12-2018

Impact of Roadside Maintenance Practices on Larinus minutus (Gyllenhal), a Biological Control Agent of Spotted Knapweed

Mary Elizabeth Ferguson
University of Arkansas, Fayetteville

Impact of Roadside Maintenance Practices on *Larinus minutus* (Gyllenhal), a Biological Control Agent of Spotted Knapweed

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Entomology

by

Mary Ferguson
Sam Houston State University, 2012
Bachelor of Science in Biology
Oklahoma State University
Master of Science in Entomology and Plant Pathology, 2014

December 2018
University of Arkansas

This thesis is approved for recommendations to the Graduate Council.

____
Robert N. Wiedenmann, Ph.D.
Dissertation Director

Timothy J. Kring, Ph.D. Committee Member	Frederick M. Stephen, Ph. D. Committee Member
Neelendra Joshi, Ph.D. Committee Member	Nilda Roma Burgos, Ph. D. Committee Member

Abstract

Spotted knapweed, *Centaurea stoebe*, is an invasive weed found throughout much of the United States. Spotted knapweed is a rangeland weed where it was originally introduced into western North America in the 1880s.Where spotted knapweed spread to the southeastern U.S., it is found mostly along roadsides. It has been the focus of a biological control program beginning in the 1960s, with 12 insects established, with the final introductions occurring in the 1990s. After the success observed in the western U.S. and Canada with one of these insects, *Larinus minutus*, this weevil was established in northwestern Arkansas. It is too early to assess the reduction of knapweed in Arkansas. Limits on the impact of this agent may result from the frequent disturbance of the plant and *L. minutus* by mowing. Field studies were conducted to measure the impact of temporal availability of floral resources on ovary maturation, egg production, and larval mortality of *L. minutus.* Presence of *L. minutus*, and spotted knapweed seed production, height, and cover were recorded. Reduced availability of floral resources and delay of access to floral resources delayed ovary development and reduced egg production. Increased larval mortality was observed in the areas of the spotted knapweed patch that had been mowed. Mowing resulted in fewer total seeds in the capitula. Both mowed and un-mowed areas had capitula containing mature seeds and seeds damaged by *L. minutus* feeding. The number of seeds damaged by *L. minutus* increased as the proportion of capitula with *L. minutus* increased in the patch. A survey of 2245km of highways in Carroll, Benton, Madison, and Washington counties found knapweed was present along 13.9% of the highway kilometers. Mowing times could be altered to avoid disturbance of *L. minutus* from late spring-summer when knapweed produces the most blooms. However, preservation of this weed biological control agent would need to become a consideration in future highway weed management decisions.

Acknowledgements

There are many people who I would like to thank for their aid in completing this dissertation. First and foremost, my advisor, Rob Wiedenmann, who was willing to take me into his lab and has provided both advice and mentorship throughout my time at Arkansas. Also, Tim Kring who laid the foundation for my research and has continued to contribute to the project and to my career path. I would also like to thank my committee members: Fred Stephen, Neelendra Joshi, and Nilda Burgos, who have offered their time, support, and wisdom to help mold me into a better scientist. Thanks to Dr. Andy Mauromoustakos for aid with statistical analysis.

I would also like to thank both the past and present graduate students in the Entomology department, especially Mark Janowiecki, Ricki Hamilton, and Matt Ethington, who have been willing to listen and discuss ideas both big and small. Additional thanks to Hayden Gore and Gabby Douglas who braved the weeds in the ditches to help collect samples.

Thank you to my family who have been a constant source of support throughout my graduate education. From moving across the country to missed holidays they have always been there with understanding. Lastly, to the family I chose, ‘The Hive’. Your friendship has been a constant through the ups and downs that makes graduate school much easier.

Dedication

I would like to dedicate this dissertation to my Nana, Esther Hope Vaught, and my Grandpa, Charles Bomar. Nana was a woman with a fiery spirit and a curiosity that could never be fulfilled. Her unwavering support and delight in the natural world and in my research were ever present. My Grandpa believed in our ability and the importance of education. He provided financial support so we could focus on educating ourselves so that we could then help others. He was fiercely supportive, providing every article and piece of news about insects to insure I had seen them, often adding commentary about what he thought. These two individuals shaped much of my character and I would not be the person or scientist I am without their love and care.

Table of Contents

Chapter 1

Literature Review

There is a long history of plant introduction (both intentional and otherwise) to the United States (Pimentel et al. 2000). Many of these plants are beneficial (crop plants, horticulture and landscaping), but approximately 5000 of these species became classified as 'invasive' (Morse et al. 1995). Many of the now-invasive plants were originally considered beneficial but were classified as weeds after escape into wild or unmanaged areas. An invasive species is defined as: "an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Executive Order 13112).

Invasive weeds become problematic due to certain characteristics in growth patterns, reproduction, defense, or competitive abilities. These characteristics were compiled from the literature involving weed characteristics that contribute to invasiveness by Bryson and Carter (2004) into 8 categories: reproduction, dispersal, habitat, interspecific interactions, phenology, physiology, protection from herbivores, and tolerance to environmental stress (Baker 1965, 1974, Meunscher 1955, Radosevich and Holt 1984, Stuckey and Barkley 1993, Westbrooks 1998). Depending on the plant, other categories include residence time (Wilson et al. 2007) and phylogeny (Duncan and Williams 2002, Proches et al. 2008). When weeds invade ecosystems the species richness and biodiversity of the habitat are often reduced. Changes in community interactions can be due to reduction in native plant growth and fitness which alters the succession of an ecosystem (Elton 1958, Simberloff 1981, Vilà et al. 2011).

In the United States, invasive weeds occupy ecosystems ranging from managed to natural. Invasive weeds were often introduced intentionally for forage or landscaping and then escaped (Hajek 2004, Pimentel 2005). Cost to control weeds in crop, pasture, and forest systems is estimated to cost $33 billion annually, with about 12% yield loss in these environments (Pimentel 1993, Pimentel et al. 2005). The overall loss due to weeds in agricultural systems -- both control costs and lost potential yield -- is thought to total more than $267 billion annually (USBC 2001), including the $15.5 billion spent on herbicides (NASS 2017).

While herbicides are often a useful tool in row crops, there are limitations to their use outside of these areas. Weeds in rangelands are often controlled through alternative methods due to the cost and logistical challenges associated with applying herbicides. Mechanical control techniques like tilling, mowing and bulldozing have proven effective against some weeds (Sheley et al. 1999). For plants that are deep-rooted, these methods will not destroy enough of the plant's reserves to be effective in reducing large populations.

Biological control is designed to be a self-sustaining method of control for target pest species. Classical methods of introducing exotic natural enemies to areas the pest has invaded operate under the concept that control can be achieved by replacing the natural enemy complex (Keane and Crawley 2002). Biological control as a term was first used in 1919 by H.S. Smith (Baker 1987) and he defined it as "the use of natural enemies (whether introduced or otherwise manipulated) to control insect pests". A commonly used biological control definition developed by DeBach (1964) is "the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence." That definition does not include the organisms used for weed biological control; a more-specific definition is "the use of an agent, a complex of agents, or biological processes to bring about weed suppression" (WSSA 2018).

Biological control is often further classified based on the actions being taken: release of commercially available organisms (augmentation), conservation of organisms in the environment (conservation), and importation of natural enemies to a location where they do not occur naturally (classical or importation biological control). The efficacy of the final outcome of biological control programs can be defined in terms of reductions of the abundance of the target pest, control costs and pesticide use, and increases in yields, profits, and interest by growers in adopting biological control (Gurr and Wratten 2000). Complete control occurs when no other control method is required or used when the agent is established; substantial control occurs when other control methods are still needed, but at a reduced level due to the activity of the natural enemy; and partial control occurs when the natural enemy exerts some effect, but other primary means of control are still necessary (van den Bosch et al. 1982, McFadyen 1998).

Classical biological control was first used successfully in the United State in the late 1890's to control the cottony cushion scale (*Icarya purchasi*) (Clausen 1978). Recommending release of insects against problem weeds was suggested by Benjamin Walsh in 1866, but widespread use in weed control programs did not begin until the 1940's (Tu and Randall 2003). Successes in biological control have been variable, with estimates of 11.2% when targeted against insects and 20.7% when targeted against weeds (Hajek 2004).

One weed that has been the target of biological control in the United States and Canada is spotted knapweed, *Centaurea stoebe* ssp. *micranthos* Gugler (Asteraceae), although successful control at local level has ranged from partial to complete (Story et al. 2006, Story et al. 2008, Myers et al. 2008, Myers et al. 2009). Spotted knapweed was introduced to the United States through contaminated ship ballast in the 1800s (Mauer et al. 2001). It established in the Pacific Northwest in vulnerable rangelands that had been overgrazed and the weed caused further reduction in plants available for forage (Lacey 1988). Spotted knapweed has since spread across the United States and is now present in most states (USDA 2013). In addition to rangelands, spotted knapweed is known to occur in grasslands, open forests and roadsides, especially in areas where the land has been disturbed (Wilson and Randall 2003, Harris and Cranston 1979, Tyser and Key 1988, Lacey et al. 1990).

Spread of spotted knapweed is passive at local levels as achenes (seeds) are released from senesced flowerheads. Long distance spread occurs through transport and movement of contaminated soil, hay, or commercial seed; livestock; or vehicles carrying seeds (Roche et al. 1986). Where it establishes, spotted knapweed's negative impacts are both economic and ecological, including reduced forage, overgrazing of native grasses, increased soil erosion and water runoff, and reduced plant biodiversity (Tyser and Key 1988, Lacey et al. 1989, Lacey et al. 1990, Ochsmann 2001).

Spotted knapweed is a short-lived perennial (7-8 years) that typically spends the first year after germination as a basal rosette. In the subsequent years the plant produces stems bearing flowers (bolts). Flowering is intermittent from April to November with the bulk of flowering occurring from June-July. Flowerheads are pink-purple in color and comprised of 30-50 florets on a composite head. Stiff bracts surround the flowers and are black-tipped giving it a spotted appearance. Once seeds have reached maturity, flowerheads senesce to release achenes around the plant. Most spotted knapweed plants have fully senesced by late October-November. At this time bolts have also died back to the crown, relying on carbohydrate stores in the roots to over-winter and produce new bolts the following season in February-March (Wilson and Randall 2003).

Spotted knapweed reproduces primarily via seed production. Capitula (senesced flowerheads) have been reported to contain 9-37 seeds (Watson and Renney 1974, Schirman 1981). Depending on plant size and density, mature plants have been estimated to produce between 5,000 and 29,600 seeds per m^2 annually (Davis et al. 1993, Sheley et al. 1998), although one study estimated an accumulation of ~146,000 seeds per m2 (Watson and Renney 1974). In addition to being a prolific seed producer, spotted knapweed seeds are capable of germination up to 8 years after deposition onto the soil surface and can accumulate in the soil seedbank (Davis et al. 1993). Although spotted knapweed typically invades sites that are already disturbed, once established, the weed has several means of out-competing the native plants present. The deep taproot and ability to shade other plants through vertical growth help the weed to increase the area it infests (Roche et al. 1986).

Spotted knapweed has been the target of many different integrated weed management strategies, including herbicide use, mechanical control, cultural practices and biological control. Herbicides have been effective in controlling spotted knapweed for 2 to 3 years, but require multiple applications in a season or over multiple years, both to prevent plants from reaching maturity and to deplete the seed bank (DiTomaso 2000). Herbicides that have been used by land managers include 2,4-D, clopyralid, dicamba, glyphosate, or a combination of dicamba + 2,4-D (Sheley et al. 2000). Continued reliance on herbicides as a sole tactic increases the risk of resistance in the weed (Green 2014). Although resistance has not yet occurred in spotted knapweed, its congener, yellow star-thistle, *Centaurea solstitialis* L., has shown some evidence of resistance to clopyralid and picloram (Sabba et al. 2003).

Mechanical control tactics include hand-pulling of small infestations or individual plants, cutting, tilling or mowing. These are often labor intensive and must be repeated frequently to ensure that plants do not reach maturity and produce new seeds (DiTomaso 2000). In areas where the soil is rocky or steeply sloped, mechanical control may not be an option due to access or limitations of equipment used.

Cultural control focuses primarily on the prevention of overgrazing in rangelands. Grazing is not an eradication method, but studies have found that cattle, goats, and sheep will all readily graze spotted knapweed. Of these, sheep have had the greatest success in reducing spotted knapweed patches because they graze early in the season when knapweed is the only plant available for forage (DiTomaso 2000). Prescribed burning is another cultural management tactic that has been used, but little information on its long-term effectiveness is available (Zouhar 2001).

Biological control has been of interest in regard to control of spotted knapweed and its congeners. A program began in the United States in the 1960s and resulted in the release and establishment of 13 species of insect natural enemies. Not all insects were established in the same area, but often multiple species were intentionally released at a location. The final species introduction and establishment of natural enemies was in 1991, after which no new insects have been approved for release in the United States.

The insects that have been used against spotted knapweed can be broadly grouped into two feeding guilds: seed feeders and root feeders. Successful reduction of spotted and diffuse knapweeds has been attributed to insects from both feeding guilds: the seed-feeding weevil, *Larinus minutus* (Myers et al. 2009), and the root-feeding weevil, *Cyphocleonus achates* Fahraeus (Story et al. 2006, Myers 2008). These successes were recorded in both short-term studies (4-5 years) (Myers et al. 2009) and longer-term (20-30 years) (Story et al. 2008, Myers et al. 2009). Where the weevils were present, knapweed patches showed fewer flowerheads and an overall reduction in plant density. At a landscape level, numbers of seeds present in the soil seedbank were reduced, leading to subsequent decreases in numbers of knapweed plants (Story et al. 2009).

Spotted knapweed also occurs in the southern and southeastern United States (USDA 2013). In the state of Arkansas, spotted knapweed is primarily a roadside weed, but is also present to a lesser degree in pastures. Spotted knapweed in Arkansas has been the focus of a biological control program since 2006. Three biological control agents are known to be present in the state as of 2018: *Urophora quadrifasciata* (Diptera: Tephritidae), and the two weevils, *Cyphocleonus achates* and *Larinus minutus*. *Urophora quadrifasciata* is believed to have arrived adventively and was detected during sweep net sampling of spotted knapweed (Kring, unpublished data). This fly develops inside the flowerhead and instigates gall formation of the developing seeds (Harris and Shorthouse 1996). A study by Duguma (2009) found that U. quadrifasciata could reduce the number of knapweed seeds in the capitula, the action of the fly was not sufficient to reduce spotted knapweed infestations.

Both *L. minutus* and *C. achates* were intentionally introduced to Arkansas after being collected from populations that had been established in Colorado. Releases of both insects occurred from 2008-2011; establishment of *L. minutus* was confirmed by Minteer et al. (2011) and establishment of C. achates was confirmed in 2014 (Ferguson and Kring, unpublished data). These weevils were chosen for release based on the success attributed to them in the western US and Canada.

Larinus minutus becomes active in early spring and begins to feed on spotted knapweed vegetation and floral parts. This weevil is univoltine and copulation occurs multiple times in a season. Female *L. minutus* chew into the flowerheads where 2-3 eggs are laid in the pappus hairs

(Groppe 1990). Developing larvae feed on the seeds inside the flowerhead and have been documented to cannibalize other larvae present. Pupation also occurs inside the same senesced flowerhead (capitulum), and adult weevils emerge, chewing distinctive holes through the top of the capitulum (Kashefi and Sobhian 1998). These emerged adults will feed on spotted knapweed vegetation and move to the soil near the plants to overwinter.

Cyphocleonus achates oviposits at the root crown where larvae mine into the roots and can induce formation of root galls. Feeding by late-instars can cause significant damage, especially in smaller plants. The larvae overwinter in the roots and adults emerge in the following July, occasionally going to the tops of the plant to find a mate. Like *L. minutus*, this weevil is univoltine and has multiple mating events throughout the growing season (Stinson et al. 1994).

