.0005), but not in 2012 (11.6 release and 12.1 control. P=0.87). Seeds were not counted in 2011, as there were a low number of seed bags that were found undamaged. There was no difference in the number of immature seeds, immature seed weights, or mature seed weights during any time of the study. There was also no difference in the change in the number of capitula during any year of study or over the entire length of the study.

Discussion

Many biological control programs take >10 years to bring the target weed under control (McFadyen 1998), and therefore, three years is not long enough to evaluate the efficacy of the spotted knapweed biological control program in Arkansas. Although three years is not a sufficient number of years to study the efficacy of *L. minutus*, these data contribute to the discussion of the Cumulative Stress Hypothesis/Lottery Model as it illustrates the ability of a

single biological control agent species (added to system with an ineffective species) to effect the density of plants, even over a short period of time. This study also provides a good foundation for longer studies of this system to determine if the presence of *U. quadrifasciata* and *L. minutus* (as opposed to many agents) will lead to effective control of spotted knapweed in the Southeast**.**

Plant density

Plant density variables at the beginning of the study were similar for release and control plots, but almost all plant variables in the plots with *L minutus* increased in density/size (non-significant) between 2010 and 2011. Although the trends seen were not significant, they are interesting to note, as control plots did not show this same trend (Figures 1 and 2). Some plants show an ability to compensate for herbivory with increased growth or reproduction (Lennartsson et al. 1998, Poveda et al. 2003)**.** This has also been shown with spotted knapweed with feeding from both *Agapeta zoegana* and *Cyphocleonus achates* (Steinger and Müller-Schärer 1992, Newingham et al. 2007, Ortega et al. 2012). The trend of almost all plant variables measured increasing the first year of study in the release plots could be evidence for compensatory growth; however, this trend for was only observed between year one and year two. During 2011-2012 most plant density variables showed a trend to decrease or stay level in both the release and control plots. This could be due to the extreme drought throughout the study area in 2012. Prior to 2011, there was a drought in northwest Arkansas in 2006 (based on the Palmer Hydrological Drought Index, which monitors long-term conditions). Following the drought in 2006, most months during the growing season were listed as moderately- to extremely-moist (2007-2009). In 2010 the Palmer Hydrological Drought Index was listed as "normal" for the study area in June, July, and August. In 2011 June was listed at "moderately moist" and July and August were classified as "normal". In June 2012 the study area was classified as "moderate drought" and as

“severe drought” in July and August. Additional years of study are needed to determine if plant compensation, due to feeding by *L. minutus,* is occurring in this system.

The only significant difference seen in plant density measurements in this study was rosette density in 2011-2012. Rosette densities decreased more in the release plots during this time period, than the control plots. This could be attributed to *L. minutus* herbivory killing rosettes, a combination of the stress of herbivory and the 2012 drought, a decrease in the number of seeds being deposited into the seed bank from the previous year (see seed production), *L. minutus* feeding on seedlings and thereby reducing the number of seedlings growing into rosettes, or it could be that the herbivory stimulated more plants to bolt, therefore “reducing” the number of rosettes present. Life table studies on spotted knapweed may help to pinpoint the plant stage that is most susceptible to *L. minutus* feeding and help investigate if this reduction in rosettes will continue.

Plant size

Although there were no differences in the change of plant size variables (rosette diameter, plant height, # of stems/plant) during 2010-2011 or 2011-2012, there was a trend seen in the change in plant height during 2010-2011. This trend was only seen the first year and was not seen the second year (2011-2012) or over the length of the study (2010-2012). This could be a response to the wide range of moisture conditions over the term of the study. Spotted knapweed plants have been shown to respond to herbivory differently over varying soil nutrient levels. Under high soil nitrogen levels herbivory reduced shoot biomass by 30%, but under lower N levels shoot biomass was reduced 63%, when compared to plants with no herbivores present (Steinger and Müller-Schärer 1992). It is possible that responses to herbivory would be varied over varying moisture levels as well. Change values from year to year were analyzed. When

control plants (no *L. minutus*) had enough moisture, they were able to thrive and increase in height by 12.34 cm (on average), while plants under pressure by *L. minutus* decreased in plant height (-8.53 cm) on average. During 2011-2012, when hydrologic conditions worsened, both control and experimental plants decreased in height (-25.17 cm and -15.34 cm respectively). Over the entire length of the study both control and release sites increased in average plant height (+11.24 cm and +4.13 cm, respectively), but the response was not significant. One of the three *L. minutus* release sites had a drastic reduction in the number of adult knapweed plants between 2011 (10.5 plants/m^2) and 2012 (0 bolted plants/m^2). There were no bolted plants in any of the quadrats surveyed. Without bolted plants in 2012 the change in plant height for the period of 2010 through 2012 could not be calculated, so there were only two release plots used during the plant height analysis. This extremely low number of replicates for the release plots could have affected the ability to achieve significance.

Seed production

Seed production was significantly lower in release plots than in control plots in 2010, but not in 2012. Variability in the release sites due to the low number of replicates could be the cause. As with the plant height analysis, one of the three release plots did not have any bolted plants present. Without bolted plants there were no capitula to dissect to determine average seeds/capitulum, thus data from only two release sites were available to analyze. I believe that there would have been a statistically significant difference in the average number of seeds produced in 2012 if there were more replicates.

Although there were not any reductions in plant density measurements or seed production over the length of the study (2010 through 2012), there were changes/reductions in several plant variables measured between years of the study. I believe that the drastic changes in the long

term hydrological conditions (represented by the Palmer Hydrological Drought Index) had a profound effect on the results seen in this study and may be evidence of varying response to herbivory over different levels of water stress. More work is needed to test this hypothesis. Although efficacy of a biological control agent cannot be determined during a three-year period, this study illustrated short-term effects of *L. minutus* on spotted knapweed infestations. Additional work to determine if *L. minutus* can reduce spotted knapweed infestations without the addition of other effective natural enemies will continue.

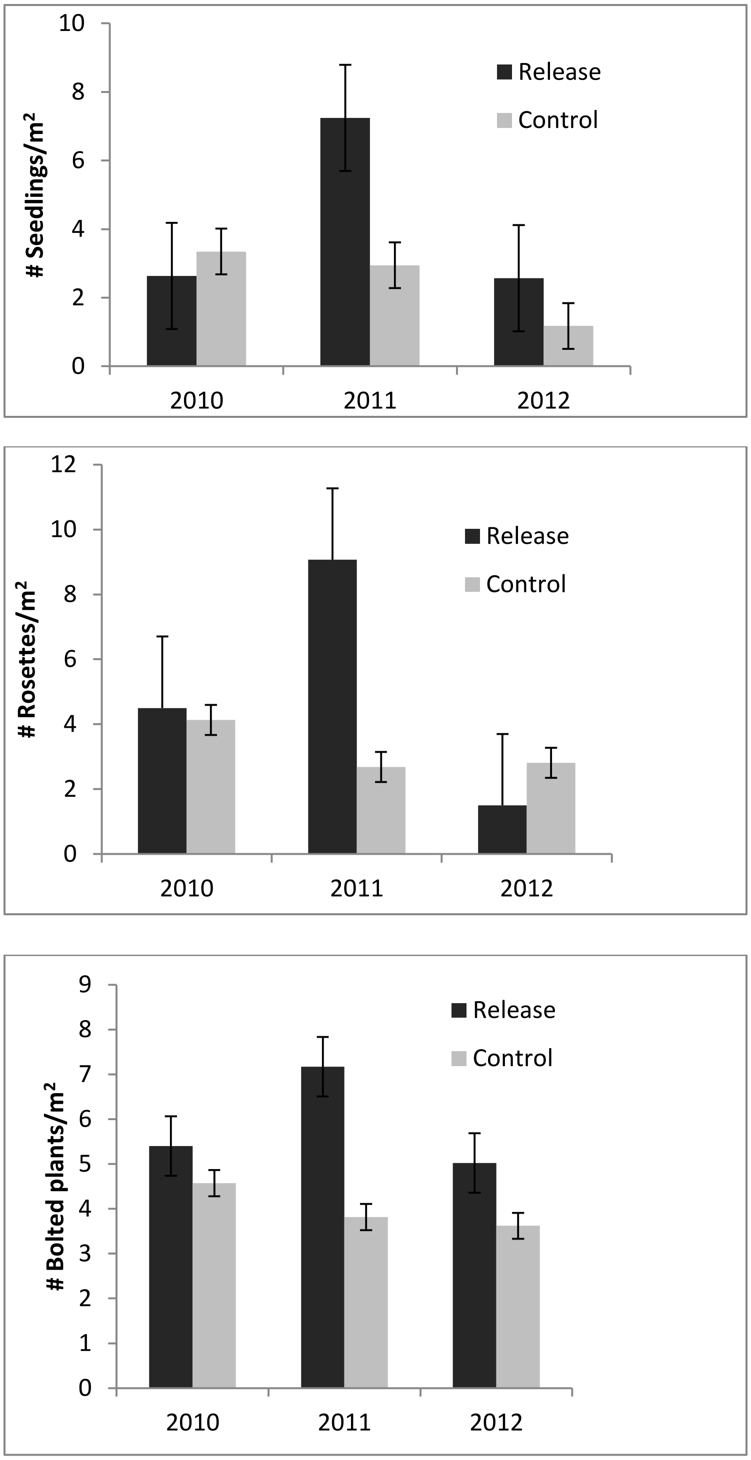


Figure 1: Spotted knapweed plant density measurements per square meter over all growth-stages of the plant (seedlings, rosettes, and bolted plants) for plots with *Larinus minutus* present (Release) and absent (Control) (with standard error bars).