At this time, neither species has exhibited large-scale success at reducing spotted knapweed in Arkansas. Initial reductions of spotted knapweed were observed at some of the original release sites, but there has been an increase in spotted knapweed infestation at many sites in recent years. Differing rainfall patterns and amounts, as well as varying winter temperatures, could be contributing to the changes in infestations observed. The occurrence of spotted knapweed on roadways and the management practices used against the weed may also be contributing to the lack of large-scale success. Because long-term control will require depleting the seed bank and the resulting decrease in numbers of spotted knapweed plants, success of the biological control program may not be seen within a couple of years, but may require decades to manifest.

Spotted knapweed along roadways in Arkansas is mowed 2-3 times a year in order to maintain aesthetics, improve visibility, and reduce risk of fire. Requirements for mowing times of highways are mandated by the Arkansas Highway and Transportation Department for three events: prior to Memorial Day, during the month of July (often prior to Independence Day), and prior to Thanksgiving. During the first two mowing events, vegetation within 5m of the road is removed, leaving 10-25cm vertical growth. The third mowing event is typically from the roadside to the fence/boundary line (AHTD).

The three mowing events correspond to major phenological stages in spotted knapweed. but mowing is not intended to control this invasive weed. The first mowing corresponds to bud production and occurs immediately prior to onset of flowering. The second mowing occurs during peak flower production by spotted knapweed. The third and final event occurs after senescence of the plant, but mowing at that time facilitates seed spread along roadsides. Knapweed plants are capable of producing flowers after the first mow and, rarely after the second event; however, flowers produced after the second mowing do not produce mature seeds (Ferguson, unpublished data).

In addition to disruption of spotted knapweed growth, mowing also has the potential to interfere with the life cycle of *L. minutus*. Mowing in May delays flowering in spotted knapweed plants, which also delays access by *L. minutus* to floral resources that are thought to be necessary for egg production (Kashefi and Sobhian 1998) and sites for oviposition. The second mowing event occurs while larval *L. minutus* are developing inside the flowerhead and are susceptible to damage by action of the mowing equipment. The third mowing, late in the season, has little impact on *L. minutus*, as the weevil adults have moved into the soil where they overwinter and are no longer feeding.

Observations about spotted knapweed have been made since the biological control program began in Arkansas, but dedicated monitoring with consistent methodology has not occurred. Monitoring involves repeated sampling from plots within populations and follows changes in trends over long periods of time (Elzinga et al. 1998). Monitoring for multiple seasons provides a means to compare and quantify changes in infestations of knapweed at different times. Monitoring also provides a measure of spread and abundance of the biological control agents and can reveal areas of abundance or areas where the agent has failed to spread and establish. Using the information about spotted knapweed and its biological control agents could present the opportunity to identify sections of highways where integrated weed management can be incorporated.

Monitoring can occur through the use of field surveys or via technology, such as satellites and remote sensing, to detect the extent of infestations. Data collected from these methods are often used to create distribution maps to understand the extent that the target organism has spread. Once the infested areas are identified, repeated surveys of the areas with the invasive plant can provide a baseline of information about spotted knapweed with which to compare in future surveys.

Surveys in Arkansas were conducted at 'permanent plots' from 2010-2012 and again in 2016 after *L. minutus* was released. Each time, the plots were sampled for parameters of plant growth (crowns, bolts, number of capitula), presence of *L. minutus*, and presence of other plant species (Minteer 2012). Additional surveys of *L. minutus* movement were conducted by Alford et al. (2016) but after these studies quantitative surveys were no longer being completed in Arkansas. Updating information through a current distribution survey and patch-level information could help determine the success of the introduced biological control agents in controlling spotted knapweed.

Objectives

In order to improve the current understanding of how roadside management is impacting spotted knapweed and *L. minutus*, this dissertation research had three main objectives.

1) The first objective was to examine the relationship of floral resources (pollen and flower petals) to ovary development and maturation of *L. minutus*. Once floral resources were determined to be important, the time of availability of these resources and any impact on ovary maturation and egg production was observed.

2) The second objective examined the relationship of spotted knapweed regrowth following the first mowing event, and the ability of larval *L. minutus* to survive to adulthood inside the flowerhead.

3) Finally, a survey was conducted in Benton, Carroll, Washington, and Madison counties in Arkansas to determine current distribution of spotted knapweed and *L. minutus*. Research in this objective also included examining spotted knapweed in distinct infestations to understand the current growth characteristics.

References

Arkansas Highway and Transportation Department. Contract Mowing Guidelines. https://www.arkansashighways.com/maintenance_division/maintenance_management.aspx

Alford, A. M., T. Kring, and S. Raghu. 2016. Spread of *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of knapweeds, following introduction to northwestern Arkansas. Florida Entomologist. 99: 593-599

Baker, H. G. 1965. Characteristics and modes of origin of weeds. In H. G. Baker and G. L. Stebbins, eds. The Genetics of Colonizing Species. New York: Academic. Pp. 147–172.

Baker, H. G. 1974. The evolution of weeds. Annual Review of Ecological Systems. 5:1–24

Baker, K. F. 1987. Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology. 25: 67-85

Bryson, C. T. and R. Carter. 2004. Biology of pathways for invasive weeds. Weed Technology. 18: 1216-1220

Clausen, C. P. 1978. Introduced parasites and predators of arthropod pests and weeds: a world review. U.S. Department of Agriculture, Agricultural Handbook. 480

Davis, E. S., P. K. Fay, T. K. Chicoine, and C. A. Lacey. 1993. Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. Weed Science 41: 57-61

Debach, P. H. 1964. Biological control of insect pests and weeds. Reinhold, New York.

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science. 48: 255-265

Duguma, D., T. J. Kring, and R. N. Wiedenmann. 2009. Seasonal dynamics of *Urophora quadrifasciata* on spotted knapweed in the Arkansas Ozarks. Canadian Entomology. 141:70-79

Duncan R. P. and Williams P. A. 2002. Ecology: Darwin's naturalization hypothesis challenged. Nature. 417:608–609

Elton, C. S. 1958. The ecology of invasions by animals and plants. Methuen, London, UK.

Elzinga, C. L., D. W. Salzer, and J. W. Willoughby. 1998. Measuring and monitoring plant populations. Denver, CO; USDI-BLM Technical Reference. 1730-1. Pp 1-10

Executive Order 13,112, 3 C. F. R. 6183-6186 (1999)

Green, J. M. 2014. Current state of herbicides in herbicide-resistance crops. Pest Management Science. 70: 1351-1357

Gurr, G. and S. D. Wratten. 2000. Biological control: measures of success. Kluwer Academic Publishers. Pp. 429

Groppe, K. 1990. *Larinus minutus* Gyll. (Coleoptera: Curculionidae), a suitable candidate for the biological control of diffuse and spotted knapweed in North America. CABI Institute of Biological Control, Delémont, Switzerland

Hajek, A. E. 2004. Natural Enemies: An introduction to biological control. Classical biological control. Cambridge University Press. Pp. 378

Harris, P. and R. Cranston. 1979. An economic evaluation of control methods for diffuse and spotted knapweed in western Canada. Canadian Journal of Plant Science. 59:375-382

Kashefi, J. M. and R. Sobhian. 1998. Notes on the biology of *Larinus minutus* Gyllenhal (Col.,Curculionidae), an agent for biological control of diffuse and spotted knapweeds. Journal of Applied Entomology. 122:547-549

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution. 17: 164-170

Lacey C. A., C. Egan, W. Pearson, and P. K. Fay. 1988. Bounty programs an effective weed management tool. Weed Technology. 2:196-197

Lacey, J., P. Husby, and G. Handl. 1990. Observations on spotted knapweed and diffuse knapweed invasion into ungrazed bunchgrass communities in western Montana. Rangelands. 12:30-32

Lacey, J. R., C. B. Marlow, and J. R. Lane. 1989. Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Technology. 3:627-631

Mauer, T., M. Russo, and M. Evans. 2001. Element stewardship extract for *Centaurea maculosa*. The Nature Conservancy, Arlington, Virginia

McFadyen, R.E.C. 1998. Biological control of weeds. Annual Review of Entomology. 43:369-393

Minteer, C. 2012. The biological control of spotted knapweed in the southeastern United States. PhD Dissertation. University of Arkansas

Minteer, C. R., T. J. Kring, J. Shen, and R. N. Wiedenmann. 2011. *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of spotted knapweed (*Centaurea stoebe* ssp. *micranthos*), established in northern Arkansas. Florida Entomologist 94: 350-351

Morse L. E., J. T. Kartesz, and L. S. Kutner. 1995. Native vascular plants. Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of US Plants, Animals, and Ecosystems. Washington (DC): US Department of the Interior, National Biological Service. Pages 205-209 in LaRoe, E. T., G. S. Farris, C. E. Puckett, P. D. Doran, and M. J. Mac, eds.

Muenscher, W. C. 1955. Weeds. 2nd ed. Ithaca, NY: Cornell University Press. 586 pp.

Myers, J. H., H. Quinn, C.A.R. Jackson, and S. R. White. 2008. Successful biological control of diffuse knapweed in British Columbia, Canada. *In* Proceedings of the XII International Symposium on Biological Control of Weeds Apr 22-27 2007, La Grande Motte, France.

Myers, J. H., C. Jackson, H. Quinn, S. R. White, and J. S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canadian Biological Control. 50: 66-72

National Agricultural Statistics Service. 2017. Total Chemical (Herbicide) Usage for the United States. https://quickstats.nass.usda.gov/results/3BB776CF-C9C7-38FE-B7C1-6A5852A77AFB

Ochsmann, J. 2001. On the taxonomy of spotted knapweed (*Centaurea stoebe* L.), pp. 33-41 *In* L. Smith (ed.), The first international knapweed symposium of the twenty-first century, March 15-16 2001, Coer d'Alene, Idaho

Pimentel, D. 1993. Habitat factors in new pest invasions. In: Kim, K.C., McPheron, B.A. (Eds.), Evolution of Insect Pests—Patterns of Variation. John Wiley and Sons, New York, pp. 165-181

Pimentel, D., 1997. Techniques for Reducing Pesticides: Environmental and Economic Benefits. John Wiley and Sons, Chichester, UK

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience. 50:1 53-65.

Pimentel, D., R. Zuniga, D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273-288

Procheş Ş., J. R. Wilson, D. M. Richardson, and M. Rejmánek. 2008. Searching for phylogenetic pattern in biological invasions. Global Ecology and Biogeography. 17:5–10

Radosevich, S. R. and J. S. Holt. 1984. Weed Ecology. J. Wiley. 265 p.

Roche, B. F., Jr., G. L. Piper, and C. J. Talbott. 1986. Knapweeds of Washington. Cooperative Extension Bulletin 1393, Washington State University, Pullman

Sabba, R. P., I. M. Ray, N. Lownds, and T. M. Sterling. 2003. Inheritance of resistance to clopyralid and picloram in yellow starthistle (*Centaurea solstitialis* L.) is controlled by a single nuclear recessive gene. Journal of Heredity. 94: 523-527

Schirman. R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. Journal of Range Management. 34: 45-47

Sheley R. L., J. S. Jacobs, and M. F. Carpinelli. 1998. Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technology. 12:353-362

Sheley, R. L., S. Kedzie-Webb, and B. D. Maxwell. 1999. Integrated weed management on rangeland. Pages 57–68 in R. L. Sheley and J. K. Petroff, eds. Biology and Management of Noxious Rangeland Weeds. Corvallis, OR: Oregon State University Press

Sheley, R. L., C. A. Duncan, M. B. Halstvedt, and J. S. Jacobs. 2000. Spotted knapweed and grass response to herbicide treatments. Journal of Rangeland Management. 53:176-182

Simberloff, D. 1981. Community effects of introduced species. Biotic crises in ecological and evolutionary time. Academic Press, New York, New York, USA. Pages 53-81

Stinson, C.S.A., D. Schroeder, and K. Marquardt. 1994. Investigations on *Cyphocleonus achates* (Fahr.) (Col., Curculionidae), a potential biological control agent of spotted knapweed (*Centaurea maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) Compositae in North America. Journal of Applied Entomology 117: 35-50

Story, J. M., N. W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced weevil *Cyphocleonus achates* (Fahraeus). Biological Control. 38:227-232

Story, J. M., L. Smith, J. G. Corn, and L. J. White. 2008. Influence of seed head-attacking biological control agents on spotted knapweed reproductive potential in western Montana over a 30-year period. Environmental Entomology. 37: 510-519

Stuckey, R. L. and T. M. Barkley. 1993. Weeds. In N. R. Morin, ed. Flora of North America. Volume 1. New York: Oxford University Press. Pp. 193– 198

Tu, M. and J. M. Randall. 2003. Biological Control. Weed Control Methods Handbook, The Nature Conservancy pp 41-44

Tyser, R. W. and C. H. Key. 1988. Spotted knapweed in natural area fescue grasslands: an ecological assessment. Northwestern Science 62:151-159.

USBC, 2001. Statistical Abstract of the United States 2001. Washington, DC: U.S. Bureau of the Census, U.S. Government Printing Office.

USDA, NRCS. 2013. The PLANTS database. https://plants.usda.gov. National Plant Data Team, Greensboro, NC USA

Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarošík, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pyšek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters. 14: 702-708

Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds, *Centaurea diffusa* and *C. maculosa.* Canadian Journal of Plant Science 54: 687-7011

Weed Science Society of America. 2018. Biological Control of Weeds (W16)

Westbrooks, R. G. 1998. Invasive Plants, Changing the Landscape of America: Fact Book. Washington, DC: Federal Interagency Committee for the Management of Noxious and Exotic Weeds. 109 p.

Wilson J. R., D. M. Richardson, M. Rouget, Ş. Procheş, M. A. Amis, L. Henderson, and W. Thuiller. 2007. Residence time and potential range: crucial considerations in modelling plant invasions. Diversity and Distributions. 13:11–22

Wilson, L. M. and Randall, C. B., 2003. Biology and Biological Control of Knapweed. Forest Health Technology Enterprise Team, Technology Transfer

Zouhar, K. 2001. *Centaurea maculosa*. In: Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer) Available: http://www.fs.fed.us/database/feis

Chapter 2

Effect of Spotted Knapweed (*Centaurea stoebe*) Pollen Availability on Ovary Maturation of *Larinus minutus* (Gyllenhal)

Abstract

Spotted knapweed is an invasive weed found throughout the United States. Where it is present in the southeast it is often a roadside weed. A biological control agent, *Larinus minutus* (Coleoptera: Curculionidae), was intentionally released and established in northwest Arkansas. *Larinus minutus* feeds on the leaves, stems, and floral parts of spotted knapweed in early spring during bud formation. Field-cage trials were conducted to examine the impact of availability of different plant resources as food on ovary development and egg production in *L. minutus* females. Adult *L. minutus* that emerged from over-wintering were collected and maintained on a diet of spotted knapweed leaves and stems. In 2015, *L. minutus* were placed onto either flowerheads or stems and leaves of spotted knapweed in the field. Collections of 10 *L. minutus* occurred every three days from each treatment group. *Larinus minutus* were then dissected and ovaries removed, the lengths of the germarium and vitellarium were measured and the presence of eggs was noted. The experiment was repeated in 2016. A control group was provided flowers from onset of the experiment through 30 days. Three treatments were established, providing flowers for three days while varying the timing of flower availability, either day 0-3, day 6-9, and day 12-15. No egg production occurred when vegetation was the only food source provided. For all treatments, egg production began as soon as 3 days after access to floral resources was initiated. The vitellarium and germarium had the greatest lengths in the control group for 2016. Delayed access to floral resources negatively impacted the ovary development and overall production of eggs in *Larinus minutus*.

Introduction

Spotted knapweed, *Centaurea stoebe* L., is an invasive weed that occurs primarily in the western United States but can be found throughout the United States and portions of southern Canada (USDA 2013). It was unintentionally introduced to North America from Europe in the 1800s along with its congener, diffuse knapweed, *Centaurea diffusa* Lamarck, likely through contaminated seed (Mauer et al. 2001). Spotted knapweed is a short-lived perennial with a lifespan of 7-8 years (Watson and Renney 1974). Areas infested by knapweed are reported to have held 146,000 seeds per square meter (Wilson and Randall 2003, Schirman 1981, Mauer et al. 2001), and the seeds can persist in the soil for up to eight years (Davis et al. 1993). In the Pacific Northwest of the United States, spotted knapweed has invaded rangelands and caused a reduction in forage usable by cattle (Lacey 1988) through competition (Martin et al. 2014; Rand et al. 2015). Spotted knapweed is also associated with increased soil erosion, greater water runoff (Lacey et al. 1989), and reduced plant diversity (Tyser and Key 1988).

Biological control has been successfully employed against both spotted and diffuse knapweeds in North America (Story et al. 2006; Myers et al. 2009). Introduction of a complex of thirteen insects from Eurasia into the northwestern United States and Canada began in the 1960s (Story 2002). Two introduced weevils, the root-feeder *Cyphocleonus achates* (Fahr.) and the seed-feeder *Larinus minutus* (Gyllenhal) (Coleoptera: Curculionidae) are considered to have varied success in reducing densities of spotted knapweed (Story et al. 2006) and diffuse knapweed (Myers et al. 2009).

Spotted knapweed has spread to southern areas of the United States. Where spotted knapweed occurs in Arkansas, it is primarily as a roadside weed that is managed to enhance highway safety. Management methods include mowing and using herbicides to maintain visibility and aesthetic standards. However, mowing has been ineffective at controlling the weed, and other management methods are necessary. A biological control program targeting spotted knapweed in Arkansas began in 2006. A survey of knapweed plants in northern Arkansas revealed the presence of a gall-forming fly, *Urophora quadrifasciata* (Diptera: Tephritidae), which was presumed to have arrived adventively (T.J. Kring, unpublished data), but did not impact knapweed populations (Duguma et al. 2009). As there were no other biological control agents present in Arkansas, two weevils, *C. achates* and *L. minutus*, were collected from locations in Colorado and moved to northwest Arkansas. *Cyphocleonus achates* was established at one site (Ferguson and Kring, unpublished data) but has not been found away from the introduction site. *Larinus minutus* was established at multiple sites (Minteer et al. 2011) and has since spread and been recovered from additional, non-release sites (Alford et al. 2016).

Larinus minutus is univoltine and overwinters as an adult. Emergence of adult *L. minutus* coincides with knapweed growth and, in Arkansas, typically precedes by 3 to 4 weeks the onset of knapweed bloom from late June to early July. Once flowering begins, the female weevils oviposit 2-5 eggs per flowerhead and can produce more than 100 eggs over the season (Kashefi and Sobhian 1998). After eggs hatch, larvae feed on developing seeds inside the flowerhead for up to 4 weeks before pupating within the same senesced flowerhead (capitulum) (Groppe 1990; Alford 2013). Adults emerge and feed on vegetation before they move to the soil in late summer to over-winter (Jordan 1995).

Access to certain food resources to facilitate ovary development or produce eggs is known in the Coleoptera and other holometabolous insect orders (Gilbert 1972, Grodowitz and Brewer 1987, Human et al. 2007). *Larinus minutus* was reported to require feeding on 'floral resources' for egg development (Groppe 1990; Kahefi and Sobhian 1998), but the identity of the resources, and when exposure to those resources is necessary, are unknown. Female *L. minutus* feed after emerging from overwintering, and the feeding is thought to affect egg production. Female *L. minutus* have paired ovaries with two ovarioles each, and changes in ovary physiology and egg production as indicators of physiological age and reproductive maturity can be assessed using measurements of the ovariole and its components (Grodowitz et al. 1997, Perez-Mendoza et al. 2004). The ovariole (distal to proximal to the common oviduct) consists of the terminal filament, germarium, vitellarium, and pedicel. Mitosis occurs in the germarium, which produces primary oocytes. The oocytes pass to the vitellarium where they grow by vitellogenesis, or yolk deposition (Bonhag 1958). This production of oocytes and eventual egg formation cause expansion of the ovary structures, which can be differentiated into distinct stages (Fig. 2.1): a lack of oocytes (stage 1), presence of oocytes and yolk and eggs without chorion (stage 2), and presence of mature eggs with chorion present (stage 3). Other cues accompanying the stage differentiation include change in color from transparent and white to opaque and yellow in color.

Due to the specificity of *L. minutus* to knapweeds, the availability of spotted knapweed as a food source is critical for *L. minutus* after post-wintering emergence (Groppe 1990). Depending on the parts of the plant needed to produce eggs, removal knapweeds on roadsides by mowing could influence the successful establishment and efficacy of the weevil. The Arkansas Highway and Transportation Department is responsible for mowing roadsides, and mowing occurs three times during the growing season: May (prior to Memorial Day), when knapweed has begun to flower; July, when knapweed has begun to senesce but continues to produce flowers; and October, when the plant is fully senesced (AHTD). The mowing in May is of concern if it removes the plant resources required for egg production by *L. minutus* and sites for oviposition. Thus, the weed management methods could conflict with each other.

A field-cage study was conducted to gain insight into the importance of spotted knapweed structures as a food resource to aspects of *L. minutus* reproduction. The field experiment examined the relationship of egg production and different food resources available from spotted knapweed. The impact of time when *L. minutus* was first exposed to floral resources was also examined for its effect on egg production. The use of morphological characteristics as indicators of ovary maturation was also tested. These different aspects could provide insight into the oviposition potential of *L. minutus* and, thus, the success of *L. minutus* as a biological control agent.

Methods

Spotted knapweed in northwest Arkansas was monitored visually in late April for presence of *L. minutus* adults, and collections were timed to coincide with emergence of weevils from overwintering. After the onset of emergence, adult weevil collections were conducted over 2-3 weeks until large buds formed on the plants. The timing of collections assured that the collected *L. minutus* had fed only on knapweed vegetation at the time of collection, and that the weevils were phenologically similar. Collections occurred by vigorously shaking the stems of a knapweed plant over an 18-L bucket to dislodge adult weevils, and collected weevils were returned to the laboratory.

After collection, *L. minutus* were kept in environmental chambers (12:12 L:D and 22±1°C) and maintained on fresh knapweed stems and leaves. Using morphological characteristics (Kashefi 1993), male and female *L. minutus* were separated, and sorted into female-male pairs. Individual pairs were placed into a single, fine-mesh bag (5x7.5cm), which was used to contain the weevils and knapweed in the field.

Bags containing weevils were randomly assigned a treatment group and individual bags were placed over 1-3 stems of a knapweed plant. Bags were secured tightly by fishing line to prevent escape by the weevils but not so tight as to cut into the stem. The duration of experiments was set at 30 days, which was less than the average 40-day lifespan for female *L. minutus* (Kashefi and Sobhian 1998) and less than the duration of blooming of spotted knapweed. Any flowers that had been used for experiments were re-covered by an empty bag to exclude access by any extant weevils. Capitula were monitored for emergence of mature adults and later dissected to detect larval presence to determine if eggs produced by weevils were viable. However, the capitula were not searched to determine presence of eggs.

In 2015, the two treatments consisted of exposing adult weevils to either a diet of floral parts (petals, bracts, pollen) or a diet of vegetation (stems and leaves). Both treatments began on May 27th and continued through June 27th. Bags containing weevils were randomly assigned to the treatments, with n=120 bags in each treatment. Weevils provided a floral diet (floral treatment) were secured over stems with 2-4 knapweed flower buds, and those provided vegetation (vegetation treatment) were placed over 2-3 stems with no flowers. Bags were moved every three days to another location on the same plant and were secured over fresh plant material of the same treatment (floral or vegetation) for the duration of the experiment. Flowers that had already bloomed were avoided to prevent contamination by extant weevils that would have had the opportunity to oviposit. The experiment began at onset of flowering and continued through peak bloom period. On the same 3-day cycle, 10 bags were randomly selected from each treatment group, removed to the laboratory, and the weevils were stored for dissection.

In 2016, three treatments consisted of exposing weevils to a floral diet for three days: floral diet from day 0-3, day 6-9, or day 12-15 of the 30-day experiment. At all other times the weevils in those treatments were placed on vegetation and moved every three days. A positive control consisted of exposing the weevils to a floral diet for the duration of the experiment (day 0-30). Results in 2015 showed that weevils exposed only to vegetation did not produce eggs, so no negative control was used in 2016. Bags containing paired weevils were randomly assigned to one of the three treatments or control, and all began with n=120 bags. All treatments for the experiment began on May 31st when at least 30% of flowers were in bloom, with that same minimum percentage of flowers present for the duration of the experiment. As in 2015, bags were moved on a 3-day cycle, and 10 bags were randomly selected from each treatment group, removed to the laboratory, and weevils stored for dissection.

All weevils that were removed for dissection were stored in 70% EtOH. Dissections for both years took place in Ringer's solution and the ovaries were removed by cutting at the base of the lateral oviduct and placed on a slide. Measurements of the length of the germarium, vitellarium, and ovariole were taken and recorded, using the Dinocapture 2.0™ (AnMo Electronics Co. 2016) system, with measurement accuracy to 1.5µm. Ovary stage was assessed using production of oocytes, mature eggs, and color (transparent, yellow, dark yellow).The numbers of mature eggs present were counted for ovaries categorized as stage 3, and gut content was assessed. Average egg counts for each collection day were calculated by dividing the number of eggs by the number of weevils dissected. Total egg counts were obtained by taking the sum of all eggs found within weevils in each treatment.

All statistical analyses were conducted using JMP Pro® 13.2 software (SAS Institute 2016). For the 2015 experiment, the numbers of eggs, and lengths of the germarium, vitellarium, and ovariole at each 3-day collection were analyzed by conducting a univariate ANOVA and Tukey's HSD for means separation. A Chi-square analysis was conducted to compare the proportion of ovaries in each of the three stages across the duration of the experiment. For the 2016 experiment, data from each collection day were analyzed using a two-factor ANOVA with 'treatment' and 'collection event' as the main effects. A mean separation of the data was conducted using Tukey's HSD. A comparison of the proportion of ovaries in each classification stage was conducted using Chi-square analysis. An ANOVA and Tukey's HSD for means separation was used to compare new eggs found in the ovaries at each collection day. The first data point for each experimental group was classified as an outlier and dropped from final analysis of fit.

Results

2015

The ovaries of weevils in the vegetation only treatment did not produce yolk or eggs, remaining transparent with no presence of oocytes in the vitellaria. The ovaries of the group in the floral treatment developed oocytes and yolk, as well as mature eggs. Additionally, ovaries of weevils in this treatment changed to yellow in color. Dissections showed there were no oocytes (stage 1) in the ovaries of any *Larinus minutus* before the insects were placed onto floral resources. Three days after exposure to floral resources all ovaries of *L. minutus* contained oocytes and mature eggs (stage 2 or 3) for the duration of the experiment. In the treatment provided vegetation all ovaries dissected throughout the experiment lacked oocytes (stage 1).

When access to floral resources (floral treatment) was provided for the duration of the experiment, *L. minutus* were capable of both ovary maturation and egg production. The length of germaria in the floral treatment averaged 1.16±0.03 mm and did not differ significantly from the average length (1.06±0.03 mm) of germaria in the vegetation treatment (df=157, F-Ratio 2.73, p=0.11). The average length of vitellaria in the floral treatment, 1.63±0.06 mm, was greater than the average length of vitellaria in the vegetation treatment, 1.31±0.07 mm (df=178, F-Ratio 11.47, $p<0.0009$). The lengths of the vitellaria were significantly different between treatments on day 15 of the experiment, with an average length of 3.06±0.33 mm in the floral treatment compared with 1.66±0.14 mm in the vegetation treatment. Within the floral resources treatment, vitellaria lengths on days 6-12 and 18-21 were shorter than on day 15 but not different from each other. Lengths were significantly shorter on day 3 and shortest on day 0, or at onset of the experiment (df=19, F-Ratio 16.77, $p<0.05$).

Dissection of the gut of weevils in the floral treatment revealed the presence of pollen in every sample, and one *L. minutus* collected on the final collection day (day 27) also had fragments of flower petals present in the gut.

2016

Ovary appearance was influenced by access to floral resources. Ovaries in all treatments lacked oocytes (stage 1) prior to exposure to floral resources. After exposure to floral resources, oocytes were present in all treatments, and all weevils contained oocytes (stage 2 and 3) for the duration of the experiment. The Chi-square test determined that the variation in the proportion of ovaries in each stage was not due to random chance, with significance in the effect of both the Treatment (df=2, X^2=141.28, $p<0.0001$) and the Collection Event (df=18, X^2=81.23, $p<0.0001$). The duration and timing of access to floral resources influenced ovary structures and egg production. The two-factor model for germarium length was significant for both Treatment (df=3, F-Ratio 8.15, $p<0.0001$) and Collection Day (df=9, F-Ratio 8.68, $p<0.0001$), but their interaction Treatment*Collection Day did not impact the model (df=27, F-Ratio 1.79, $p=0.10$). Mean germarium length was greater in the control group Floral Diet Day 0-30 than the three treatments (df=39, F-Ratio 3.87, $p<0.0001$), but germarium length was not significantly different among the three treatments (Table 2.1).

Length of vitellarium increased after exposure to floral resources in all treatments and the control group. The model using Treatment and Collection Day as the main factors showed a significant effect of Treatment (df=3, F-Ratio 46.12, $p<0.0001$), Collection Day (df=9, F-Ratio 29.44, $p<0.0001$) and the interaction of Treatment*Collection Day (df=27, F-Ratio 4.68, $p<0.0001$) (Table 2.2).

The numbers of mature eggs in the ovaries of *L. minutus* varied over time and were significantly impacted by Collection Day (df=9, F-Ratio 10.67, $p<0.0001$), Treatment (df=3, F-Ratio 42.01, $p<0.0001$), and the interaction of Collection Day*Treatment (df=27, F-Ratio=6.78, $p<0.0001$) (Table 2.3). The average number of eggs was greatest in the control group, with 1.2 ± 0.10 eggs/weevil (df=12, F-Ratio 13.18, $p<0.0001$). Numbers of eggs in the ovaries for weevils in the remaining three treatments were: Floral Diet Day 0-3, 0.35±0.07eggs/weevil; Floral Diet Day 6-9, 0.47±0.10 eggs/weevil; and Floral Diet Day 12-15 0.21±0.06 eggs/weevil.

Production of mature eggs for all treatments was first recorded 3 days post-access to floral resources (Table 2.4). When comparing the total number of eggs present in the ovaries, the positive control group Floral Diet Day 0-30 had significantly more eggs than the three treatment groups (df=3, F-Ratio 17.58, $p<0.0001$) (Table 2.2). The percentage of *L. minutus* producing eggs in the control group (68%) was significantly different from the three treatments (df=3, X^2=76.04, $p<0.0001$). Eggs were produced by 25% of *L. minutus* in both the Floral Diet Day 0-3 and Floral Diet Day 6-9 treatments, whereas 14% of *L. minutus* in the Floral Diet Day 12-15 treatment produced eggs.

The number of mature eggs present in the ovaries at each collection day was related to access to floral resources. The regression line for the control (Floral Diet Day 0-30) had a positive slope and was significantly different than the treatment groups for the number of eggs present in the ovaries at each collection day (df=25, F-Ratio 53.98, p<0.001). The remaining three treatments had negative slopes that did not differ significantly from each other. There also was no significant difference between the y-intercepts of the regression lines (df=3, F-Ratio 1.13, p=0.36) (Fig. 2.2).

Discussion

The parts of the plant comprising the diet of *L. minutus* impacted the production of eggs. No *L. minutus* that were given access to only vegetative plant parts (leaves, stems) produced eggs, whereas those given access to floral resources did produce eggs. Floral resources for spotted knapweed include components of the flower itself (e.g., pollen, petals, nectar) and the structural bracts that support the composite head. All the 100 dissected *L. minutus* from the floral diet treatment in 2015 had pollen present in the gut. Only one weevil from the 100 dissected in 2015 contained fragments of a flower petal in the gut; the bright pinkish-purple color was clearly visible and contrasted with the dull yellow color of pollen and dark green of vegetation. Finding pollen in all weevils that produced eggs leads to the conclusion that pollen is the critical floral resource necessary for initiation of oocyte production and development of eggs in *L. minutus*. Although some necessary proteins and amino acids can be found in nectar, the chewing mouthparts of *L. minutus* suggest that nectar would not be the source of those nutrients.