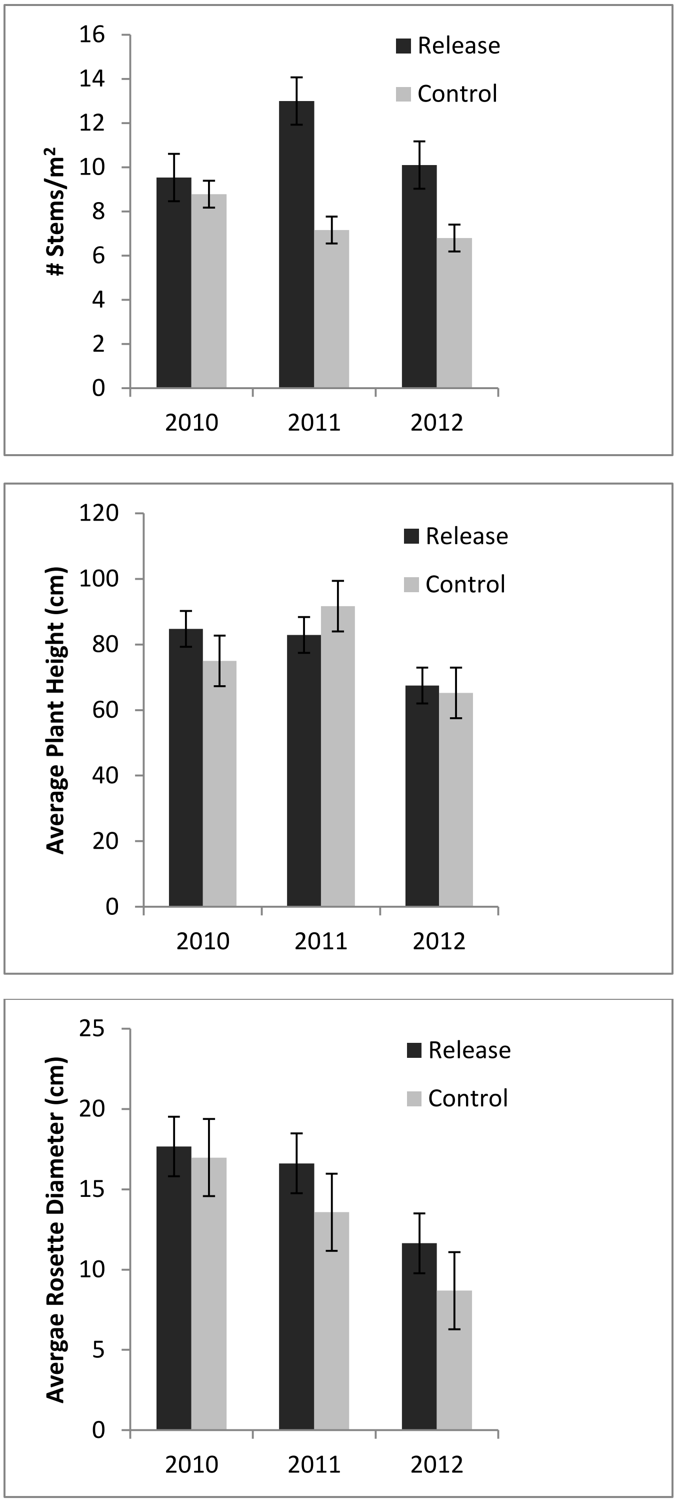


Figure 2: Spotted knapweed plant-size measurements (# stems, plant height, rosette diameter) for plots with *Larinus minutus* present (Release) and absent (Control) from 2010 through 2012 (with standard error bars).

Chapter 4

Interactions between Two Seed-Feeding Insects in the Capitula of Spotted Knapweed

Abstract

Spotted knapweed is an invasive plant from Eurasia that causes severe damage in rangelands and pastures, where it can reduce forage by up to 90%. In Arkansas, two seed-feeding biological control agents for spotted knapweed, *Larinus minutus* and *Urophora quadrifasciata* are established. The larvae of both *U. quadrifasciata* and *L. minutus* feed on the developing seeds inside the capitula of spotted knapweed. This seed-feeding habit of both species results in interactions between *U. quadrifasciata* and *L. minutus* larvae. It is currently unknown if the interaction between these two natural enemies is synergistic, antagonistic, or neutral when it pertains to spotted knapweed seed production. Spotted knapweed capitula were collected weekly from three sites in northwest Arkansas over a period of two years. Capitula were monitored for emergence of *L. minutus* and *U. quadrifasciata*. Data were split into two groups: capitula with and without seeds present upon dissection. Frequency of the presence or absence of *L. minutus* and *U. quadrifasciata* was analyzed for both groups. Seed counts were analyzed by year and time of season (early-, mid-, and late-season). A Fisher's exact test determined that the occurrences of the *L. minutus* and *U. quadrifasciata* are not independent of each other in either group (capitula with or without seed). There were no differences in seed production (for either year) in the early- or late- season based on the presence or absence of *L.*

minutus, *U. quadrifasciata*, or both species in the capitula. There was a difference in the number of seeds produced between capitula with *L. minutus* and capitula without *L. minutus* during mid-season of both years. This result indicates that *L. minutus* should be an effective biological control agent of spotted knapweed in Arkansas. It is clear from these results that there is an interaction between the presence of *Larinus minutus* and *Urophora quadrifasciata* inside the capitula of spotted knapweed, but is unclear if this interaction will have any effect on seed production.

Introduction

Spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek is an invasive perennial plant from Eurasia that was accidentally introduced into North America in the 1890s. Spotted knapweed now infests over 3 million ha in the United States (DiTomaso 2000). Infestations of spotted knapweed increase soil surface runoff and stream sediment yields (Westbrooks 1998), reduce native plant diversity, and can contribute up to a 90 % loss of available forage, making invaded rangelands less valuable because cattle avoid feeding on bolted plants (Sheley and Barko 1999, MacDonald et al. 2003).

A biological control program for diffuse (*Centaurea diffusa* L.) and spotted knapweeds was started in North America in the 1960s. Since that time 13 different insect species have been released and established in the United States and Canada (Story 2002). A survey in 2006 of biological control agents in spotted knapweed infestations in Arkansas was conducted (Kring, unpublished data). Only one natural enemy, *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) was found in this survey. Insecticidal exclusion studies demonstrated that *U. quadrifasciata* does not reduce spotted knapweed seed production during the peak of the growing season (late June through July). However, *U. quadrifasciata* does reduce seed production later in the growing season, although seed production is presumably lowest at this time (Duguma 2008). These studies suggest that *U. quadrifasciata* alone does not reduce spotted knapweed infestations effectively, so addition of another natural enemy is warranted.

Larinus minutus Gyllenhal (Coleoptera: Curculionidae) was selected for release based on evidence from British Columbia and the northwestern United States that it is effective in reducing knapweed infestations (Myers et al. 2009). *Larinus minutus* was released in Arkansas

from 2008 through 2012 and established in several counties (Minteer et al. 2011). The larvae of both *U. quadrifasciata* and *L. minutus* feed on the developing seeds in spotted knapweed capitula. This seed-feeding habit results in interactions between *U. quadrifasciata* and *L. minutus* larvae. It is currently unknown if the interaction between these two natural enemies is synergistic, antagonistic, or neutral when it pertains to spotted knapweed seed production.

Urophora quadrifasciata

Urophora quadrifasciata oviposit under the bracts of developing spotted knapweed flower buds (Rees and Story 1991, Burkhardt and Zwölfer 2002). Eggs hatch in three to four days and larval development (3 instars) takes approximately 3 weeks (Harris and Shorthouse 1996, Nowierski et al. 2000). Larvae feed and develop inside the ovary of the capitulum and form a thin, papery gall within the ovary (Harris 1980). In Arkansas the majority of *U. quadrifasciata* emerge between June and October, but 38% enter diapause and overwinter in capitula, emerging as adults the following year (Duguma et al. 2009). *U. quadrifasciata* has up to three generations in Arkansas. The second and third generations attain a peak of adult numbers around the end of June and July, respectively, and up to 12 flies emerge per capitulum (Duguma et al. 2009)**.**

Larinus minutus

Larinus minutus is a univoltine species that is native to Europe and was first released into the United States in 1991 (Story 2002). In Arkansas, *L. minutus* emerges from overwintering sites and becomes active in late-April to early May (Chapter 1). Adult weevils feed on spotted knapweed vegetative stems until flowering occurs. Weevils prefer to feed on flowers once they are available (Groppe 1990). Mating occurs throughout the growing season and oviposition occurs in newly opened flower heads. During laboratory studies, oviposition occurred for 11

weeks (Groppe 1990). Eggs hatch in three to four days (under laboratory conditions, 25° C). Larva1 development takes approximately four weeks and larvae go through three instars (Groppe 1990). Larvae feed on the developing seeds and pupate in the capitulum, making a cocoon out of the seed head material (Kashefi and Sobhian 1998). The adults emerge in mid-July in the southeastern United States and feed on plants until winter, when they overwinter in the leaf litter and emerge in late-April to early May (Chapter 1). The overlap of adult emergence of *L. minutus* and the high numbers of *U. quadrifasciata* emerging from capitula throughout the summer greatly increase the chance for interaction.

Interactions between *Larinus minutus* and *Urophora* spp.

With the temporal overlapping of larval *L. minutus* (emerge in mid-July) and *U. quadrifasciata* (emerge in late June and late July) within the capitula, it is possible that interactions between the two species occur. If the presence of *U. quadrifasciata* negatively impacts the oviposition or the seed feeding of *L. minutus*, which has been shown to be a more efficient natural enemy (Minteer *in review*, Duguma 2008), interactions between these two biological control agents could lead to a less effective biological control program.

No studies on interactions between *U. quadrifasciata* and *L. minutus* have been reported. This is probably because *U. quadrifasciata* and *Urophora affinis* Frfld. (Diptera: Tephritidae) are both located in the areas where any interaction studies have been conducted and *U. affinis* reduces the ability of the first generation of *U. quadrifasciata* to reproduce (Berube 1980). Studies on the interactions between *L. minutus* and *U. affinis,* as well as *L. minutus* and *Urophora* spp. (*U. affinis* and *U. quadrifasciata*), however, have been conducted. Like *U. quadrifasciata, U. affinis* is not very effective in reducing knapweed densities. *Larinus minutus* consumes up to 40% of *Urophora* species encountered in the capitula of spotted knapweed

(Seastedt et al. 2007). Crowe and Bourchier (2006) found that *U. affinis* negatively affects the attack rate of *L. minutus*, but *L. minutus* does not affect *U. affinis* density. Capitulum attack rates are higher with both *L. minutus* and *U. affinis* present. However, seed destruction in areas with both *U. affinis* and *L. minutus* present is reduced compared to areas with *L. minutus* alone (Crowe and Bourchier 2006)**.**

The objective of this study was to determine if the interaction between *L. minutus* and *U. quadrifasciata* is antagonistic, synergistic, or neutral in regard to seed destruction in spotted knapweed. Based on the results of the studies on the interactions between *L. minutus* and *U. affinis*, I hypothesize that spotted knapweed capitula with only *L. minutus* present will produce fewer seeds than capitula with no natural enemies emerged, *U. quadrifasciata* only, or both *L. minutus* and *U. quadrifasciata.* This study has important implications for the biological control of spotted knapweed in the southeastern United States, as the results will determine if the interaction of *U. quadrifasciata* and *L. minutus* is negative. This will allow biological control practitioners in areas where the weed or the biological control agents are not present the ability to reduce the number of agents released by only releasing effective agents that do not interfere with the effectiveness of another.

Materials and Methods

Three sites were chosen for this study. All sites were located in Washington County, Arkansas. Releases of *L. minutus* were made at these sites in 2008 or 2009. *L. minutus* was confirmed at each of the sites prior to data collected. *Urophora quadrifasciata* was present at all three sites before *L. minutus* releases were made.