The combination of morphological characteristics of ovaries allowed classifying the ovaries of *L. minutus* into three distinct stages of development: stage 1, with immature ovaries that lacked oocytes and eggs; stage 2, with mature ovaries that contained oocytes but no mature eggs; and stage 3, with mature ovaries that contained mature eggs. Those weevils that were not given access to floral resources lacked oocyte production and had ovaries that were transparent, indicating the ovaries did not progress past the first stage. That lack of oocyte production contrasts with those weevils that were provided floral resources at any time during the experiment, with visible changes to ovary size and color. In both the 2015 and 2016 experiments, ovaries in stage 2 and 3 were found after *L. minutus* was provided floral resources. Additionally, in the treatments in 2016 when floral resources were later removed, the ovaries continued to have at least some oocytes present and remained in stage 2 at a minimum. Whether maintaining a stage 2 ovary status would continue to hold true if the experiment were increased in duration is not known. However, exposure to floral resources, regardless of when, was critical to ovary maturation.

With any access to floral resources, *L. minutus* began production of oocytes in the germaria. Those *L. minutus* with access to floral resources had larger germaria, with the largest occurring in those weevils that had continual access to floral resources. Similarly, weevils with continued access to floral resources also had the longest vitellaria. Weevils given any access to floral resources had longer vitellaria than the *L. minutus* without that access, and those having any access to floral resources also contained developing oocytes and mature eggs. Those *L. minutus* given floral resources between 0-3 or 6-9 days had vitellaria of intermediate length, and the weevils with the shortest vitellaria were those given access after 12 days.

Production of mature eggs within three days of access to floral resources was observed regardless when flowers were provided. The sampling interval of 3 days did not allow for measuring the initiation of egg production on a finer temporal scale. The numbers of eggs found in the vitellaria of the ovaries were greatest in the group with continual access to floral resources. Those with access only from days 12-15 had the fewest eggs present and those with access from days 0-3 and 6-9 had egg numbers that were intermediate between the two. The time of floral resource availability and duration were also important in determining the cumulative number of eggs produced. Continued access resulted in significantly more eggs than those with limited access. Numbers of eggs found at each dissection increased for *L. minutus* with continual access to floral resources. In contrast, the numbers of eggs produced at each dissection decreased in all other treatments. The rate of reduction in eggs was similar in the three treatment groups. Additionally, the intercepts for all the regression lines were not statistically significant with an extrapolated range of 9.3-13.3 eggs predicted at the onset of egg production. Therefore, while all groups were initially capable of producing similar numbers of eggs, the impact of food source impacted the number of eggs produced in each treatment over time. It appears that there was a cost to the fecundity of the weevil with a delay in access and duration of the floral resources. Whether the decrease is due to a depletion of pollen provided only for a limited duration is not known.

The findings from this experiment have additional consequences when considering the biology of *L. minutus*. This weevil is univoltine and overwinters as an adult in the soil near the base of the plants. When the adult weevils emerge in the spring, they feed on vegetation and mate until flowers become available in late May to mid-June

Experiments with controlled diets isolating the components of 'floral resources' would need to be conducted to confidently determine pollen as the critical component required for egg production. Isolating pollen, controlling for the amount provided to individual *L. minutus,* examining weevils and capitula for eggs daily, would provide a more complete picture of the relationship between resource and egg production.

References

Alford, A. M., T. Kring, and S. Raghu. 2016. Spread of *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of knapweeds, following introduction to northwestern Arkansas. Florida Entomologist. 99: 593-599

Bonhag, P. F. 1958. Ovarian structure and vitellogenesis in insects. Annual Review of Entomology. 3: 137-160

Davis, E. S., P. K. Fay, T. K. Chicoine, and C. A. Lacey. 1993. Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. Weed Science. 41: 57-61

DinoCapture 2.0. 2016. AnMo Electronics Corporation version 1.5.28.A

Duguma, D., T. J. Kring, and R. N. Wiedenmann. 2009. Seasonal dynamics of *Urophora quadrifasciata* on spotted knapweed in the Arkansas Ozarks. Canadian Entomology. 141:70-79

Gilbert, L. E. 1972. Pollen feeding and reproductive biology of *Heliconius* butterflies. Proceedings of the National Academy of Sciences (USA). 69: 1403-1407

Grodowitz, M. J. and F. D. Brewer. 1987. Ovarian anatomy and physiological age-grading of the female boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). Annals of the Entomological Society of America. 80: 642-651

Grodowitz, M. J., T. D. Center and J. E. Freedman. 1997. A physiological age-grading system for *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), a biological control agent of water hyacinth, *Eichhornia crassipes* (Mart.) Solms. Biological Control. 9: 89-105

Groppe, K. 1990. *Larinus minutus* Gyll. (Coleoptera: Curculionidae), a suitable candidate for the biological control of diffuse and spotted knapweed in North America. CABI Institute of Biological Control, Delémont, Switzerland

Human, H., S.W. Nicolson, K. Strauss, C.W.W. Prik, and V. Dietemann. 2007. Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellate*). Journal of Insect Physiology. 53: 649-655

Jordan, K. 1995. Host specificity of *Larinus minutus* Gyll. (Col., Curculionidae), an agent introduced for the biological control of diffuse and spotted knapweed on North America. Journal of Applied Entomology. 119: 689-693

Kashefi, J. M. 1993. A new method for determining the sex of living weevils. Proc. Entomolical Society of Washington. 95: 511-512

Kashefi, J. M. and R. Sobhian. 1998. Notes on the biology of *Larinus minutus* Gyllenhal (Col., Curculionidae), an agent for biological control of diffuse and spotted knapweeds. Journal of Applied Entomology. 122: 547-549

Lacey C. A., Egan C., Pearson W., Fay P. K. 1988. Bounty programs an effective weed management tool. Weed Technology. 2:196-197

Lacey, J. R., C. B. Marlow, and J. R. Lane. 1989. Influence of spotted knapweed (Centaurea maculosa) on surface runoff and sediment yield. Weed Technology. 3:627-631.

Martin, L. M., N. W. MacDonald, and T. E. Brown. 2014. Native plant establishment success influenced by spotted knapweed (*Centaurea stoebe*) control method. Ecological Restoration. 32: 282-294

Mauer, T., M. Russo, and M. Evans. 2001. Element stewardship extract for *Centaurea maculosa*. The Nature Conservancy, Arlington, Virginia

Minteer, C. 2012. The biological control of spotted knapweed in the southeastern United States. PhD Dissertation. University of Arkansas

Minteer, C. R., T. J. Kring, J. Shen, and R. N. Wiedenmann. 2011. *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of spotted knapweed (*Centaurea stoebe* ssp. *micranthos*) established in northern Arkansas. Florida Entomologist. 94: 350-351

Myers, J. H., C. Jackson, H. Quinn, S. R. White, and J. S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control. 50: 66-72

Perez-Mendoza, J., J. E. Throne, and J. E. Baker. 2004. Ovarian physiology and age-grading in the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). Journal of Stored Products Research 40: 179-196

Rand, A. T., S. M. Louda, K. M. Bradley, and K. K. Crider. 2015. Effects of invasive knapweed (*Centaurea stoebe* subsp. *micranthos*) on a threatened native thistle (*Cirsium pitcher*) vary with environment and life stage. Botany. 93: 543-558

Schirman, R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. Journal of Range Management. 34: 45-47

Smith, E. H. and E. H. Salkeld. 1964. Ovary development and oviposition rates in the plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae). Annals of the Entomological Society of America. 57: 781-787

Story, J. M. 2002. Spotted knapweed, pp. 169-180. In R. G. Van Driesche, B. Blossey, M. Hoddle, S. Lyon and R. Reardon (eds.), Biological control of invasive plants in the Eastern United States. USDA Forest Service, Morgantown, West Virginia

Story, J. M., N. W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control. 38: 227-232

Tyser, R. W. and C. H. Key. 1988. Spotted knapweed in natural area fescue grasslands: an ecological assessment. Northwest Science. 62: 151-160

USDA, NRCS. 2013. The PLANTS database.https://plants.usda.gov. National Plant Data Team, Greensboro, NC USA

Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds. *Centaurea diffusa* and *C. maculosa*. Canadian Journal of Plant Science. 54: 687-7011

Wilson, L. M. and C. B. Randall. 2003. Biology and Biological Control of Knapweed. USDA Forest Service. Forest Health Technology Enterprise Team, Technology Transfer

Table 2.1. Lengths of the germarium present in *Larinus minutus* in 2016 experiments. Treatments consisted of providing access to floral resources for three days, either days 0-3, 6-9, or 12-15. The positive control consisted of *L. minutus* given continual access to floral resources for the duration of the 30-day experiment. Columns with figures followed by different letters are significantly different ($p<0.05$).

Treatment Days *L. minutus* exposed to floral diet	Germarium Length (mm) Mean±SE
Control (Day 0-30)	1.49±0.03a
Day 0-3	1.36±0.03b
Day 6-9	1.34±0.02b
Day 12-15	1.37±0.03b

Table 2.2. Lengths of the vitellarium present in *Larinus minutus* at collection dates at three-day intervals from day 0 to day 27 in 2016 experiments. Treatments consisted of providing access to floral resources for three days, either days 0-3, 6-9, or 12-15. The positive control consisted of *L. minutus* given continual access to floral resources for the duration of the 30-day experiment. Figures followed by different letters are significantly different ($p<0.05$).

	Experimental Days provided Floral Resources (Mean±SE)			
Collection Day	Day 0-30 (Control)	Day 0-3	Day 6-9	Day 12-15
3	1.61±0.09l	1.58±0.11l	1.60±0.08l	1.59±0.10l
6	2.24±0.22ijk	2.43±0.19ijk	1.42±0.09l	1.59±0.08l
9	3.33±0.12abc	2.38±0.12ijk	2.09±0.07jk	2.12±0.07jk
12	3.26±0.18bcde	3.35±0.19ab	3.72±0.22a	2.06±0.12k
15	3.28±0.21bcd	2.28±0.17ijk	2.50±0.10ghij	2.09±0.10jk
18	3.08±0.15bcde	2.24±0.16ijk	2.42±0.12ijk	2.47±0.27hijk
21	2.93±0.16cdef	2.34±0.17ijk	2.44±0.10ijk	2.36±0.25ijk
24	3.18±0.16bcde	2.40±0.08ijk	2.45±0.10hijk	2.32±0.10ijk
27	2.85±0.16efgh	2.16±0.15jk	2.63±0.19fghi	2.27±0.11ijk
30	2.89±0.15defg	2.49±0.12ghij	2.34±0.16ijk	2.14±0.12jk

Table 2.3. Average numbers of eggs present in ovaries of *Larinus minutus* at collection dates at three-day intervals from day 0 to day 27 in 2016 experiments. Treatments consisted of providing access to floral resources for three days, either days 0-3, 6-9, or 12-15. The positive control consisted of *L. minutus* given continual access to floral resources for the duration of the 30-day experiment. Figures followed by different letters are significantly different ($p<0.05$).

	Experimental Days provided Floral Resources (Mean±SE)			
Collection Day	Day 0-30 (Control)	Collection Day	Day 0-30 (Control)	Collection Day
3	0.00±0.00j	0.00±0.00j	0.00±0.00j	0.00±0.00j
6	0.20±0.20hij	0.40±0.22ghij	0.00±0.00j	0.00±0.00j
9	1.30±0.40bcd	1.00±0.39cdef	0.00±0.00j	0.00±0.00j
12	1.40±0.27bc	0.80±0.20defg	3.10±0.38a	0.00±0.00j
15	1.20±0.25bcde	0.40±0.22ghij	0.70±0.21efgh	0.00±0.00j
18	1.50±0.31bc	0.20±0.13hij	0.30±0.15ghij	0.70±0.33efgh
21	1.70±0.50b	0.30±0.21ghij	0.20±0.13hij	0.60±0.34fghi
24	1.50±0.27bc	0.10±0.10ij	0.20±0.13hij	0.40±0.16ghij
27	1.50±0.27bc	0.10±0.10ij	0.10±0.10ij	0.10±0.10ij
30	1.70±0.21b	0.20±0.20hij	0.10±0.10ij	0.30±0.21ghij

Table 2.4. Cumulative totals of mature eggs present in the ovaries of female *Larinus minutus*. Eggs were counted at three-day intervals for the duration of the 30-day experiment. First day exposed to floral resources is in relation to experiment start time.

Treatment	Day First Exposed to Floral Resources	Day First Eggs Found	Total Number of Eggs Produced
Floral Day 0-30 (Control)	0	3	120
Floral Day 0-3	0	3	35
Floral Day 6-9	6	9	42
Floral 12-15	12	15	21

Figure 2.1. Development of ovaries in female *Larinus minutus* (Coleoptera: Curculionidae) pre- and post-consumption of floral resources from spotted knapweed, and classification of ovary development by stages. Germarium is labelled as I. and vitellarium II. (A) Stage 1: ovarioles are translucent and oocytes are not present in the vitellaria; (B) Stage 2: ovarioles have increased opacity with yellow yolk present in the vitellaria. Egg lacks chorion, indicating it is not yet fully developed; (C) Stage 3: yolk is present in the vitellarium and mature eggs have chorion around their exterior, indicating full development, and are deeper yellow in color than still-developing oocytes.

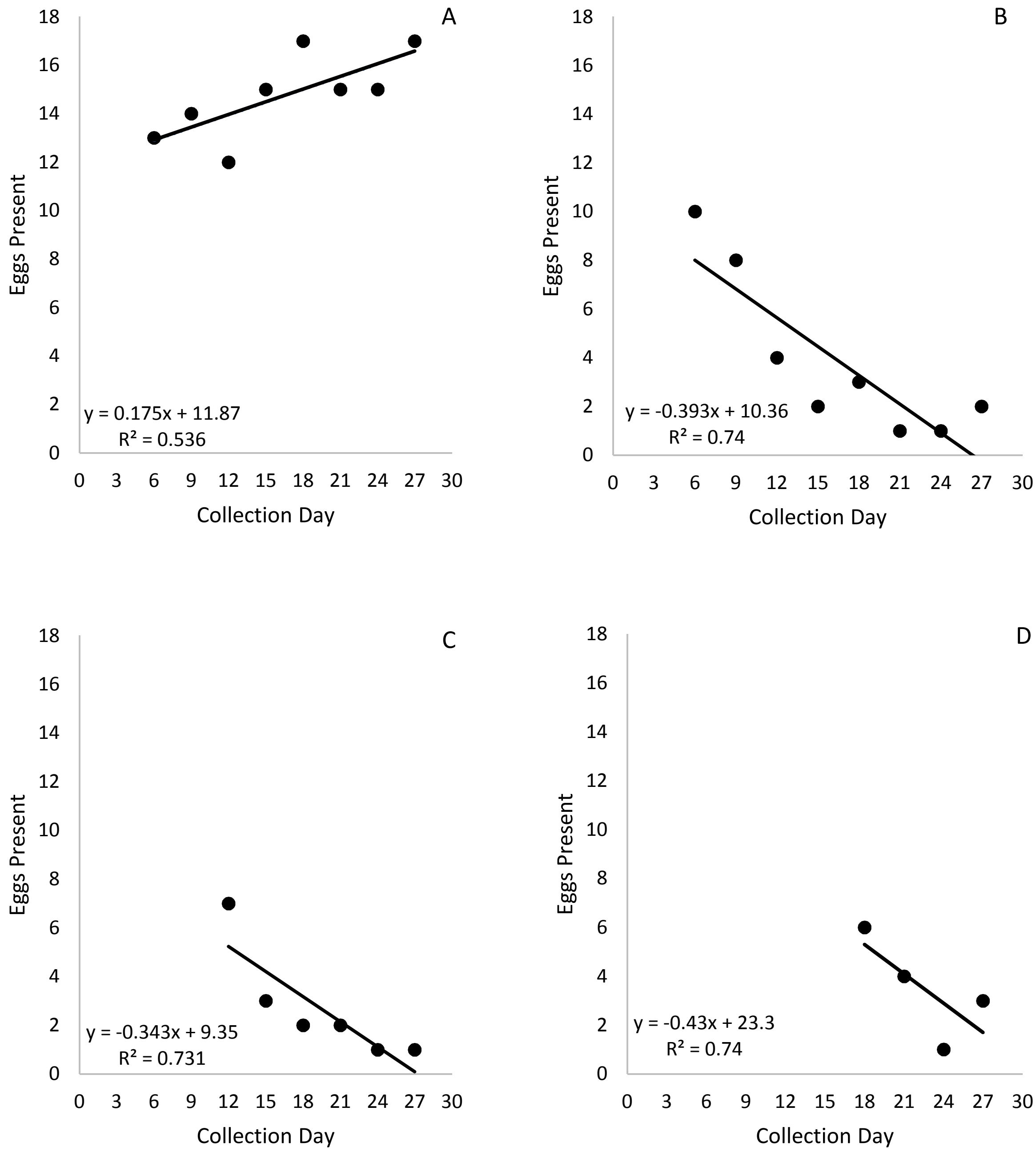

Figure 2.2. New eggs present in the ovaries in *Larinus minutus* at each collection day for the control ‘Flower Day 0-30’ (A), treatments ‘Flower Day 0-3’ (B), ‘Flower Day 6-9’ (C), and ‘Flower Day 12-15 (D). Trend lines were constructed using ‘Fit Model’ and ‘Line of Fit’.

Chapter 3

Mortality of *Larinus minutus* Gyllenhaal due to roadside mowing of spotted knapweed

Abstract

In the southeastern U.S., the invasive weed, spotted knapweed is often found along roadsides. Due to its location along roadsides in Arkansas, spotted knapweed is frequently mowed for highway maintenance. Spotted knapweed has been a target of biological control, and one of the biological control agents established in Arkansas, *Larinus minutus*, feeds on the vegetation and flowers of spotted knapweed as an adult and on the seeds inside the flowerhead as a larva. The entire life cycle from egg to adult emergence occurs within the same capitulum. During the immature stages the biological control agent is susceptible to any disturbance, such as mowing, of the plant. The purpose of this experiment was to determine if mowing spotted knapweed resulted in higher mortality of *L. minutus* and if there was any impact on seed production within individual capitula. Roadside sites infested with knapweed were stablished, with 8 sites in 2017 and 23 sites in 2018. All sites contained two treatments: un-mowed spotted knapweed and mowed spotted knapweed, both in adjacent plots, 5x25m in area. From each treatment area 50 capitula were collected (if fewer were available, all capitula per plot were collected) either the knapweed plants (un-mowed) or ground (mowed). After 6 weeks, capitula were dissected and seed types (mature, immature, and damaged) and presence or evidence of *L. minutus* in the capitulum was recorded. In the un-mowed treatment, fewer dead *L. minutus* were found inside the capitulum for both years. The un-mowed treatment also had greater emergence both prior to mowing and after mowing than in the mowed treatment. There was a strong correlation both years between the proportion of capitula containing *L. minutus* and average damaged seeds present in a capitulum. The disturbance of spotted knapweed by mowing is

negatively impacting *L. minutus* in its larval stage. Altering mowing strategies may reduce the observed difference in mortality between un-mowed and mowed spotted knapweed.

Introduction

Spotted knapweed, *Centaurea stoebe*, is an invasive Eurasian weed that was unintentionally introduced to the United States in the 1800s. The original introduction, through contaminated seed, occurred in the Pacific Northwest where the plant was able to establish, especially in rangelands that had been subjected to over-grazing (Mauer et al. 2001). Over time, spotted knapweed has spread so that it is now found in most states in the continental U.S., with size and severity of infestations varying greatly (USDA 2013).

Spotted knapweed is a short-lived perennial plant that reproduces through seed production (Watson and Renney 1974). Local spread occurs passively as the achenes (mature seeds) are released from the flowerheads, whereas long-distance spread occurred through movement of contaminated hay or commercial seed, or by livestock or vehicles that carry the seeds (Roche et al. 1986). The means of the long-distance movement of spotted knapweed to the southeastern U.S. is not understood, nor when that spread occurred. Spotted knapweed is primarily a weed of rangeland in the western U.S. but, in the southeastern U.S., the weed is primarily found along roadsides. Similar to the disturbance by over-grazing in the western U.S., roadsides in the southeast are regularly disturbed by equipment used in road construction and by repeated mowing of vegetation.

Knapweed typically spends the first year after spring germination as a basal rosette before producing bolts (flower-producing stems) in subsequent years. In the southeastern U.S., bolt production begins in early spring with bud formation occurring in the last half of April. Flowering begins in late May and continues to late October, with the peak in blooms occurring in early June. In the fall, flowerheads senesce and release mature seeds in the area around the plant, and above-ground portions die back to the roots. In the spring, bolts are again produced from the roots, with an increasing number produced by individual plants each year for the 7-8 year lifespan (Watson and Renney 1974). Knapweed seeds have the potential to remain viable for up to 8 years (Davis et al. 1993). Seed numbers found within the capitulum ranged from 9-37 seeds (Watson and Renney 1974, Schirman 1981).

Management strategies for spotted knapweed infestations vary depending on land use. In rangelands, successful control methods have been primarily cultural, focusing on reducing grazing and alternating the species grazing early in the season, mowing (DiTomaso 2000), or long-term programs of prescribed burns (Zouhar 2001). Management of spotted knapweed that occurs along roadsides is mostly by mowing, and this tactic is used primarily to maintain safety and aesthetic standards. Although less common, herbicides are used for weed control in areas difficult to reach with mowing equipment.

Biological control has been used successfully against spotted knapweed in the Pacific Northwest of the U.S. and western Canada (Story et al. 2006; Myers et al. 2009). The biological control program that began in the 1960s resulted in establishment of 13 introduced species of insects throughout the western U.S. and Canada (Story 2002). Of these, the seed-feeding weevil, *Larinus minutus* (Coleoptera: Curculionidae), successfully reduced infestations of spotted and diffuse knapweed (*Centuarea diffusa*) (Story et al. 2006; Myers et al. 2009). Given its past success and potential for use against spotted knapweed in Arkansas, *L. minutus* was collected from Colorado and moved to Arkansas where it was established in the northwestern portion of the state (Minteer et al. 2011).

Larinus minutus is a univoltine insect whose lifecycle is tied to spotted knapweed. Once knapweed flowerheads (capitula) begin to bloom, *L. minutus* oviposits 2-3 eggs inside a capitulum, where the weevil larvae feed on the seeds. Female *L. minutus* can produce more than

100 eggs over the duration of the growing season (Kashefi and Sobhian 1998). Weevil larvae remain in the same flowerhead for the duration of their larval stages and pupate within the capitulum (Groppe 1990; Alford 2013). After pupation, the new adult weevil chews an exit hole through the top of the now-senesced flowerhead. Typically, only one adult weevil will emerge from a single flowerhead, although occasionally two adults can be produced (Kashefi and Sobhian 1998). The newly emerged adults feed on spotted knapweed vegetation to build fat stores before going into the soil to over-winter (Jordan 1995). In the subsequent spring, the adults emerge from overwintering, feed on knapweed vegetation and flowers, mate, and begin producing eggs.

Because the life cycle of *L. minutus* is closely tied to spotted knapweed, disturbing knapweed at critical times, such as by mowing, could negatively impact biological control as a management tactic. The immature weevil spends its larval stages within a capitulum, so mowing has the potential to kill any weevils present in the capitulum. Mowing along roadsides occurs two to three times per year and its purpose is to reduce vegetation to maintain visibility, reduce fire hazards, and maintain aesthetic standards as mandated by the state highway departments. Mowing that occurs at inopportune times can remove the resources that are needed by adult weevils as oviposition sites and by immature *L. minutus* as a food source.

This study was conducted to assess the potential conflict between management approaches, to determine if mowing has a deleterious impact on *L. minutus* and, if so, whether that is interfering with the ability of *L. minutus* to increase in number. Another planned outcome for this study was to develop recommendations for optimizing the timing of mowing to enhance compatibility with the phenology of the biological control agent and, thus, provide better control of the weed by combining tactics.

Methods

Surveys for experimental sites were conducted in early spring of 2017 along state highways in Washington County, Arkansas. Criteria for sites included spotted knapweed in a patch with a minimum size of 10m in width, perpendicular to the road, and at least 25m in length, parallel to the road. The management standard mandated by the Arkansas Highway and Transportation Department is to mow a swath 5 m wide. As a result, all sites selected had infestations that were at least 10 m wide, measured perpendicular to the roadway, which allowed placing both mowed and un-mowed treatments proximally. At each site, a 10m by 25m area was subdivided into two 5m x 25m rows, each called a plot and each containing five 5x5m subplots. The 5x25m plot closest to the highway contained the mowed treatment, whereas the 5x25m plot farther from the highway contained the un-mowed plot.

In 2017, 8 sites were established along State Highway 45, with at least 20m separating each site. An additional 23 sites were established in 2018 along State Highway 45 and U.S. Highway 412. Mowing was conducted by companies contracted through the Arkansas Highway and Transportation Department. All mowed areas extended 5m from the roadside as mandated by the highway department, with mowing occurring in May and July. Mowing typically consisted of one pass and left 15-25cm of vegetation standing whereas un-mowed plants were 65-70cm in height.

No samples were taken in association with the May mowing, as spotted knapweed had just produced buds, but flowering had not yet begun. Sampling in July occurred when flowers were present and consisted of collecting knapweed capitula within 24 hours of mowing. Capitula from the mowed plots were collected from the ground and only capitula that were undamaged by the mowing equipment were selected. Samples from the un-mowed plots were collected directly from the plants.

From each of the five subplots per treatment, a sample of 10 capitula was collected, with a maximum of 50 per plot. If there were fewer than 50 capitula in a plot, as many as were available were collected. Sampling consisted of walking through each subplot and collecting capitula in an unguided (not truly random) manner. Data from the subplots were combined to yield a total per plot for each treatment at each site.

Samples were sorted into 29-ml, clear plastic cups with lids, so each cup contained a single capitulum. Cups were kept at 22°C and 16:8 L:D for four weeks and monitored for emergence of adult *Larinus minutus*. After four weeks, the capitula were dissected and counts were made of the number of *L. minutus* in the capitulum (dead larvae, pupae, and/or adults), adult *L. minutus* in the container (indicating the weevils emerged after mowing), or emergence holes with no weevil present (indicating the weevils emerged before mowing). Additionally, the numbers of mature seeds, immature seeds, and seeds damaged by *L. minutus* feeding were counted. No seeds damaged by mowing were found and none were considered for this study.

All data were analyzed using JMP Pro ® 13.2 software (SAS Institute 2018). A univariate ANOVA was conducted for all data classifications. The 'Treatment' was used as the main effect and Student's t-test was used to separate means between the treatments if the model was found to be significant. Statistics for *L. minutus* were calculated as proportions due to presence of multiple weevils in $<0.01\%$ of capitula sampled. Regression lines were constructed using 'Line of Best Fit', which produced exponential curves fitting mowed and un-mowed treatments.

Results

2017

Larinus minutus, or evidence of its presence, was found in both treatments (Table 3.1). No capitula were found with evidence that weevils emerged prior to mowing in either the un-mowed or mowed plots. Un-mowed plots had a significantly greater proportion of capitula with *L. minutus* (0.18±0.02) than mowed plots (0.04±0.02) (df=14, t=4.24, p<0.0008). No capitula in the un-mowed plots contained dead weevils, whereas 0.04±0.02 capitula in mowed plots contained dead weevils. An average of 0.18±0.02 of the capitula in un-mowed plots had weevil emergence after the mowing event, whereas no weevils emerged after mowing from capitula collected from the mowed plots (df=14, t=8.24, p<0.0001).

A total of 400 capitula were examined in the un-mowed treatment and 266 capitula total were examined in the mowed treatment, and both treatments yielded mature, immature and damaged seeds. The parameters measured differed significantly between the mowed and un-mowed plots with the exception of the average number of damaged seeds present in the capitula (Table 3.2). The average number of seeds (7.87±0.28) in the un-mowed treatment was significantly greater than the number (2.31±0.26) in the mowed treatment (df=14, t=5.00, p<0.0001). The average number of mature seeds (6.03±0.24 seeds/capitulum) in un-mowed plots was significantly greater than the average number of mature seeds (1.90±0.24 seeds/capitulum) in the mowed plots (df=14, t=5.16, p<0.0001). Average counts of immature seeds for the un-mowed treatment (1.84±0.13 seeds/capitulum) was significantly greater than the average immature seeds in the mowed plots (0.57±0.07 seeds/capitulum) (df=14, t=3.28, p<0.006). The un-mowed treatment had 0.03±0.01 damaged seeds/capitulum and the mowed treatment had no damaged seeds.

2018

Both treatments yielded evidence of *Larinus minutus*, either that emerged prior to mowing, emerged after mowing or died while still in the capitula (Table 3.3). The proportion of capitula with *L. minutus* in the un-mowed treatment was greater (0.38±0.01) than the mowed treatment (0.26±0.01) (df=44, t=2.65, p<0.011). The proportion of capitula with dead *L. minutus* in the un-mowed treatment (0.07±0.01) was significantly less than the mowed treatment (0.21±0.01) (df=44, t=5.05, p<0.0001). The proportion of capitula in the un-mowed treatment with weevil emergence before mowing (0.18±0.01) was greater than the mowed treatment (0.03±0.01) (df=1, 44, t=7.59, p<0.0001). The un-mowed treatment had 0.11±0.00 capitula with evidence of emergence, which was greater than the mowed treatment, which had 0.02±0.01 (df=44, t=4.06, p<0.0001).

A total of 1150 capitula was examined in the un-mowed treatment and 1096 capitula in the mowed treatment, and both treatments yielded capitula with mature, immature and damaged seeds (Table 3.4). The un-mowed treatment had more seeds/capitulum (10.45±0.22) than the mowed treatment (7.57±0.22) (df=44, t=2.31, p<0.025). Similarly, the number of mature seeds differed significantly between treatments (df=1, 44, F=7.48, p<0.009). The average number of mature seeds in un-mowed plots (5.54±0.18 seeds/capitulum) was significantly greater than in mowed plots (3.78±0.18 seeds/capitulum) (df=44, t=2.74, p<0.009). The number of immature seeds per capitulum (1.30±0.065) for the un-mowed treatment was significantly less than number from mowed plots (1.80±0.09 (df=44, t=2.73, p<0.009). The average number of damaged seeds per capitulum for the un-mowed plots (3.60±0.16) was significantly greater than in mowed plots (1.99±0.13) (df=44, t=2.22, p<0.031).

Of the total seeds within a capitulum, on average the proportion damaged by *L. minutus* differed significantly between the treatments (df=1, 2187, F=32.46, p<0.0001) where 0.29±0.01 of the total seeds were damaged in the un-mowed and 0.19±0.01 seeds were damaged in the mowed treatment (df=2187, t=5.70, P<0.0001). The numbers of damaged seeds per capitulum and proportions of capitula per plot with *L. minutus* were moderately to strongly correlated in both the un-mowed (Spearman's ρ=0.93, p<0.0001) and mowed treatments (Spearman's ρ=0.97, p<0.001). The regressions for both the 'un-mowed' model (df=2, 20, F=63.26, p<0.0001) and the 'mowed' model (df=2, 20, F=23.18, p<0.0001) were significant and were fitted with exponential curves (Fig. 3.1). Both treatments exhibited a positive response in number of seeds damaged as the proportion of *L. minutus* increased.

Discussion

The presence of *L. minutus* in both mowed and un-mowed plots demonstrated the use of the entire patch as an oviposition source. Both the mowed and un-mowed portion of the patch were capable of producing mature seed despite spotted knapweed being shorter and with less flowers in the mowed portion of the patch. There were sufficient resources for the completion of the lifecycle of *L. minutus* to adulthood in both the un-mowed and mowed sections of the patch.

The average immature seeds/capitulum varied year to year. This category of seed type is not contributing to the seed bank and is a lower priority as a biological control target for reducing spotted knapweed populations. There was a possibility that these seeds had the potential to reach maturity but were removed from resources and growth halted. In both years the average number of mature seeds was greater in the un-mowed plots than the mowed plots. The presence of mature seeds in mowed knapweed indicates the ability of plants to maintain the seedbank after mowing. The average number of damaged seeds was also greatest in the un-mowed group in

2018 and was likely the result of feeding by *L. minutus* that emerged either prior or after mowing or that died while inside the capitula. Neither immature or damaged seeds contribute to the seedbank. Although finding fewer mature seeds in the mowed treatment could be beneficial, there were still sufficient numbers of seeds to maintain knapweed patches, as knapweed has been present at moderate densities for at least 3-4 years.