In 2010 fifteen mature capitula were randomly collected weekly from each site beginning the first week that mature capitula were available until no suitable capitula were available. A

capitulum was considered mature when the bracts had started to turn brown and flowers in the head had senesced, but the head had not yet opened. The number of capitula collected was increased to 30 per site per week in 2011. Capitula were placed individually into plastic diet cups and covered with a lid and held at room temperature. Emergence from capitula was monitored weekly during periods of high emergence and every other week during periods of low emergence. If an insect emerged from a capitulum, the insect was removed from the cup, the species was recorded, and the lid was replaced. Capitula were monitored for emergence of insects starting the week after collection until spring of the following year. Capitula with un-emerged natural enemies inside were not used in the analysis because it was unknown how long these larvae fed and, therefore, how the feeding would affect seed count.

Data were split into two groups: capitula without seeds present upon dissection and capitula with seeds upon dissection. Frequency of the presence or absence of *L. minutus* and *U. quadrifasciata* was analyzed for both groups. A Fisher's exact test was performed on frequencies of *L. minutus* and *U. quadrifasciata* in both capitula with and without seeds to determine if the presence of these biological control agents in the capitulum was independent of each other. Data for capitula (both with and without seeds) were analyzed by year and time of season: early season (start of collection until third week in June), mid-season (forth week in June through end of July), and late season (August through end of the season) to determine frequencies of biological control agents by time of season.

Presence/absence data were collected and an analysis of variance was run as a two-factor factorial (presence of fly and weevil) on total seed counts for capitula that had seeds observed upon dissection. Multiples of either species in a single seed head were treated as the same as I

was only concerned with presence/absence of the natural enemies. Total seed counts were natural log transformed prior to analysis so that the distribution was normal.

Results

Capitula without seeds

In capitula that had zero seeds upon dissection in 2010 (23) 17% had *L. minutus* only, 35% had *U. quadrifasciata* only, and 47% had neither *L. minutus* nor *U. quadrifasciata*. No capitula with zero seeds in 2010 had both *L. minutus* and *U. quadrifasciata* in a single capitulum. In 2011 62% of capitula without seeds (166) had *L. minutus* only, 10% had *U. quadrifasciata* only, 2% had both *L. minutus* and *U. quadrifasciata,* and 26% had neither *L. minutus* nor *U. quadrifasciata* (Figure 1). A Fisher's exact test indicated that the occurrences of *L. minutus* and *U. quadrifasciata* in capitula with zero seeds were not independent of each other ($P<0.0001$).

Capitula without seed by season

There was only one capitulum present during the early portion of the growing season that had no seeds upon dissection, so data are not presented. In the middle of the season (87 capitula), 60% of capitula without seeds upon dissection had *L. minutus* emerge, 15% *U. quadrifasciata*, 3% *L. minutus* and *U. quadrifasciata*, and 22% with no natural enemies. Fifty-three percent of late season capitula with zero seeds had *L. minutus* emerge, 11% *U. quadrifasciata*, 2% both *L. minutus* and *U. quadrifasciata* and 34% neither *L. minutus* nor *U. quadrifasciata* (Figure 2).

Capitula with seeds present

In capitula that had seeds present upon dissection in 2010 (278) 15% had *L. minutus* alone, 38% *U. quadrifasciata,* 3% both *L. minutus* and *U. quadrifasciata,* and 44% no natural enemy species. In 2011 the capitula that had seed present upon dissection (466) 34% had

Larinus alone, 19% *U. quadrifasciata* alone, 2% both *L. minutus* and *U. quadrifasciata*, and 45% had neither species (Figure 3). Occurrences of *L. minutus* and *U. quadrifasciata* in capitula with seeds present upon dissection (2010 and 2011) were not independent of each other (Fisher's Exact Test, P<0.0001).

Capitula with seed by season

Early in the growing season 6% of the capitula that had seeds upon dissection had *L. minutus* emerge, 41% *U. quadrifasciata*, 1% both *L. minutus* and *U. quadrifasciata*, and 52% neither *L. minutus* nor *U. quadrifasciata*. In the middle of the growing season capitula with seeds showed 27% with an emerged *L. minutus*, 22% *U. quadrifasciata*, 3% both *U. quadrifasciata* and *L. minutus*, and 48% neither *U. quadrifasciata* nor *L. minutus*. At the end of the growing season *L. minutus* emerged from 25% of the capitula with seeds, 24% *U. quadrifasciata*, 1% both *L. minutus* and *U. quadrifasciata*, and 49% neither *L. minutus* nor *U. quadrifasciata* (Figure 4).

All capitula

Of all the capitula dissected (both with and without seed) in 2010 (300) 16% had *L. minutus* emerge, 38% *U. quadrifasciata*, 3% both *L. minutus* and *U. quadrifasciata*, and 44% neither species. In 2011 632 capitula were dissected. Forty percent of these capitula had *L. minutus* emerge, 17% *U. quadrifasciata*, 2% both *L. minutus* and *U. quadrifasciata*, and 41% neither species (Figure 5).

Presence/absence and the effect on seed production

There were no differences in seed production among sites (ANOVA, P=0.35), so all sites were analyzed together. There was, however, a difference in seed production between years (P<0.0001), so 2010 and 2011 data were analyzed separately.

2010

There were no differences in seed production in the early season based on the presence or absence of *L. minutus*, *U. quadrifasciata*, or both species in the capitula (P=0.17) (Figure 6). During the middle of the season there was a difference in the seeds produced as a result of natural enemies present (P<0.0001). There were no differences in seeds produced between capitula with *U. quadrifasciata* or without (P=0.08), or among capitula with *U. quadrifasciata* alone, *L. minutus* alone, or both *U. quadrifasciata* and *L. minutus* (P=0.49). There was, however a difference in seed production between capitula with *L. minutus* and capitula without *L. minutus* (P<0.0001) (Figure 6). There were no significant differences in seed production at the end of the season (P=0.53) (Figure 6).

2011

There were no differences in seed production in the early season between capitula with or without *U. quadrifasciata* (P=0.5) (Figure 7). No *L. minutus* emerged from capitula collected during this time. During the middle of the season there was a significant difference in the seeds produced depending on the natural enemies present (P<0.0001). There were no differences in seed production between capitula with *U. quadrifasciata* or without (P=0.9), or among capitula with *U. quadrifasciata* alone, *L. minutus* alone, or both *U. quadrifasciata* and *L. minutus* (P=0.07). There was, however a difference in seed production between capitula with *Larinus* and capitula without *L. minutus* (P=0.008) (Figure 7). There were no significant differences in seed production at the end of the season (P=0.63) (Figure 7).

Discussion

Capitula with no seeds

In dissected capitula, when no seeds were found, all combinations of *L. minutus* and *U. quadrifasciata* (alone and together) were found. This indicates that both of the biological control agents were able to consume all of the seeds in a capitulum. The lack of seeds in capitula in which no natural enemies emerged indicated that seeds did not develop in all capitula. This could be an artifact of my definition of mature capitula. It is possible that some of the capitula collected did not have sufficient time to develop seeds or were not pollinated. The percentage of capitula with *L. minutus* present and with both *L. minutus* and *U. quadrifasciata* present increased between 2010 and 2011. However, the percentage of capitula with just *U. quadrifasciata* decreased between the two years. This was the same trend as seen in the capitula with seeds. Releases of *L. minutus* at these sites occurred in 2008 and 2009. This increase in the prevalence of *L. minutus* in the capitula without seeds was likely a result of the density of *L. minutus* increasing. The associated decrease in the presence of *U. quadrifasciata* suggested that the occurrences of the two species are not independent, and the Fisher's exact test confirmed this. This could be due to predation of *U. quadrifasciata* by *L. minutus*, as seen with predation of *U. affinis* by *L. minutus* (Seastedt et al. 2007). Specific tests would need to be conducted to determine if this is the case.

The occurrence of both *L. minutus* and *U. quadrifasciata* in capitula (without seeds) collected both in the mid- and the late-season suggest that the biological control agents were still active and able to destroy 100% of the seeds in a capitulum, even late in the season. This has good implications for the efficacy of the biological control program, particularly when considering that spotted knapweed re-sprouts and produces viable seed after mowing in the south (Chapter 2).

Capitula with seeds present

Frequencies of *L. minutus* and *U. quadrifasciata* changed throughout the growing season. Early in the growing season the percentage of capitula (with seeds) with *L. minutus* emerged is lower than at any other time during the growing season. This is expected as adult *L. minutus* don't become active until late-April to early May and feed on flowers (available in mid-May to early June) before ovipositing (Groppe 1990). Flowers were first seen on June 3 in 2010 and June 8 in 2011 (Chapter 1), therefore, there was a limited amount of time for female *L. minutus* to oviposit in early-season capitula. *Urophora quadrifasciata* was prevalent in the early season (when compared to mid- and late-season). *U. quadrifasciata* is multivoltine with the first generation reaching peak numbers around the end of May (Duguma et al. 2009). Both species were still active inside capitula at the end of the growing season.

Presence/absence and the effect on seed production

Differences in number of seeds/capitulum were found between years of this study, even in capitula without either natural enemy. The differences between years could be due to drought conditions experienced during 2011. The drought likely had some impact on the plant, pollinators and the biological control agent. It is not known what caused this difference, but the years were analyzed separately, so that the results would not be confounded by this difference.

No differences in the number of seeds/capitulum based on the presence or absence of *L. minutus or U. quadrifasciata* were seen during the beginning or the end of the growing seasons in 2010 or 2011, but there were differences seen between capitula with *L. minutus* and without *L. minutus* during mid-season of both years, when *L. minutus* is most active in the capitula (Chapter 1). *Larinus minutus* can destroy up to 100% of the seeds in a capitulum (Kashefi and Sobhian 1998), so this result was not surprising. This result, along with significant seed reduction seen in

the efficacy trials (Chapter 3), indicates that *L. minutus* should be an effective biological control agent of spotted knapweed in Arkansas.

It is clear from these results that there is an interaction between the presence of *Larinus minutus* and *Urophora quadrifasciata* inside the capitula of spotted knapweed. It is unclear if this interaction will have any effect on seed production. There were extremely low numbers of capitula with both *L. minutus* and *U. quadrifasciata* present. Additional studies are needed to determine if there is extrinsic competition between adults or changes in oviposition sites based on prior presence of the other species.

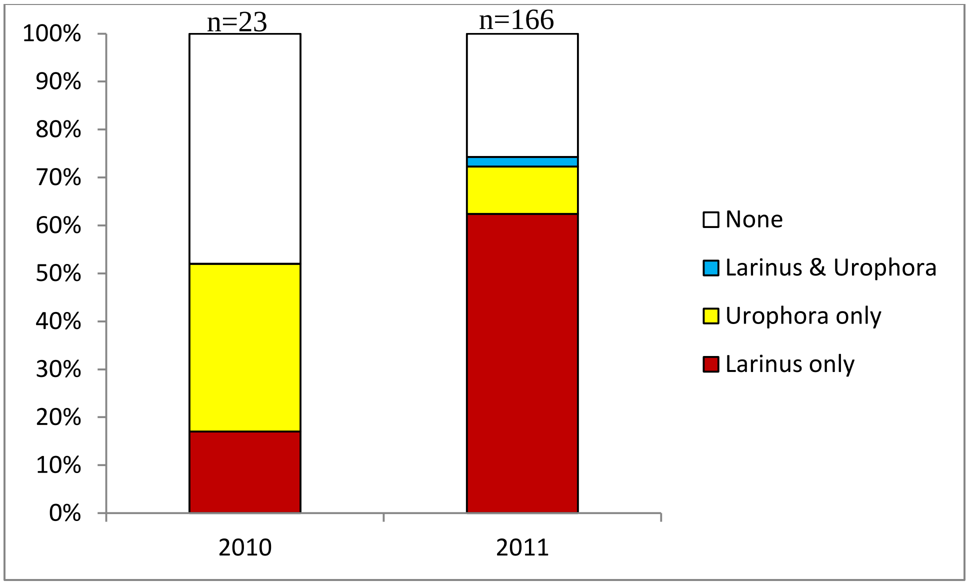


Figure 1: Capitula collected from three sites in Northwest Arkansas that had zero seeds upon dissection with the percentages of *Larinus minutus, Urophora quadrifasciata*, both *L. minutus* and *U. quadrifasciata*, or no natural enemies that emerged from the capitula.

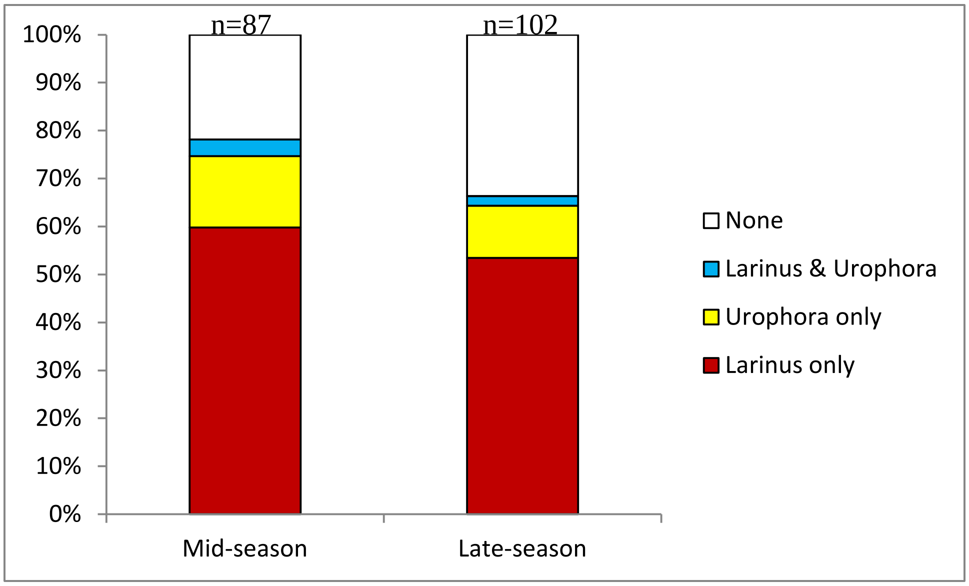


Figure 2: Capitula collected from three sites in Northwest Arkansas during mid-season (fourth week of June through end of July) and late-season (August through the end of season) of 2010 and 2011, that had zero seeds upon dissection with the percentages of *Larinus minutus*, *Urophora quadrifasciata*, both *L. minutus* and *U. quadrifasciata*, or no natural enemies that emerged from the capitula. There was only one capitulum with zero seeds early in the year, so it was not included.

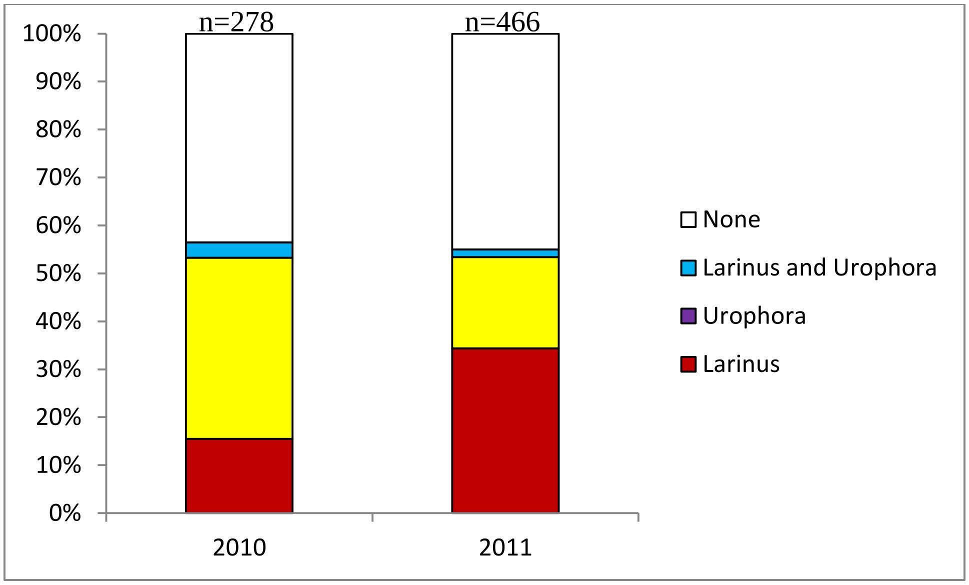


Figure 3: Capitula collected from three sites in Northwest Arkansas in 2010 and 2011 that had seeds upon dissection with the percentages of *Larinus minutus, Urophora quadrifasciata*, both *L. minutus* and *U. quadrifasciata*, or no natural enemies that emerged from the capitula.

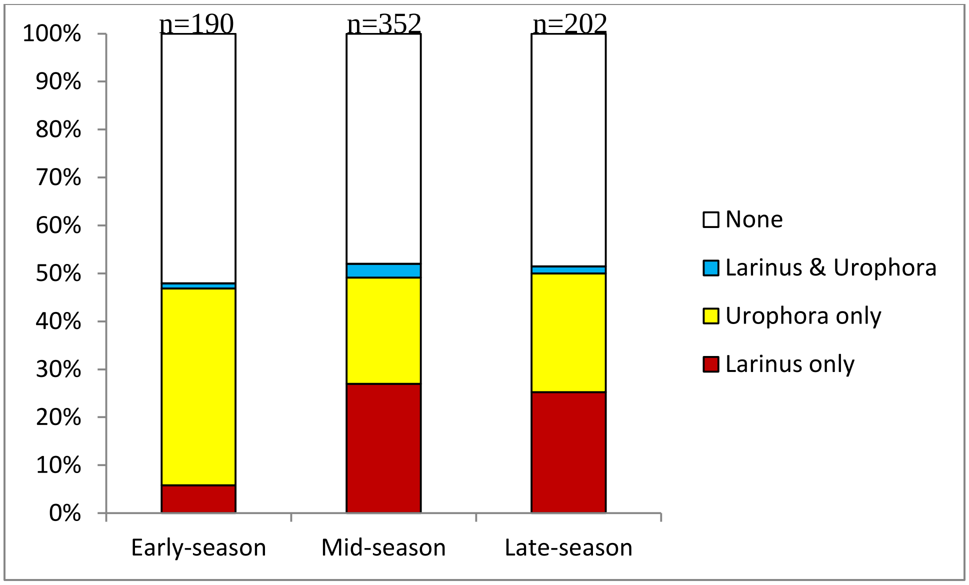


Figure 4: Capitula collected from three sites in Northwest Arkansas during early-season (start of flowering through third week in June), mid-season (fourth week of June through end of July) and late-season (August through the end of season) of 2010 and 2011, that had seeds present upon dissection with the percentages of *Larinus minutus, Urophora quadrifasciata*, both *L. minutus* and *U. quadrifasciata*, or no natural enemies that emerged from the capitula.

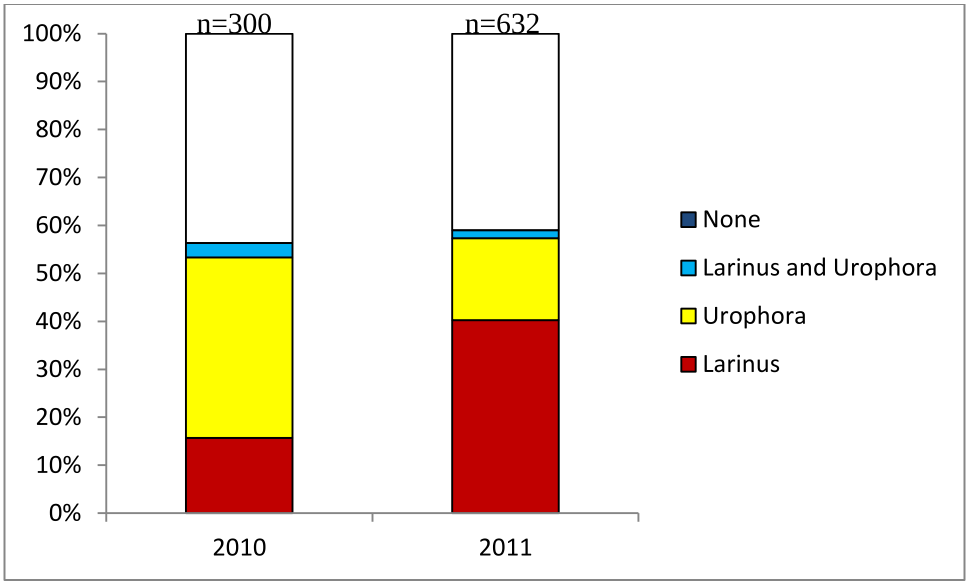


Figure 5: Percentages of *Larinus minutus*, *Urophora quadrifasciata*, both *L. minutus* and *U. quadrifasciata*, or no natural enemies that emerged from the capitula (both with and without seeds present) collected from three sites in Northwest Arkansas from 2010 and 2011.