In 2017 there were no dead weevils found in the un-mowed treatment and no weevils in the mowed treatment that emerged after mowing. This may have been due to the limited number of sites sampled across a relatively small area and higher *L. minutus* numbers in 2018. Patterns observed in 2018 were similar to the prior year and, with more capitula dissected, the presence of weevils that died in the capitula and occurrence of *L. minutus* emerging after mowing were detected in both treatments. In both years, the mowed treatment had a greater proportion of capitula containing dead *L. minutus*. Although we cannot assume all of the *L. minutus* in this category would successfully emerge as adults without interference from mowing, it does indicate that mowing is having a negative impact on the populations sampled. It is also important to note that, in both the categories of emergence prior to mowing and emergence after mowing, the un-mowed treatment had more *L. minutus*. These adults will over-winter and comprise the next generation that emerges in the spring.

The potential for *L. minutus* to reduce seed densities was illustrated by the increase in damaged seeds as the proportion of capitula with *L. minutus* increased. This relationship shows an exponential increase in damaged seeds in relation to increasing proportion with *L. minutus*. This can be simplified to an increased chance of finding a damaged seed because the chance of finding a capitulum with *L. minutus* increases as more capitula contain larvae.

The factor impacting these results is the regrowth from the mowing event in May and secondarily the July mow. Spotted knapweed that is mowed in May has delayed re-growth and must replace lost stems and buds before flowering. Mowed plants are shorter in height and produce flowers 2-3 weeks after peak flowering occurs in the un-mowed patches of spotted knapweed. It is likely that, if left undisturbed in July, plants mowed only in May would be capable of producing more mature seeds than what was observed and could better support complete development of *L. minutus* within a capitulum. There is also the possibility of a roadside effect on spotted knapweed in the mowed area. Trahan (2005) found that plants closer to the road have increased damage, decreased photosynthesis, and the soil is often poor in nutrients.

Although *L. minutus* has been successfully released and established across northwest Arkansas (Minteer et al. 2011), it has not yet been as effective in reducing spotted knapweed as was seen in the western U.S. and Canada (Story et al. 2006, Myers et al. 2009). There, the rangelands were able to be left undisturbed (other than by grazing), unlike roadsides that are repeatedly maintained. It is likely that numbers of *L. minutus* in western areas are greater than what would be observed in the southeastern U.S., due to the difference in disturbance of the plant and weevils. Additionally, changes in knapweed infestations in the western U.S. were not seen until at least 4-5 years after release of biological control. At some locales, changes in spotted knapweed were not recorded for over a decade. Those findings suggest that it is still too early to detect landscape level changes in spotted knapweed infestations in Arkansas. Given the loss of potential adults in mowed areas, it is not unreasonable to assume current numbers of *L. minutus* are smaller than would be seen without disturbance. Because of management methods, the experimental areas contained both mowed and un-mowed plots. It is likely that the proportion of capitula with *L. minutus* in the un-mowed part of the plot was smaller than would be observed in a patch with no close proximity to mowed knapweed. Mowing strategies presently used in the southeast are incompatible with *L. minutus* and, ironically, are potentially preventing successful control of the invasive weed.

Mowing of roadsides in Arkansas (and other areas in the southeast) is designed to maintain aesthetics, reduce fire risks, and increase visibility. Early-season mowing that occurs in mid-May removes vegetation as well as buds that have formed and the very few flowers that have developed early in the season. These flowers are a necessary resource for adult *L. minutus* in order to produce eggs (Groppe 1990). Mowing delays access to an important resource for the adult and loss of sites for oviposition and larval development. Spotted knapweed is able to recover from removal of bolts and produce more flowers. As this regrowth is in the flowering stage, it is once again mowed. Removal of flowers later in the season and well after the average recorded lifespan of the overwintered adult leaves little opportunity for replacing any larvae that were present in capitula. Spotted knapweed very rarely produces a third set of flowers; if so, typically, no seeds reach maturity (personal observation). A final mowing event occurred in late October-early November after all spotted knapweed had senesced and *L. minutus* were overwintering in the soil. That mowing event covers a wider swath along roads but has little impact on the spotted knapweed which has (typically) died back to the roots.

It is not reasonable to forego all mowing on areas of roadsides where spotted knapweed is present. Mowing allows better visibility (thus increasing safety) and reduces risk of fires. Although the schedule for mowing is designed to curb vertical plant growth, mowing is not effective for controlling spotted knapweed (Minteer 2012). Spotted knapweed can overcome mowing through lateral growth and production of new flowers after early and mid-season mows.

Any portions of the plant that were too low to the ground to be removed by the first mowing likely remain undamaged by future mowing events (Rinella et al. 2001). Mowing late in the season occurs well after any plant growth or seed production and has no effect on knapweed plants, but movement of seed by equipment can exacerbate further spread of spotted knapweed.

Eliminating mowing is not an option and is not recommended, because of highway department standards and potential loss of income for mowing contractors. However, adjusting the timing of mowing could still allow meeting mandated requirements while improving the compatibility of mowing with biological control efforts. The approach most beneficial to all interests would be to alter mowing times, with the first mowing preferably occurring before buds are formed and the second occurring after peak bloom. In practice, this would move the May mowing event earlier by 2-3 weeks. Spotted knapweed will have begun to produce bolts, but the majority of vertical growth and bud formation will not have occurred. Additionally, if the second mowing is delayed 2-3 weeks so it occurs after peak flowering, the delayed mowing would avoid killing developing *L. minutus* larvae in capitula.

In addition to altered mowing times, protecting "reservoirs" -- patches of knapweed outside the mowed areas -- should be considered. These patches are often mowed as a result of a ‘good neighbor’ policy, by which the roadside area mowed extends to the fence line of any property owner who also mows to their side of the fence. Areas with heavy knapweed infestations could benefit from exemption from this policy, leaving reservoirs for oviposition and larval development. Although leaving weed patches may seem counterintuitive because these plant reservoir areas have the potential to produce more mature seeds than if they were mowed, the potential long-term benefit from an increase in numbers of *L. minutus* could outweigh the short-term increase in seed production.

Further investigation into how altered timing of mowing could impact knapweed seed production and *L. minutus* would need to be conducted to insure these policies are both successful at reducing knapweed and do not promote growth of other invasive weeds. In areas where other invasive weeds are known to be present, it would be prudent to determine if any native plants can be competitive and be used to prevent further invasion.

References

Alford, A. M. 2013. The life history of *L. minutus*, a biological control agent of invasive knapweeds, and its dispersal from release sites in Arkansas. Master's Thesis. University of Arkansas

Davis, E. S., P. K. Fay, T. K. Chicoine, and C. A. Lacey. 1993. Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. Weed Science. 41: 57-61

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science. 48: 255-265

Groppe, K. 1990. *Larinus minutus* Gyll. (Coleoptera: Curculionidae), a suitable candidate for the biological control of diffuse and spotted knapweed in North America. CABI Institute of Biological Control, Delémont, Switzerland

JMP® Pro 14.0.0. 2018. SAS Institute Inc., Cary, NC

Jordan, K. 1995. Host specificity of *Larinus minutus* Gyll. (Col., Curculionidae), an agent introduced for the biological control of diffuse and spotted knapweed on North America. Journal of Applied Entomology. 119: 689-693

Kashefi, J. M. and R. Sobhian. 1998. Notes on the biology of *Larinus minutus* Gyllenhal (Col., Curculionidae), an agent for biological control of diffuse and spotted knapweeds. Journal of Applied Entomology. 122: 547-549

Mauer, T., M. Russo, and M. Evans. 2001. Element stewardship extract for *Centaurea maculosa*. The Nature Conservancy, Arlington, Virginia

Minteer, C. R., T. J. Kring, J. Shen, and R. N. Wiedenmann. 2011. *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of spotted knapweed (*Centaurea stoebe* ssp. *Micranthos*) established in northern Arkansas. Florida Entomologist. 94: 350-351

Myers, J. H., C. Jackson, H. Quinn, S. R. White, and J. S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control. 50: 66-72

Rinella, M. J., J. S. Jacobs, R. L. Sheley, and J. J. Borkowski. 2001. Spotted knapweed response to season and frequency of mowing. Journal of Range Management Archives. 54: 52-56

Roche, B. F., Jr., G. L. Piper, and C. J. Talbott. 1986. Knapweeds of Washington. Cooperative Extension Bulletin 1393, Washington State University, Pullman

Schirman. R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. Journal of Range Management. 34: 45-47

Story, J. M. 2002. Spotted knapweed, pp. 169-180. In R. G. Van Driesche, B. Blossey, M. Hoddle, S. Lyon and R. Reardon (eds.), Biological control of invasive plants in the Eastern United States. USDA Forest Service, Morgantown, West Virginia

Story, J. M., N. W. Callan, J. G. Corn, L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control. 38: 227-232

Trahan, N. and C. Perterson. 2005. Factors impacting the health of roadside vegetation. Report CDOT-DTD-R-2005-12; Prepared for the Colorado Department of Transportation by the Department of Biological Sciences, University of Colorado. Greeley, CO

USDA, NRCS. 2013. The PLANTS database.https://plants.usda.gov. National Plant Data Team, Greensboro, NC USA

Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds. *Centaurea diffusa* and *C. maculosa*. Canadian Journal of Plant Science. 54: 687-7011

Wilson, L. M. and Randall, C. B., 2003. Biology and Biological Control of Knapweed. Forest Health Technology Enterprise Team, Technology Transfer

Zouhar, K. 2001. *Centaurea maculosa*. In: Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer) Available: http://www.fs.fed.us/database/feis

Table 3.1. Total numbers of spotted knapweed capitula, total proportion of capitula with evidence of *Larinus minutus*, proportion of capitula containing dead *L. minutus*, and proportion of capitula from which *L. minutus* adults emerged after mowing. Shown are means ±SE. Numbers within a column followed by different letters are significantly different[1].

Treatment	Total Capitula	Proportion of capitula with evidence of *L. minutus*	Proportion of capitula with dead *L. minutus*	Proportion of capitula from which *L. minutus* emerged after mowing
Un-mowed	n=400	0.18±0.02 a	0.00 a	0.18±0.02 b
Mowed	n=266	0.04±0.02 b	0.04±0.02 a	0.00 a

[1] Capitula were collected from eight mowed and un-mowed plots along roadsides in Washington County, Arkansas, in July 2017. No *L. minutus* emerged from capitula pre-mowing.

Table 3.2. Total numbers of spotted knapweed capitula sampled, and average numbers of total seeds, mature seeds, immature seeds, and damaged seeds counted in eight mowed and un-mowed plots along roadsides in Washington County, Arkansas, in July 2017. Shown are means ±SE. Numbers within a column followed by different letters are significantly different

Treatment	Total Capitula	Total Seeds	Mature Seeds	Immature Seeds	Damaged Seeds
Un-mowed	n=400	7.87±0.28 a	6.03±0.24 a	1.84±0.13 a	0.00 a
Mowed	n=266	2.31±0.26 b	1.90±0.24 b	0.57±0.07 b	0.03±0.01 a

Table 3.3. Total numbers of spotted knapweed capitula, total proportion of capitula with evidence of *Larinus minutus*, proportion of capitula containing dead *L. minutus*, proportion of capitula from which *L. minutus* adults emerged pre-mowing and proportion of capitula from which *L. minutus* adults emerged after mowing. Shown are means ±SE. Numbers within a column followed by different letters are significantly different[2].

Treatment	Total Capitula	Proportion of capitula with evidence of *L. minutus*	Proportion of capitula with dead *L. minutus*	Proportion of capitula from which *L. minutus* emerged pre-mowing	Proportion of capitula from which *L. minutus* emerged post-mowing
Un-mowed	1150	0.38±0.01 a	0.07±0.01 b	0.18±0.01 a	0.11±0.00 a
Mowed	1096	0.26±0.01 b	0.21±0.01 a	0.03±0.01 b	0.02±0.01 b

[2] Capitula were collected from 23 mowed and un-mowed plots along roadsides in Washington County, Arkansas, in July 2018.

Table 3.4. Total numbers of spotted knapweed capitula sampled, and average numbers of total seeds, mature seeds, immature seeds and damaged seeds counted in 23 mowed and un-mowed plots along roadsides in Washington County, Arkansas, in July 2018. Shown are means ±SE. Numbers within a column followed by different letters are significantly different

Treatment	Total Capitula	Total Seeds	Mature Seeds	Immature Seeds	Damaged Seeds
Un-mowed	1150	10.45±0.22 a	5.54±0.18 a	1.30±0.06 b	3.60±0.16 a
Mowed	1096	7.57±0.22 b	3.78±0.18 b	1.80±0.09 a	1.99±0.13 b

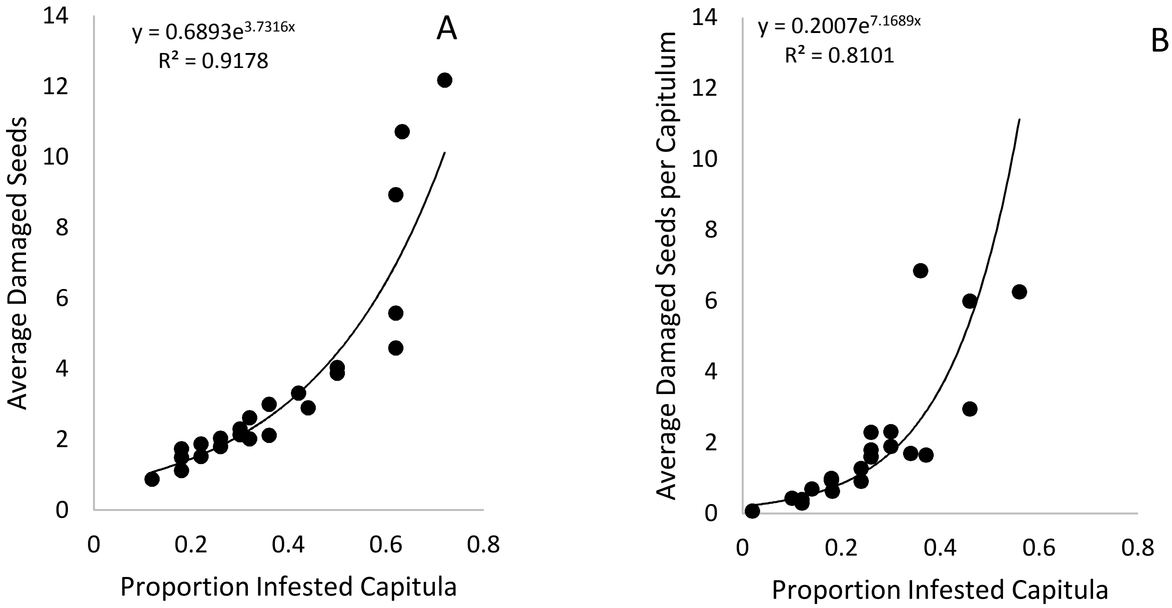

Figure 3.1. Relationship of average damaged seeds/capitulum per plot and proportion of capitula within a plot with some evidence of infestation by *Larinus minutus* for 'un-mowed' treatment (A) and 'mowed' treatment (B) in 2018.

Chapter 4

Landscape and patch level data for spotted knapweed (*Centaurea stoebe* Gugler) patches in northwest Arkansas

Abstract

A biological control program for spotted knapweed, *Centaurea stoebe*, was initiated in 2006 in Arkansas with the purposeful introduction of *Larinus minutus* (Coleoptera: Curculionidae). While introduction and establishment were occurring, surveys of spotted knapweed and the presence of the biological control agent were conducted. After the initial establishment, limited follow-up surveys were conducted to determine spread of either spotted knapweed or *L. minutus*. A survey of four counties in Arkansas was conducted in 2017 to determine the distribution of spotted knapweed and *L. minutus*. Measures of spotted knapweed height, density of bolts and crowns, patch size, and seed counts were taken at discrete patches in addition to recording evidence of *L. minutus.* From these data maps were generated showing the spotted knapweed distribution in the four counties and areas of clustering. Although measures of spotted knapweed, seeds, and proportion of capitula containing *L. minutus* varied across patches, no variation was seen between years.