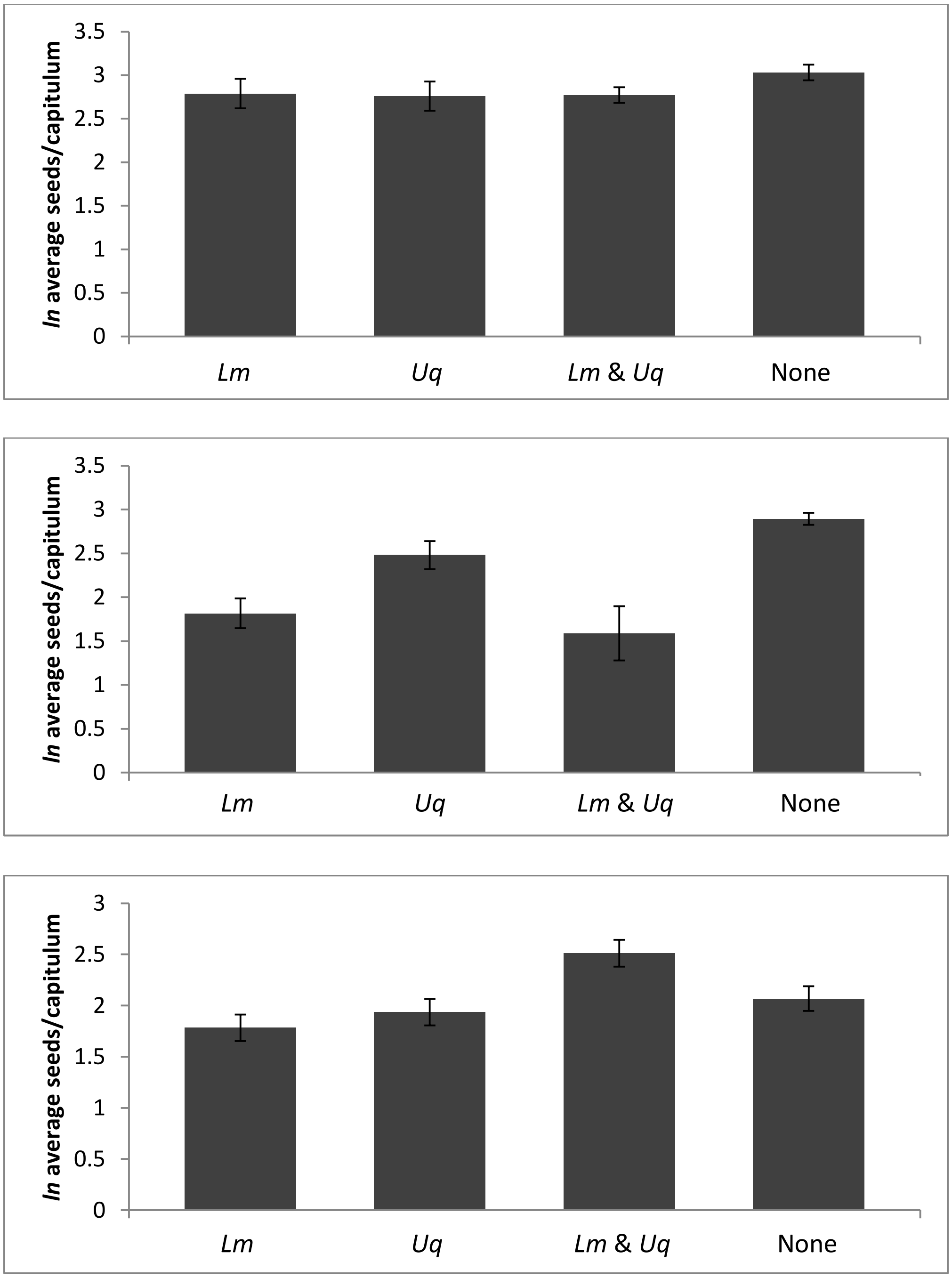


Figure 6: Natural log of the average number of seeds/capitulum for capitula that *Larinus minutus* (*Lm*), *Urophora quadrifasciata* (*Uq*), both *L. minutus* and *U. quadrifasciata*, or no natural enemies have emerged. Averages were calculated from capitula that were collected weekly during 2010 in Washington County, Arkansas during early (top), mid (middle) and late (bottom) season with standard error bars.

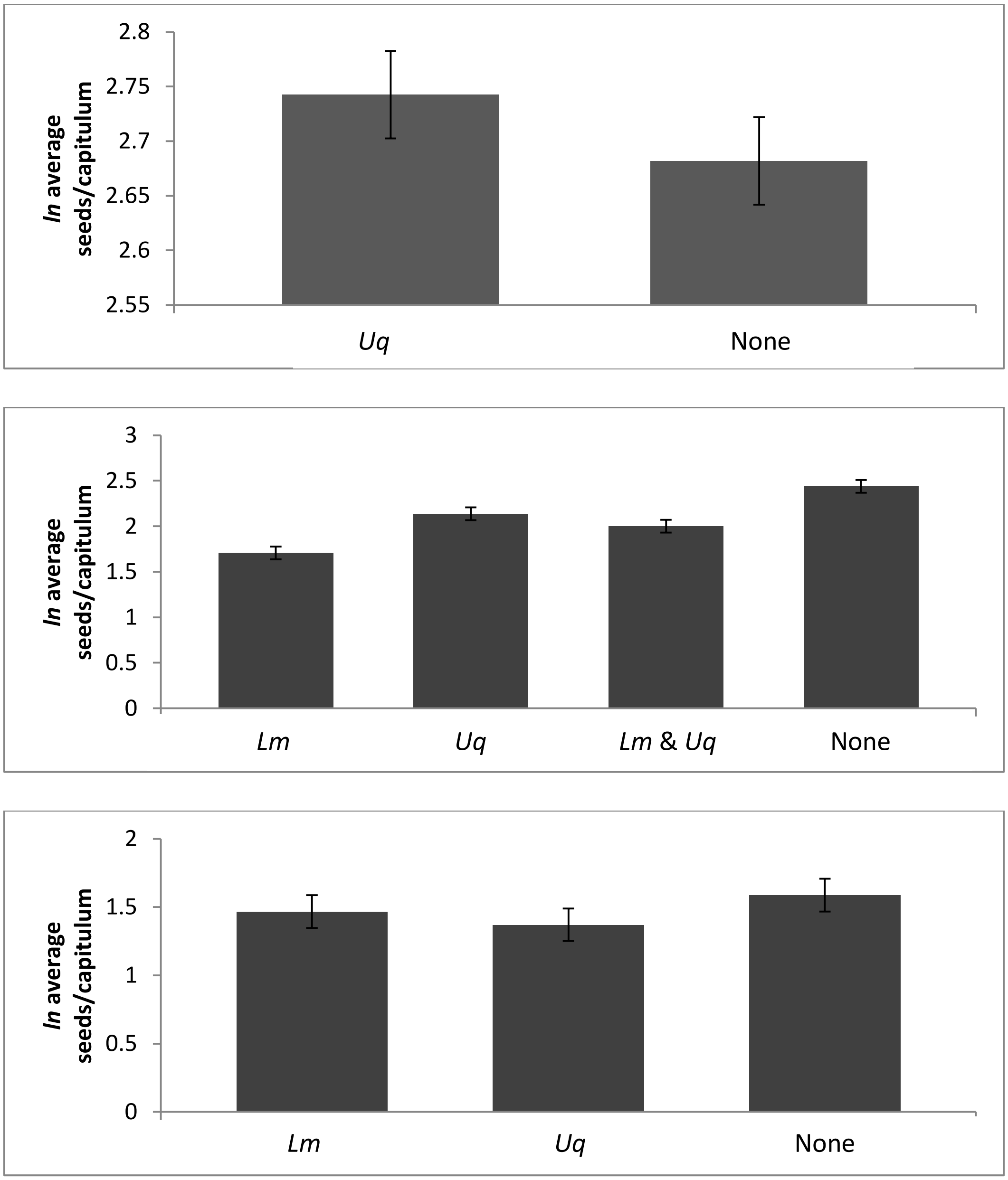


Figure 7: Natural log of the average number of seeds/capitulum for capitula that *Larinus minutus* (*Lm*), *Urophora quadrifasciata* (*Uq*), both *L. minutus* and *U. quadrifasciata*, or no natural enemies have emerged. Averages were calculated from capitula collected weekly in 2011 in Washington County, Arkansas during early (top), mid (middle) and late (bottom) season with standard error bars. *Larinus minutus* was absent from early season collections and the combination of *L. minutus* and *U. quadrifasciata* was absent from late season collections.

Chapter 5

Feasibility of Using Multispectral Remote Sensing to Monitor Spotted Knapweed (*Centaurea stoebe* ssp. *micranthos*)

Abstract

Biological control by arthropods is a cost-effective, environmentally friendly, and safe means to control invasive weeds. An often over- looked facet of a biological control program is long-term monitoring of invasive plant populations after the arthropod agent is established. Monitoring is rarely continued for long periods of time, because of the expense associated with it and the short-term nature of most funding opportunities. Remote sensing and GIS technologies can decrease both the time and costs usually seen with traditional monitoring and mapping activities. Our research investigates the feasibility of using multispectral remote sensing (e.g., WorldView-2) to monitor spotted knapweed populations. I measured *in situ* spectral reflectance curves from spotted knapweed at three cover class levels (0-10%, 11-40%, and 41-100%) during three phenological time frames (pre-flowering, flowering, and senescence) and compared these curves to reflectance curves from 36 different plant species and species mixtures. During flowering, differences in spotted knapweed spectral curves are seen between the high cover class (41-100%) and the mid and low cover classes (1-10% and 11-40%), but not between the lower cover classes. However, there are differences in the three cover classes while spotted knapweed is pre-flower and post-senescence. Spectral curves from spotted knapweed overlap with the spectral curves of other plant species in some of the spectral bands in which the WorldView-2

satellite collects data; however, there are differences between spotted knapweed spectral curves and curves from all other plant species measured in at least one WorldView-2 band. I conclude, based on this data, that it is feasible to use the WorldView-2 satellite to map spotted knapweed populations.

Introduction

Approximately 50,000 non-native species have been introduced into the United States. Many new species are introduced every year and, as global trade increases, so does the threat of introduced species. Approximately 15% of introduced species in the United States have become major problems and are considered invasive (Eav 1999). Invasive plants and animals can cause significant environmental and economic damage and costs associated with these species have been estimated at nearly $120 billion per year in the United States (Pimentel et al. 2005).

Non-native, weedy plants are considered to increase their spread into wildlife areas in the United States at a rate of approximately 700,000 ha/year (Babbitt 1998). With this rate of spread, land-managers need accurate information about weed distribution to make better and quicker management decisions. Locating new and small infestations quickly, before the populations increase, makes management easier and more effective. Traditional methods of weed mapping (field surveys with hand-held GPS devices), while potentially accurate, are time consuming and expensive. Accurate, fast, and cost-effective weed mapping strategies are required for proper management of invasive weed species. The need for such strategies suggests the need to carefully examine the current remote sensing process for possibly improved weed mapping techniques.