Introduction

One of the major challenges facing society in the early 21st century is mitigating the effects of invasive species. Among other taxa of invaders, invasive weeds are known to cause ecological and economic harm when they establish and spread (Pimentel et al. 2005). Conservative estimates for number of invasive weeds species established in the United States are ~5000 species (Morse et al. 1995). Spotted knapweed (*Centaurea stoebe*) is one such weed of concern, due to its establishment and subsequent extensive spread (Harris and Cranston 1979; Lacey 1988). The original introduction into the Pacific Northwest area of the U.S. and Canada occurred in the late 1800s as a result of contaminated seeds or seeds in ship's ballast (Mauer et al. 2001). Spotted knapweed and its congener, diffuse knapweed (*Centaurea diffusa* Lamarck), have invaded susceptible rangelands, often those that have been disturbed by over-grazing by livestock (Mauer et al. 2001). Spotted knapweed has now spread to nearly all the states in the contiguous United States (USDA 2013).

Where spotted knapweed has spread to the southeastern U.S., the weed is often found along roadsides or areas of disturbance, such as by road construction. Spotted knapweed is a prolific seed producer, with infested areas yielding estimates of 146,000 seeds per square meter (Wilson and Randall 2003, Schirman 1981). Although the accumulation of large numbers of knapweed seeds can cause localized management problems, a greater issue is spread of spotted knapweed seeds, particularly by moving heavy machinery or roadside mowing (Roche et al. 1986).

Cultural (grazing) and chemical (herbicide) control have been used to in attempts to reduce spotted knapweed populations, but the success and economic feasibility of these management tools have been hampered by the vast areas infested, and the variable grazing methods and species grazed (cattle and sheep) (Griffith and Lacey 1991). A biological control program that began in the 1960s and continued through the late 1990s led to the establishment of 12 species of insects in areas of the western United States and Canada (Story 2002). One of the first insects established was a gall-inducing fly, *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), which attacks the developing seeds. *Urophora quadrifasciata* readily spreads to uncolonized knapweed patches (Story et al. 1987) but it is not a highly effective biological control agent (Harris and Shorthouse 1996, Duguma et al. 2009). One of the later species to be introduced, *Larinus minutus* (Gyllenhal) (Coleoptera: Curculionidae), is a seed-feeding weevil that was successful in reducing spotted (Story et al. 2006) and diffuse knapweeds (Myers et al. 2009) in western North America.

Arkansas is one of the states in the southeastern U.S. that has been invaded by spotted knapweed (Fig. 4.1), and a biological control program against spotted knapweed was initiated in 2006. A survey of spotted knapweed at multiple locations in Arkansas detected *U. quadrifasciata*, which is thought to have arrived adventively in the state (T.J. Kring, unpublished data). Based on the successes in western North America, *Larinus minutus* was selected as a potential agent to deploy against knapweed. Adult weevils collected in 2008 and 2009 from populations in Colorado were released at 40 sites in Arkansas, which resulted in establishment of the weevil at 38 sites across the state (Minteer et al. 2011). Monitoring of *L. minutus* and spotted knapweed occurred at a local level for 2-3 years to confirm establishment and incipient spread (Minteer 2012) and continued spread in Arkansas (Alford et al. 2016).

Monitoring is a crucial, but often minimized, aspect of many weed biological control programs. Unlike projects using biological control against insect pests, weed biological control projects can be monitored visually. Monitoring can, first and foremost, affirm establishment of released agents, detect the spread of agents from the release sites, and determine whether the weed has been affected at a local level. Monitoring that is coupled with large-scale mapping can improve the understanding of the success of a biological control program and impacts against a target weed beyond the local level. Maps can provide a baseline from which change in areas infested by a weed can be measured, and they can help in making informed decisions on management practices.

Two important considerations in monitoring efforts are the cost and time needed to complete a survey (Hauser et al. 2006). Because monitoring occurs at the last stages of a biological control program, funds are often limited. Also, because the interactions between a target weed and the released agent are dynamic, surveys that require a long time can be outdated by the time of completion. Advances in spatial technology have made monitoring simpler and quicker, allowed better visualization and interpretation of large-scale data collections, and have the potential to be effective to use for communicating with the scientific community and the public.

Applying spatial technology to the biological control program against spotted knapweed can show the severity of infestations and the weed's spread into new areas. In addition, spatial technology can illustrate the spread of *L. minutus* from original release sites and quantify the impact of the insect against knapweed. In this study, data gathered was combined via visual surveys and sampling with spatial analytical methods to provide a better picture of knapweed distribution in northwest Arkansas. The spatial analysis can aid in understanding the impacts that *Larinus minutus* has had against spotted knapweed, both of which could better inform knapweed management decisions.

Methods

Surveying. A visual survey was conducted in 2017 in Benton, Carroll, Madison, and Washington Counties, in northwest Arkansas, during peak flowering of spotted knapweed (approximately June-July). Identification of knapweed is easier during the peak flowering period due to the distinct pink-purple color of the flowerheads. The 2017 survey covered 2741.5 km of 29 roads, including interstate, U.S. and state highways and associated scenic byways, frontage roads, and rights of way. Surveys consisted of identifying continuous patches of knapweed along the roadsides. Patches were considered continuous if there was a least a single bolted knapweed plant of any size within 50m of the nearest neighboring plant. Once a patch was located, the beginning and endpoint of the patch were recorded as waypoints, using a Garmin Montana 650t®. Additional waypoints were placed at spaces of ~10m within the patch with continuous knapweed if present in areas where accurate measure was restricted due to road conditions such as construction.

Sampling. Knapweed-infested patches was selected to sample intensively. Nineteen patches were selected for sampling in 2017. Twelve of those patches were sampled again in 2018, and four new plots were added in 2018. Selected plots were at least 30m in length parallel to the roadside. At each sampling site, the length (parallel to road) and width (perpendicular to road) of the patch were measured. Measurements for width were taken at the ends of the sampled area and up to two additional measurements at 30m and 70m depending on patch length. A linear transect was established through the approximate center of the patch and parallel to the road to collect up to 10 samples from a $1m^2$ area, beginning at the edge of the patch and occurring every 10m. Each sample consisted of recording number of crowns (base of plant where stems join the roots), number of bolts (flower-bearing stems), and canopy height. Canopy height was measured from the soil surface to the highest portion of the plant within the sampling area. In addition, at each 10m point, a 1m^2 effective canopy cover, measured as the proportion of 1m^2 area covered by spotted knapweed canopy, was assessed by visual estimate and checked by processing photos taken of each sample area using ImageJ (Rasband 2018).

A collection of 30 capitula was taken from the field sites and transported to the lab to determine the presence and impact of *L. minutus.* To determine presence and impact of biological control agents, 30 capitula (flowerheads) were collected from plants at each 10m point and transported them to the lab. Capitula were dissected, and the data recorded included the number of mature, immature and damaged seeds; presence of *Larinus minutus* emergence hole(s); presence of *L. minutus* larvae, pupae or adults within the capitulum; and presence of *Urophora quadrifasciata* larvae or puparia within the capitulum.

Mapping. All maps were created using Arcmap™ 10.4 software (ESRI© 2015). Map layers utilized included 'USA Counties' (ESRI, US Department of Commerce), TIGER/Line® Shapefile (US Census Bureau, Geography Division), and point data, or 'Waypoints', collected from the survey. All map layers were projected using the Arkansas State Plane North coordinate system. Maps illustrating the state of Arkansas were projected using the World Geographic System 1984.

Waypoints were processed by constructing a ring buffer using the 'Buffer' tool. The buffers were 30m in diameter with the waypoints as the center. Overlapping buffers were merged and designated continuity within a plot. Buffers were converted to linear data for visualization.

To determine the degree of clustering of spotted knapweed in the intensively surveyed counties, vector (point) data was converted to a raster data set. This was completed by constructing a ‘Fishnet’ with grid size 1x1 km (84 rows x 126 columns) that encompassed the surveyed counties. To combine the waypoints with the Fishnet grid, a ‘spatial join’ was used, which placed the points within the grid and converted them to raster data. A ‘Getis-Ord Hotspot Analysis’ was conducted on the raster data set to detect areas where spotted knapweed locations were numerous and located proximally.

Statistics. Univariate statistics were used to analyze differences between plots for all data measures collected. Averages per m^2 were calculated for crowns, bolts, canopy height, and effective canopy cover. Averages per capitulum were calculated for seed types and proportions of capitula per plot were calculated for *L. minutus* emergence holes, *L. minutus* larvae present, and *U. quadrifasciata* present. Exploratory statistics using a Multivariate analysis were conducted in JMP Pro® 13.2 software (SAS Institute 2016). Spearman’s Rank was used to determine the coefficient of correlation between bolts and effective canopy cover, canopy height and effective canopy cover, and emergence holes and damaged seeds.

Results

Spotted knapweed, *Larinus minutus*, and *Urophora quadrifasciata* were present throughout the four counties surveyed. Total distance surveyed was 2245.5 km, of which 295.72 km had spotted knapweed present (13.9%) (Fig. 4.2). In roads that had spotted knapweed present, the percent of the road length with spotted knapweed ranged from 0.3-50.0% (Table 4.1).

The Getis-Ord Hotspot Analysis showed significant clustering that occurred at multiple locations across the four counties surveyed (Fig. 4.3). Counties had clustering of spotted knapweed that was significant at α=0.01 (GI Z-score >2.67, $p<0.008$) and α=0.05 (GI Z-score=2.08, $p<0.037$).

Measures of plant growth (Table 4.2) and size in 2017 showed variation across the patches sampled. The average number of crowns was 1.5/ m^2(range 0.3-3.0). The number of bolts averaged 6.1/ m^2 (range 0.9-13.1) and the average canopy height was 68.6cm (range 30.2-141.6. The mean effective canopy cover was 0.3 (range 0.01-0.6).

Evidence of *L. minutus* (insect in capitula or emergence holes) was found at 18 of the 19 patches sampled in 2017. The proportions of capitula with *L. minutus* emergence holes within a patch averaged 0.11 (range 0.00-0.23). The mean proportion of capitula containing *L. minutus* larvae was 0.01 (range 0.00-0.07).

An average of 2.57 mature seeds (range 0.61-4.05) was found in all patches sampled in 2017. Damaged seeds (mean 2.00, range 0.55-4.71) were also found in all patches sampled.

In 2018, the patches sampled exhibited differences among patches in all the plant measures (Table 4.2). The mean numbers of crowns averaged 2.2 per m^2 (range 0.6-6.8), mean number of bolts was 8.4/m^2 (range 1.7-30.2), and the average canopy height was 68.9 cm (range 29.5-112.7 cm). The effective canopy cover was 0.30 (range 0.04-0.57).

In 2018, 15 of 16 plots sampled contained evidence of *L. minutus*. The proportion of capitula with *L. minutus* emergence holes was 0.16 (range 0.00-0.55). The proportion of capitula containing larval *L. minutus* averaged 0.01 (range 0.00-0.04)

Patches sampled in 2018 had mature and damaged seeds present in all patches (Table 4.7). Average mature seeds per capitulum were 1.86 (range 0.3-4.30) and damaged seeds averaged 2.00. Of the patches, 11 were sampled in 2017 and 2018. A single patch lacked *L.*

minutus in both years sampled. *Larinus minutus* was found in spotted knapweed patches outside of the original release sites (Minteer et al. 2011) (Fig. 4.4). Distance from original release sites varied between 0.01-40km with an average of 34km between sites with known *L. minutus* and original release sites.

In 2017, significant correlations were observed for bolts and effective canopy cover ($\rho=0.948$, $p<0.0001$), canopy height and effective canopy cover ($\rho=0.861$, $p<0.0001$), and emergence holes and damaged seeds ($\rho=0.780$, $p<0.0001$). Similarly, significant correlations were also observed in 2018 for bolts and effective canopy cover ($\rho=0.900$, $p<0.0001$), canopy height and effective canopy cover ($\rho=0.77$, $p<0.0001$), and emergence holes and damaged seeds ($\rho=0.8340$, $p<0.0001$). Regression equations (Fig. 4.4) for 2017 were significant and showed a moderate trend for bolts and effective canopy cover ($F_{(1,17)}=17.79$, $p<0.0006$) and canopy height and effective canopy cover ($F_{(1, 17)}=55.75$, $p<0.0001$). The regression for emergence holes and damaged seeds in 2017 was also significant ($F_{(1,17)}=7.28$, $p=0.015$) but the resulting correlation was weak ($R^2=0.30$). The regression equations for 2018 (Fig. 4.5) data were moderate to strongly correlated and significant for bolts and effective canopy cover ($F_{(1,13)}= 20.64$, $p<0.0006$), canopy height and effective canopy cover ($F_{(1, 13)}= 26.05$, $p<0.0002$), and emergence holes and damaged seeds ($F_{(1, 13)}= 74.67$, $p<0.0001$).

Discussion

Survey methods for plants vary depending on the type of information needed. While exploratory surveys have been conducted, to provide a ‘baseline’ of data for future research more intensive sampling methods were needed. Spotted knapweed was present along all highway types surveyed: interstate, state, and US highways. Significant clustering of discreet patches occurred across highway type and in all four counties surveyed. While the average length of roadside with spotted knapweed was 13%, when considering individual roads, the amount with knapweed present was much higher than average (e.g. I-540). These clusters are primarily outside of city limits, but have no apparent similarity based on variables measured to explain the patterns observed.

Sampling of plants at the local level often involves plant height, density, seed production, etc. Similar variables were measured in spotted knapweed patches to examine patterns in plant growth. These variables differed between patches both years but did not vary between years. These measures inform us on the predominance of spotted knapweed in the system where present. While there is variance across a landscape, there appears to be little change year-to-year in the knapweed patches sampled. This was a short-term study, so year-to-year variation could be greater than what was capture. The relationship of bolts to effective canopy cover and canopy height to effective canopy cover is not entirely unexpected. Effective canopy cover is measured so gaps in the foliage are a part of the surface area measured, making it less conservative than foliar cover but simpler to process. With increasing stem number and increasing height, it is logical that the surface area covered by spotted knapweed would also increase as spotted knapweed has some structural complexity.

Measures of seed load within capitula was highly variable between patches both for mature and damaged seeds. Although the relationship between damaged seeds and proportion of capitula with *L. minutus* was only weakly correlated in 2017, a moderate correlation was seen in 2018. The damage *L. minutus* does to seeds is part of why this weevil has had success in reducing spotted knapweed, but it is thought numbers must be high enough that significant damage is happening to the vegetation (Myers et al. 2009).

Overall, data collected should serve as a baseline for future research and monitoring of spotted knapweed. It is recommended that monitoring of sites free of spotted knapweed be incorporated into future monitoring to determine differences in site disturbance, soil type, plant community composition, etc. This information coupled with proper management strategies could lead a solution to removing knapweed from Arkansas roadsides.

References

Alford, A. M., T. Kring, and S. Raghu. 2016. Spread of *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of knapweeds, following introduction to northwestern Arkansas. Florida Entomologist. 99: 593-599

Duguma, D., T. J. Kring, and R. N. Wiedenmann. 2009. Seasonal dynamics of *Urophora quadrifasciata* on spotted knapweed in the Arkansas Ozarks. Canadian Entomologist. 141:70-79

Griffith, D. and J. R. Lacey. 1991. Economic evaluation of spotted knapweed [*Centaurea maculosa*] control using Picloram. Journal of Range Management. 44: 43-47

Harris, P. and R. Cranston. 1979. An economic evaluation of control methods for diffuse and spotted knapweed in Western Canada. Canadian Journal of Plant Science. 59: 375-382

Harris, P. and J. D. Shorthouse. 1996. Effectiveness of gall inducers in weed biological control. The Canadian Entomologist. 128: 1021-1055

Hauser, C. E., A. R. Pope, and H. P. Possingham. 2006. Should managed populations by monitored every year? Ecological Applications. 16: 807-819

JMP® Pro 14.0.0. 2018. SAS Institute Inc., Cary, NC

Lacey C. A., C. Egan, W. Pearson, P. K. Fay. 1988. Bounty programs an effective weed management tool. Weed Technology. 2:196-197

Mauer, T., M. Russo, and M. Evans. 2001. Element stewardship extract for *Centaurea maculosa*. The Nature Conservancy, Arlington, Virginia

Minteer, C. 2012. The biological control of spotted knapweed in the southeastern United States. PhD Dissertation. University of Arkansas

Minteer, C. R., T. J. Kring, J. Shen, and R. N. Wiedenmann. 2011. *Larinus minutus* (Coleoptera: Curculionidae), a biological control agent of spotted knapweed (*Centaurea stoebe* ssp. *Micranthos*) established in northern Arkansas. Florida Entomologist. 94: 350-351

Morse L. E., J. T. Kartesz, L. S. Kutner. 1995. Native vascular plants. Our Living Resources: A Report to the Nation on the Distribution, Abundance, and Health of US Plants, Animals, and Ecosystems. Washington (DC): US Department of the Interior, National Biological Service. Pages 205-209 in LaRoe ET, Farris GS, Puckett CE, Doran PD, Mac MJ, eds.