Mapping of invasive plant populations with remote sensing techniques has become more prevalent in recent years (Lass and Callihan 1997, Lamb and Weedon 1998, Lass et al. 2002,

Lawrence et al. 2006, Asner et al. 2008). Weber et al. (2006) evaluated the detection accuracy, ease of processing, and cost effectiveness of hyperspectral (HyMap) and multispectral (SPOT) satellites used for the remote sensing of leafy spurge (*Euphorbia esula* L.). In this particular study, producer and overall accuracies for leafy spurge were greater for the multispectral platform than for the hyperspectral platform, but user's accuracy was higher for hyperspectral. Multispectral images are easier to process, as there is a steep learning curve for processing hyperspectral images; however, once methods for processing hyperspectral images are learned, the process is smooth and repeatable. Weber et al. (2006) also estimated the cost effectiveness of HyMap (hyperspectral) and SPOT (multispectral) platforms by using an effectiveness rating defined as:

$$\text{Cost per km}^2/\text{User accuracy} = \text{Cost effectiveness}$$

Where user accuracy is a measure of how accurate the map classification performs in the field by map category. Using this formula a more cost-effective option will have a lower cost effectiveness rating and a less cost-effective option will have a higher cost-effectiveness rating. The cost effectiveness of the multispectral platform was 0.01 and hyperspectral was 1.70 (Weber et al. 2006). Both hyperspectral and multispectral platforms can be used to map certain invasive plants, but a cost/benefit analysis should be done to determine the best satellite for the task. The hyperspectral sensor (HyMap) had significantly higher spatial resolution, which could be an advantage for early detection of new plant infestations. However, the multispectral (SPOT) had clear advantage over HyMap in cost effectiveness and ease of processing (Weber et al. 2006). The first step in the process to determine a cost-effective monitoring program is to investigate if either hyper and/or multispectral remote sensing can be used to detect plant populations. These

issues have yet to be evaluated for the invasive spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek).

Spotted knapweed is a Eurasian native in the family Asteraceae. Seeds were accidentally introduced into North America in the 1890s in contaminated alfalfa seed and ship ballast (Tutin et al. 1976, Mauer et al. 2001). In the United States, spotted knapweed is a short-lived perennial that can survive up to 9 years (Boggs and Story 1987). Spotted knapweed is often found on disturbed sites, such as roadsides; however, in the Pacific Northwest the weed is known to invade rangelands and pastures where it causes the overgrazing of native grasses, replaces indigenous plants, and increases surface runoff and sediment yield (Tyser and Key 1988, Lacey et al. 1989, Lacey et al. 1990, Kedzie-Webb et al. 2001, Ochsmann 2001). Plants produce an average of 1,000 seeds per plant or 5,000 to 40,000 seeds/m^2, with the seeds remaining viable for up to 8 years (Sheley et al. 1998, Davis et al. 1993). The prolific nature of spotted knapweed and the longevity of the seed in the seed bank make this plant extremely difficult to control.

Thirteen arthropod biological control agents have been introduced into North America in an attempt to control spotted knapweed (Muller and Schroeder 1989, Story 2002). Weed biological control programs require long term monitoring to determine success or failure, as well as to determine the cost: benefit ratio of the program. Many challenges exist in long-term monitoring of biological control of weeds. Monitoring projects are time consuming and expensive, with many programs taking >10 years to control the target weed (McFadyen 1998). Quick detection of new populations of target invasive weeds is also important in the evaluation of any management practice. This often means surveying in remote areas where the weed has not been previously found, as well as continuing to monitor areas where the weed is under control, as weeds can re-invade after control efforts have been completed. Effective monitoring

and mapping activities can also increase the efficacy of other control efforts, if populations are found while they are small. Although long-term monitoring is important, the short-term funding cycles of most funding agencies result in few long-term evaluations. Remote sensing could provide a solution to the challenges faced by biological control researchers, while trying to conduct long-term monitoring or mapping of target weeds.

Lass et al. (2002) reported an early, if not the first, attempt to use remote sensing techniques to detect populations of spotted knapweed. A hyperspectral sensor (Probe 1) with 5 x 5 m spatial resolution was used in conjunction with the Spectral Angle Mapper (SAM) algorithm to successfully map spotted knapweed populations in Montana and Idaho. Infestations with 70 - 100% cover of at least 0.1 ha in size were successfully detected regardless of classification angle; however, an 11% classification angle reduced overall detection error the most and was determined to be the best angle for general surveys. Infestations with 1 - 40% spotted knapweed cover were detected with a 1% ommisional error (the proportion of an image category that is not classified correctly on the image as it is seen on the ground) and a 6% commisional error (the proportion of an image category that is present on the image but not on the ground) . They also found that multiple signature files are needed to detect populations of spotted knapweed in Idaho because of vast differences in plant height, stage, and color at different sites (Lass et al. 2002). Depending on a variety of factors from characteristics of the remote sensor data to location or environmental conditions, the SAM algorithm may not always be the best classification method for detecting spotted knapweed populations. Lawrence et al. (2006) found that it did not accurately classify spotted knapweed populations in Madison County, Montana, using the same sensor (Probe 1) as used by Lass et al. (2002). Lawrence et al. (2006) found similar challenges while using logistic regression, classification trees, and stochastic gradient boosting. They did

find that the Beiman Cutler classification provided an overall accuracy of 84% (Lawrence et al. 2006). Data from hyperspectral sensors, while effective in detecting spotted knapweed populations, are usually expensive and more difficult to analyze (Weber et al. 2006). This is clearly illustrated in the two aforementioned studies that, while using the same sensor to detect the same plant, there were still difficulties in determining the best classification method to properly analyze the data. Our study examined the feasibility of using the WorldView-2 multispectral satellite to detect populations of spotted knapweed.

WorldView-2 is a multispectral satellite that was launched in 2009 that offers high spatial and spectral resolutions. This satellite offers a high revisit rate (1.2 days), 2 x 2 m spatial resolution across eight multispectral bands, and 0.46 x 0.46 m panchromatic resolution. Four new bands not previously seen on any satellite-based multispectral platforms are found on WorldView-2, with each of the new bands specifically centered on key areas of the spectrum to help detect and identify vegetation characteristics: Coastal Band (400-450 nm), Yellow Band (585-625 nm), Red Edge Band (705 - 745 nm), and the Near Infrared 2 Band (860 - 1040 nm). It was anticipated that WorldView-2's higher spatial and spectral resolutions would provide the opportunity to successfully utilize orbital multispectral remote sensing to detect populations of spotted knapweed.

Materials and Methods

Spectral characteristics of spotted knapweed were measured *in situ* using a FieldSpec HH UV/VNIR handheld spectroradiometer (Analytical Spectral Devices Inc., 2800 Shirlington Road, Suite 800 Arlington, VA 22206) and RS^3 Spectral Acquisition Software (Analytical Spectral Devices Inc., 2800 Shirlington Road, Suite 800 Arlington, VA 22206). Measurements were taken at three phenological times throughout the growing season -pre-flowering (April - May), flowering (June - July), and senescence. Three different spotted knapweed population sizes

(<10%, 10-40%, and 40-100%) were measured in each of the aforementioned phenological time frames to determine differences in spectral reflectance curves among varying cover classes. Cover is defined as the percent of ground that is covered by spotted knapweed in the instantaneous field of view (IFOV) of the spectroradiometer. Measurements were taken at several different locations to take into consideration different soil types. Measurements of other vegetation (not spotted knapweed) were also recorded to determine the *separability* of the spectral reflectance curves. Vegetation measurements were limited to plants that occur in habitats similar to spotted knapweed and a few crop species. A total of 36 species and species mixtures (where there were more than one species in the IFOV of the spectroradiometer) were measured. All measurements were acquired in the spring and summers of 2010-2012.

Measurements were taken at a height of 1.5 m using a 25° foreoptic, which provided a ground projected IFOV with a diameter of approximately 0.6 m^2. Measurements were taken on cloud-free days within two hours of solar noon (Salisbury 1999, McCoy 2005). Twenty spectral curves were measured for each individual “scene” and were averaged to compensate for movement of the plants by the wind (Lord et al. 1985). For each plant species measured, two to four individual “scenes” of the same species were collected and then averaged to compensate for variation among individual plant spectral curves. Spectral curves were visually assessed for differences using ViewSpec™ Pro (Analytical Spectral Devices Inc., 2800 Shirlington Road, Suite 800 Arlington, VA 22206). A white reference measurement was taken every 30 minutes or less during periods of data collection using a Spectralon panel (Labsphere, Inc., PO Box 70, 231 Shaker Street North Sutton, NH 03260). This process allowed the spectroradiometer to remain calibrated to changes in the illumination levels or the changing angle of the sun.

Results and Discussion

Clear differences were observed among the three spotted knapweed cover classes (0-10%, 11-40%, and 41-100%) during pre-flower and senescence; however, the low- and mid-cover class spectral reflectance curves overlapped significantly during spotted knapweed flowering in the bands used by WorldView-2 (Figure 1). This indicates that WorldView2 might be useful to distinguish low, medium, and high populations of spotted knapweed during the spring, when spotted knapweed is pre-flower and in the late summer and winter, when spotted knapweed has senesced. Even though WorldView-2'spectra did not successfully distinguish between low- and mid- cover classes while spotted knapweed was in flower, it might distinguish both of these cover classes from the high cover class. The ability to distinguish between the three cover classes suggests that it would be feasible to use WorldView-2 spectral data to detect changes in spotted knapweed cover.

Differences are also seen among the three phenological time frames of bud, flower, and senescence over all three cover classes (Figure 2). This indicates that detecting spotted knapweed throughout the growing season will require the use of different spectral signatures. This also suggests the need to compare all spectral signatures from the differing phenological time frames with other plant species.

Table 1 lists all 36 non-spotted knapweed-plant species and species mixtures measured. Several areas of the spectral curves for these species and species mixtures overlapped. All non-spotted knapweed species measured are shown graphically (Figure 3). Whereas all the spectral curves of living, green vegetation are the same general shape, spotted knapweed reflectance values and even the most similar of the reflectance curves seen from other plant species do show differences in the regions in which WorldView-2 collects data (Table 2). Different spectral

characteristics would be needed to find spotted knapweed populations of varying cover and phenological stage on satellite images. But, it should be possible to separate spotted knapweed from non-spotted knapweed vegetation while spotted knapweed is actively growing (non-senesced). It should also possible to separate senesced spotted knapweed populations from other plant species using WorldView-2 imagery over all three cover classes (Figure 4). Good, clear, cloud-free imagery is often difficult to collect, especially during a narrow time frame such as the knapweed growing season. With the ability to detect spotted knapweed during senescence it greatly increases the amount of time available for collecting satellite imagery. Table 2 shows that there is at least one WorldView-2 band that is able to separate spotted knapweed from the other plants measured, at all spotted knapweed cover classes, but there are multiple bands which have the ability to separate spotted knapweed during bud from other plant species. This may indicate that satellite imagery collected when spotted knapweed is in bud may be more accurate at mapping spotted knapweed than satellite imagery collected at other times of the year. Further research is needed to support this.

Several spectral curves exhibit a lot of noise in the higher wavelengths, so wavelengths over 930 nm are not shown (Figures 1-4). This noise is seen in all spectra that were measured on hot days (> 35 C), and is consistent over all spectra measured during that those conditions. I believe the noise could be the effect of the heat on the spectroradiometer and I do not believe that this noise affects the validity of our findings.

I have shown that it is possible to differentiate spotted knapweed in all three phenological times frames, across varying cover, and among the 36 plant species and species mixtures measured using a handheld spectroradiometer. As the spotted knapweed spectral curves vary over cover class and phenological time, I assume that other plants will also (Leitea et

al. 2008). Some of these differences have been accounted for in our data (chicory, mixed grasses, and diffuse knapweed), but further research needs to be done using satellite-collected data to determine the errors associated with using multispectral remote sensing to map and monitor spotted knapweed. With these data, I conclude that there is strong evidence of the feasibility of using the WorldView-2 satellite to detect infestations of spotted knapweed and the changes in spotted knapweed infestations. WorldView-2 data needs to be collected to confirm these findings.

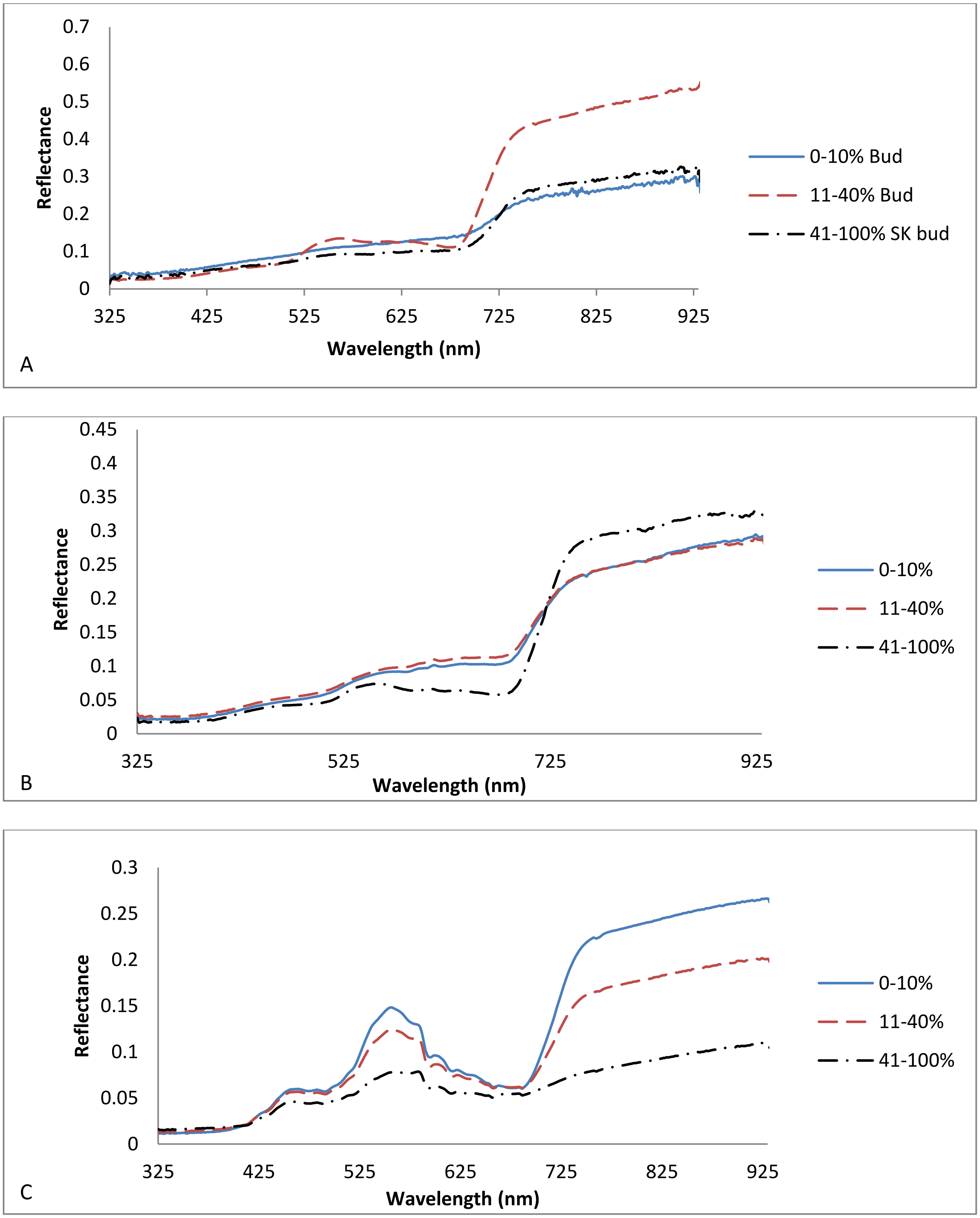


Figure 1: Spectral readings (reflectance) among three cover classes over the three phenological time frames a) bud b) flowering c) senescence.

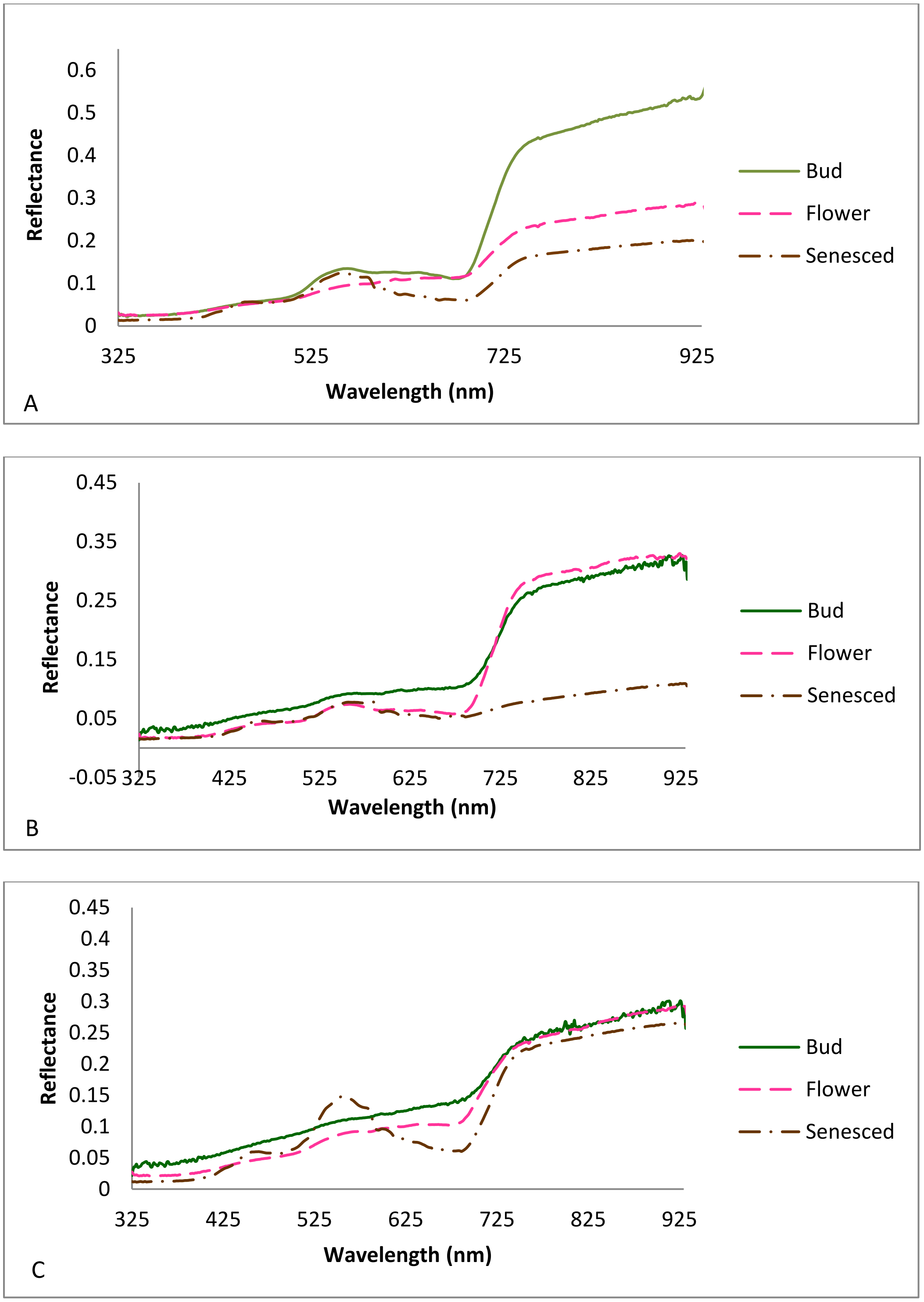


Figure 2. Spectral readings (reflectance) for three different phenological time frames (bud, flower, and senescence) over the three cover classes a) 0-10%, b) 11-40%, and c) 41-100%

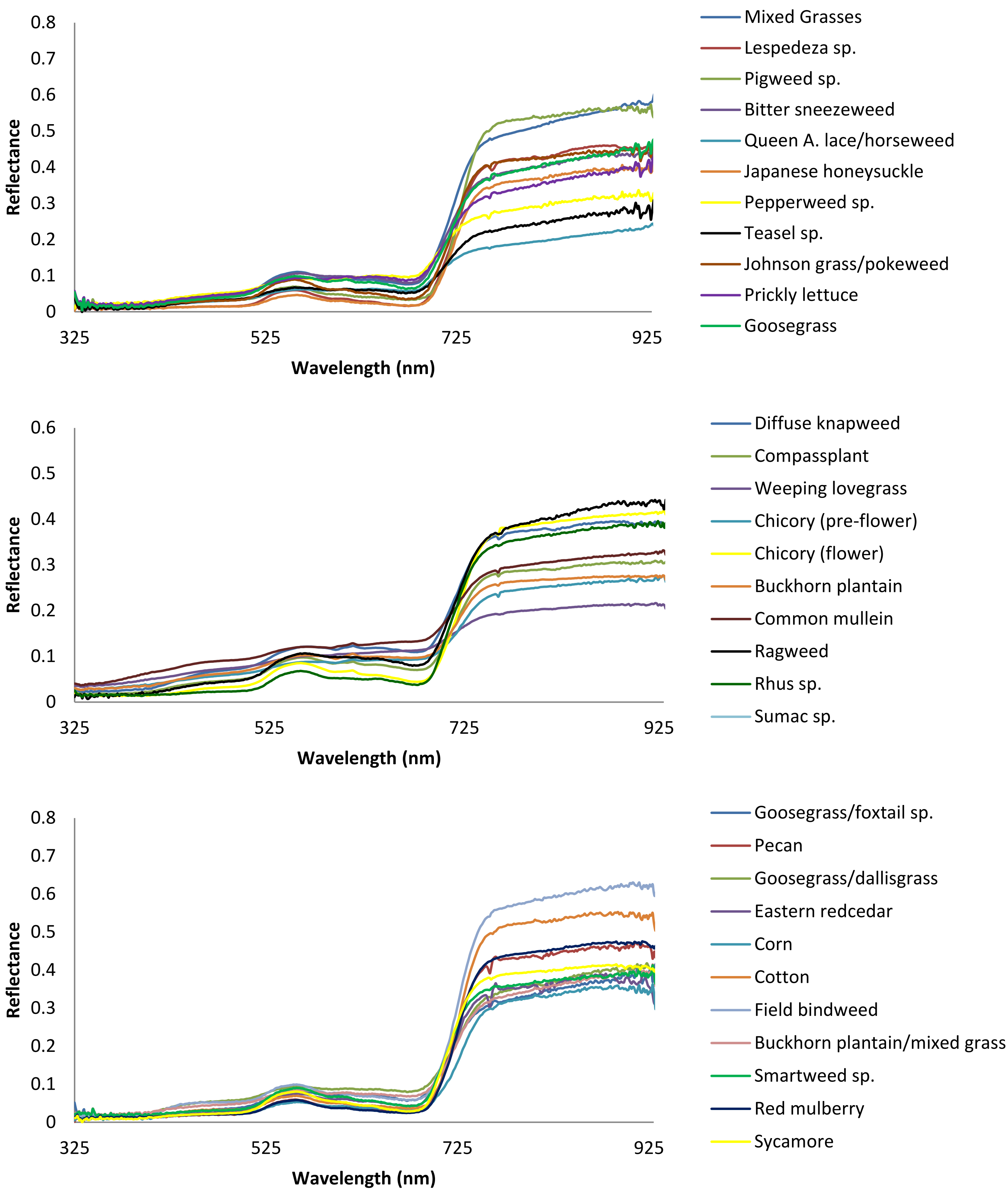


Figure 3. Spectral curves of healthy, green non-spotted knapweed vegetation measured.

Table 1. Plant species and species mixtures (36) measured to compare with spotted knapweed reflectance values.

Common name	Scientific name	Phenological stage
Tree of heaven	*Ailanthus altissima* (Mill.) Swingle	Pre-flower
Common ragweed	*Ambrosia artemisiifolia* L.	Pre-flower
Pigweed sp.	*Amaranthus sp.*	Fruit
Pecan	*Carya illinoinensis* (Wangenh.) K. Koch	Pre-flower
Diffuse knapweed	*Centaurea diffu*sa Lam.	Pre-Flower
Diffuse knapweed	*Centaurea diffusa* Lam.	Flower
Chicory	*Cichorium intybus* L.	Flower
Chicory	*Cichorium intybus* L.	Pre-flower
Field bindweed	*Convolvulus arvensis* L.	Flower
Queen Anne's lace	*Daucus carota* L.	Senesced
Common teasel	*Dipsacus fullonum* L.	Flower
Fleabane	*Erigeron* sp.	Senesced
Goosegrass	*Eleusine indica* (L.) Gaertn.	Flower
Weeping lovegrass	*Eragrostis curvula* (Schrader) Nees	Flower
Cotton	*Gossypium* L.	Flower
Bitter sneezeweed	*Helenium amarum* (Raf.) H. Rock	Flower
Eastern red cedar	*Juniperus virginiana* L.	Pre-flower
Prickly lettuce	*Lactuca serriola* L.	Flower
Virginia pepperweed	*Lepidium virginicum* L.	Flower/fruit
Sericea lespedeza	*Lespedeza cuneata* (Dumont) G. Don	Flower
Japanese honeysuckle	*Lonicera japonica* Thunb.	Flower
Red mulberry	*Morus rubra* L.	Fruit

Buckhorn plantain	*Plantago lanceolata* L.	Flower
Sycamore	*Platanus occidentalis* L.	Fruit
Smartweed	*Polygonum* sp.	Flower
Sumac	*Rhus* sp.	Fruit
Curly dock	*Rumex crispus* L.	Senesced
Compassplant	*Silphium laciniatum* L.	Pre-flower
Common mullein	*Verbascum thapsus* L.	Flower
Corn	*Zea mays* L.	Flower
Mixed Grasses	_	Vegetative
Mixed grasses	_	Senesced
Queen Anne's lace/horseweed[1]	_	Flower
Pigweed/horseweed	_	Fruit
Johnsongrass[2]/pokeweed[3]	_	Fruit
Goosegrass/foxtail[4]	_	Fruit
Fleabane/dallisgrass[5]	_	Fruit
Buckhorn plantain/mixed grass	_	Fruit

[1]*Conyza canadensis* (L.) Conronquist,[2] *Sorghum halepense* (L.) Pers., [3] *Phytolacca americana* L.,[4] *Setaria* sp.,[5] *Paspalum dilatatum* Poir.

Table 2. Spotted knapweed cover classes (0-10%, 11-40%, 41-100%) over three phenological times frames (pre-flower, flower, senesced) and the WorldView-2 bands that offer the best separation of spectral curves among spotted knapweed and the other 36 plant species and species mixtures measured.

	Phenological Time		
	Bud	Flower	Senescence
Spotted knapweed cover			
0-10%	Blue, NIR II	NIR II	Green
11-40%	Green, Yellow, Red, NIR I, NIR II	Blue	Red-edge
41-100%	NIR I, NIR II, Red	NIR I	Red edge, NIR I, NIR II

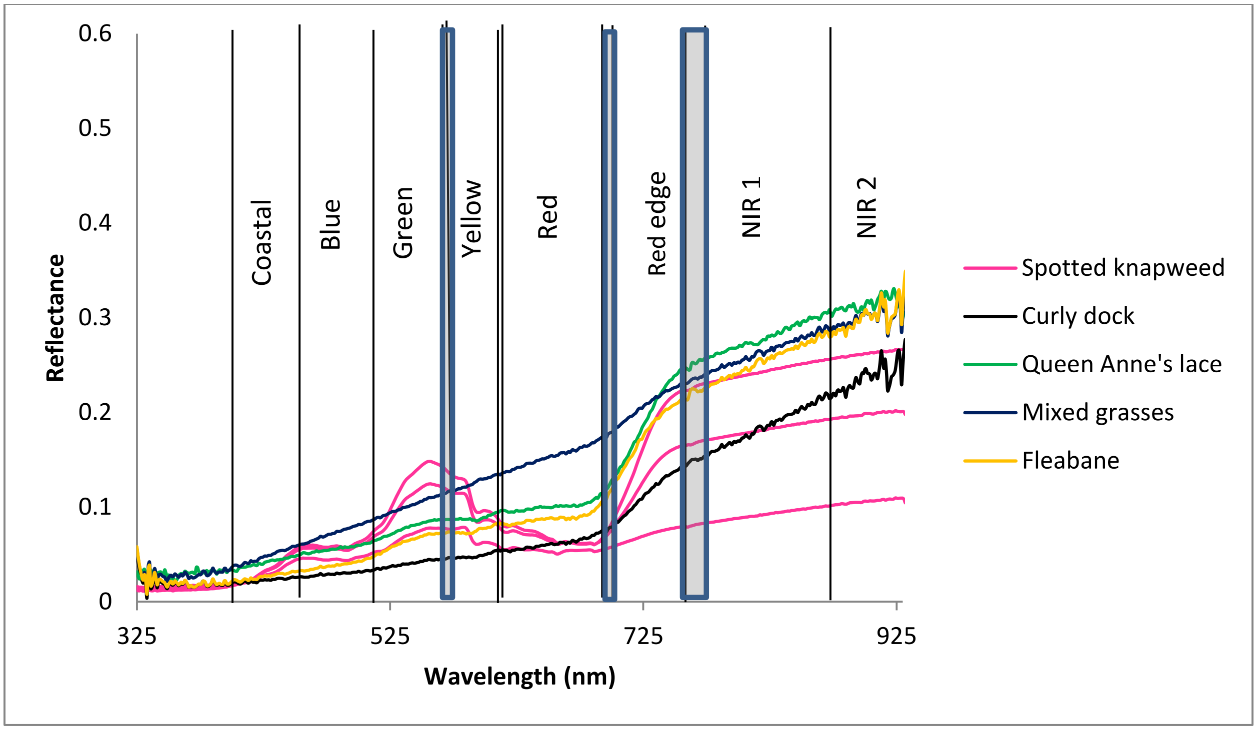


Figure 4**.** Senesced vegetation and spotted knapweed* (senesced at all 3 cover classes) shown with a WorldView-2 band overlay. Areas between bands are shaded and wavelengths over 930 are not shown due to a low signal to noise ratio. *All spotted knapweed phenological times and cover classes are seen in the same color for easier differentiation.

III. Conclusion

The knapweed biological control program in the western United States and Canada is a very long-standing and successful program. The start of the spotted knapweed biological control program in Arkansas shows some promise. *Larinus minutus* was released and established in the state, and although the weevils were active for a longer period of the season (compared to the native range of the weevil), there was no increase in the number of generations per year. Sex ratios and adult activity of *L. minutus* in Arkansas were aligned with previous studies done in the native range. I hypothesized that because of a longer growing season in the southeastern United States, additional generations of *L. minutus* (and, therefore, an increase in the level of control) may be observed than in more northern latitudes. Based on my results here, I expect that the weevil will provide control similar to that seen in the western United States and Canada.

Mowing activities for the maintenance or reduction of spotted knapweed infestations should be completed at different times dependent on the presence of *L. minutus*. When *L. minutus* is not present mowing should be completed in May to minimize the spread of viable seed into the system twice in a single season. If weevils are present in high numbers, the most opportune time for mowing is in July, so that weevils have a chance to complete development, and therefore, reduce seed in the growing plants. If control of spotted knapweed in Arkansas becomes a priority, then mowing guidelines for the state should be revised.

Larinus minutus has only been established in Arkansas since 2010, but there was some reduction in spotted knapweed seed production and rosette densities. Although these differences were not seen over the entire course of study, but they indicate that *L. minutus* has the capacity to impact spotted knapweed infestations, even in a short period of time. I believe that the presence of *L. minutus* in the state will eventually lead to the reduction of spotted knapweed populations

based on these data, along with data that show that there are significantly fewer seeds in the capitula when *L. minutus* is present. In future studies, I suggest that biomass measurements be taken in addition to individual plant measurements (e.g., plant height, number of stems). Measuring biomass could reduce some of the variability seen with the changes in spotted knapweed life stages and may give a more definitive picture of the effect of *L. minutus* or mowing on spotted knapweed.

Based on spectroradiometer data compiled in this study, it is feasible to monitor declines in knapweed populations with the WorldView-2 satellite. Collection of satellite data should begin as soon as possible, so that it can be determined if WorldView-2 will be an acceptable tool for monitoring spotted knapweed in the region. Early and regular collection of these satellite data over the same regions will be most beneficial in determining efficacy of the biological control program if data is collected closer to the start of the release program (2008).

Restoration efforts should be conducted in areas where/when spotted knapweed starts to decline, so that the areas left open by the reduction in spotted knapweed are filled with beneficial native plant species, and not invasive species.

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Appendix I

Greenland, Arkansas

July 1, 2011

July 14, 2012

Washington County Fairgrounds, Fayetteville, Arkansas

July 1, 2011

June 14, 2012

West Fork, Arkansas

June 12, 2012