Myers, J. H., C. Jackson, H. Quinn, S. R. White, and J. S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control 50: 66-72

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52: 273-288

Rasband, W. S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018.

Roche, B. F., Jr., G. L. Piper, and C. J. Talbott. 1986. Knapweeds of Washington. Cooperative Extension Bulletin 1393, Washington State University, Pullman

Schirman. R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. Journal of Range Management 34: 45-47

Story, J. M. 2002. Spotted knapweed, pp. 169-180. In R. G. Van Driesche, B. Blossey, M. Hoddle, S. Lyon and R. Reardon (eds.), Biological control of invasive plants in the Eastern United States. USDA Forest Service, Morgantown, West Virginia

Story, J. M., R. M. Nowierski, K. W. Boggs. 1987. Distribution of *Urophora affinis* and *U. quadrifasciata*, two flies introduced for biological control of spotted knapweed (*Centaurea maculosa*) in Montana. Weed Science Society of America 35: 145-148

Story, J. M., N. W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control. 38: 227-232

USDA, NRCS. 2013. The PLANTS database.https://plants.usda.gov. National Plant Data Team, Greensboro, HC 27401-4901 USA

Wilson, L. M. and Randall, C. B., 2003. Biology and Biological Control of Knapweed. Forest Health Technology Enterprise Team, Technology Transfer

Table 4.1. Roads in northwest Arkansas surveyed, distance (km) of roads surveyed, distance in which spotted knapweed was present, and the percent of sampled road that that had spotted knapweed present. Road distances include scenic byways, business routes, and associated bypasses.

Road Name	Road Length (km)	Spotted Knapweed Present (km)	Percent of Road with Spotted Knapweed
Interstate Highway 540*	82	37	45
Fulbright Expy	30	3	9
US Highway 62	235	28	12
US Highway 71	217	35	16
US Highway 412	200	25	13
State Highway 12	94	2	2
State Highway 16	136	15	11
State Highway 21	76	4	6
State Highway 23	110	6	6
State Highway 45	60	15	25
State Highway 59	105	6	6
State Highway 68	35	6	18
State Highway 72	67	4	7
State Highway 74	95	21	22
State Highway 102	29	1	5
State Highway 103	55	9	17
State Highway 112	42	2	4
State Highway 127	58	1	2
State Highway 143	16	6	36
State Highway 156	19	1	10
State Highway 170	36	12	34
State Highway 187	28	4	14
State Highway 221	33	0	0
State Highway 264	35	6	17

Table 4.1 continued.

Road Name	Road Length (km)	Spotted Knapweed Present (km)	Percent of Road with Spotted Knapweed
State Highway 265	61	13	22
State Highway 279	20	1	6
State Highway 295	74	4	5
State Highway 303	36	7	21
State Highway 311	36	18	50

*Renumbered as Interstate 49 in 2014

Table 4.2. Indices measured for patches in 2017 and 2018. With the exception of *L. minutus*, values are listed as mean and standard error. *Larinus minutus* is listed as a proportion. Values within a column with different letters are significantly different ($p<0.05$)

	2017		2018	
	Mean±SE	Range	Mean±SE	Range
Crown	1.52±0.14	0-7	2.26±0.26	0-25
Bolt	6.41±0.80	0-50	8.64±1.17	0-110
Effective Canopy Cover	0.28±0.03	0-1	0.31±0.03	0-1
Canopy Height (cm)	66.75±4.81	0-190.5	69.26±4.55	0-170.18
Mature Seeds	2.43±0.22	0-14.5	1.86±0.16	0-8.89
Damaged Seeds	1.66±0.17	0-15	2.00±0.16	0-0.78
L. minutus	0.11±0.01	0-0/63	0.15±0.02	0-0.77

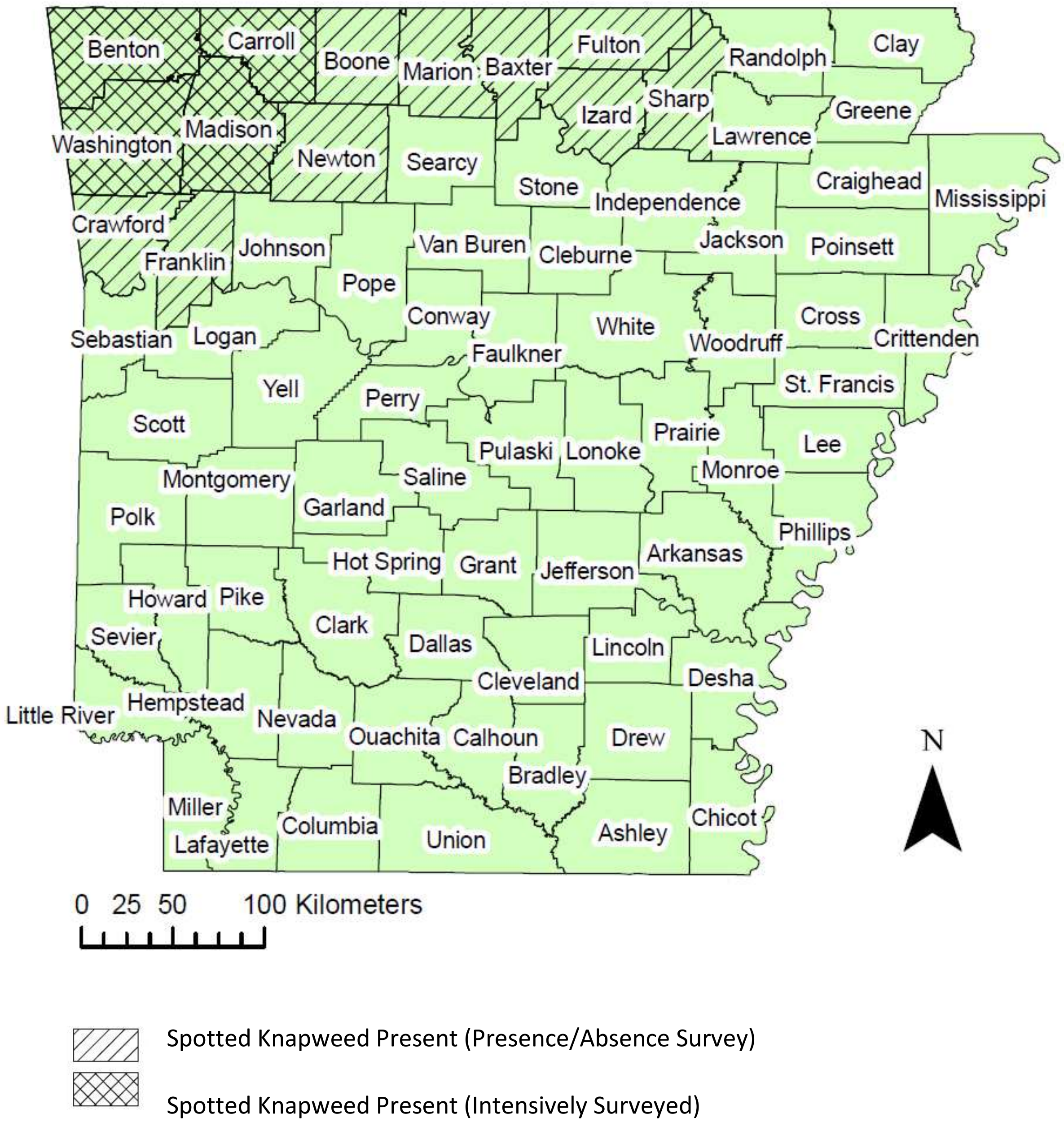

Fig 4.1. Map of the counties of Arkansas, showing counties surveyed and counties with infestations of spotted knapweed. Map projected using geographic coordinate system WGS 1984.

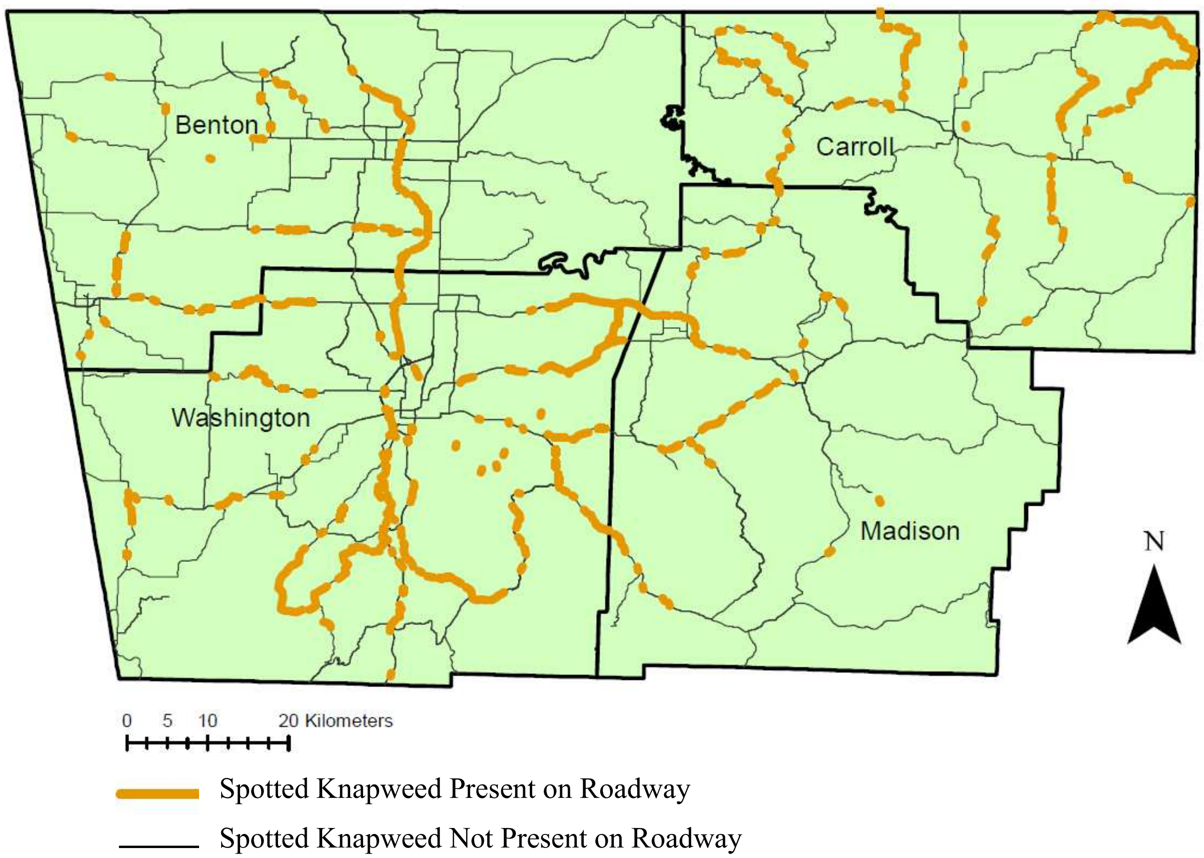

Figure 4.2. Map of Benton, Carroll, Washington and Madison Counties, in northwest Arkansas, showing roads sampled and locations of spotted knapweed infestations in 2017 and 2018. Map projection in Arkansas North State Plane.

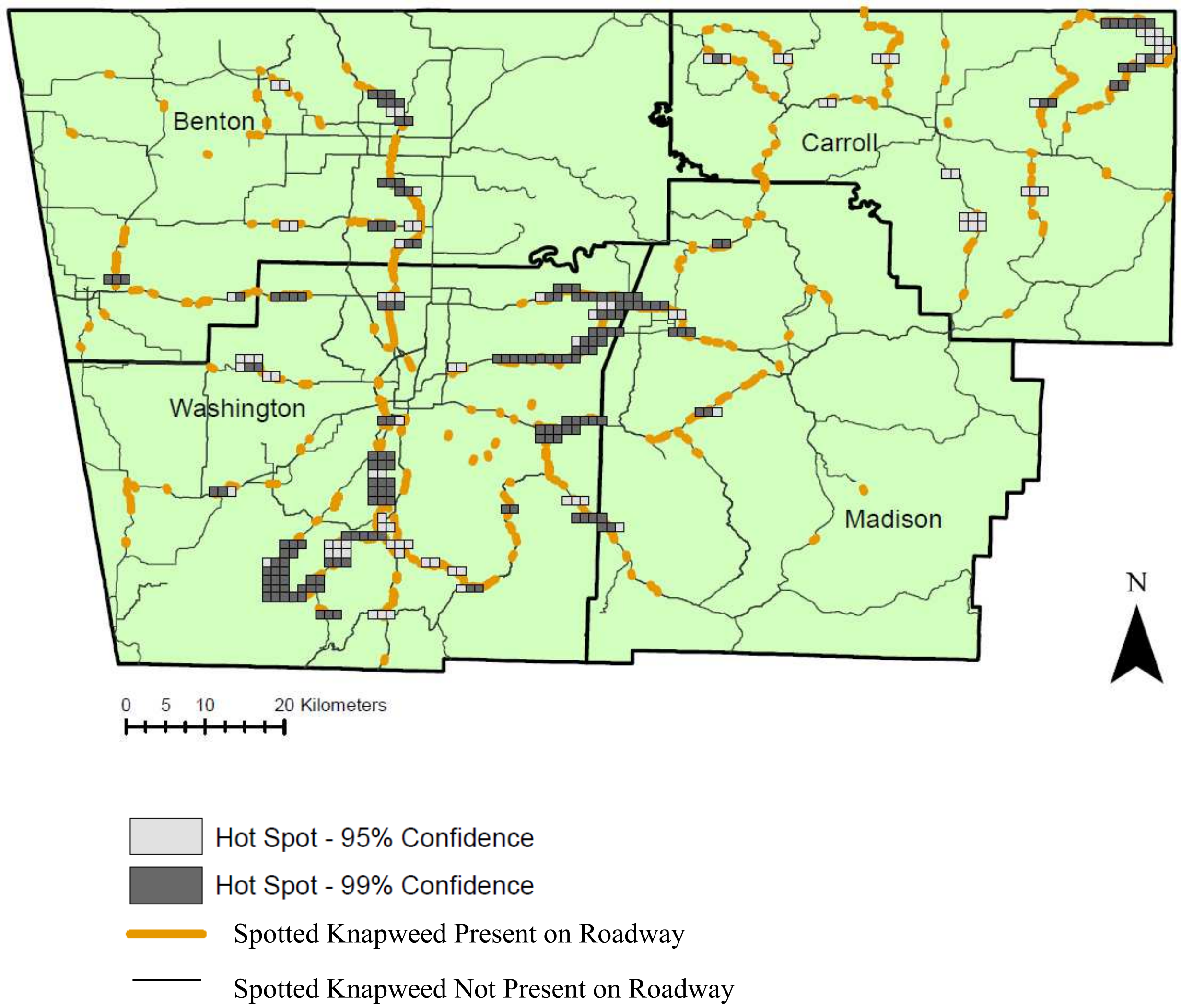

Figure 4.3. Getis-Ord Hot Spot Analysis displayed on map of surveyed roads in Benton, Carroll, Washington and Madison Counties, in northwest Arkansas. Boxes present indicate areas of high clustering of spotted knapweed and represent 1x1 km^2 area.

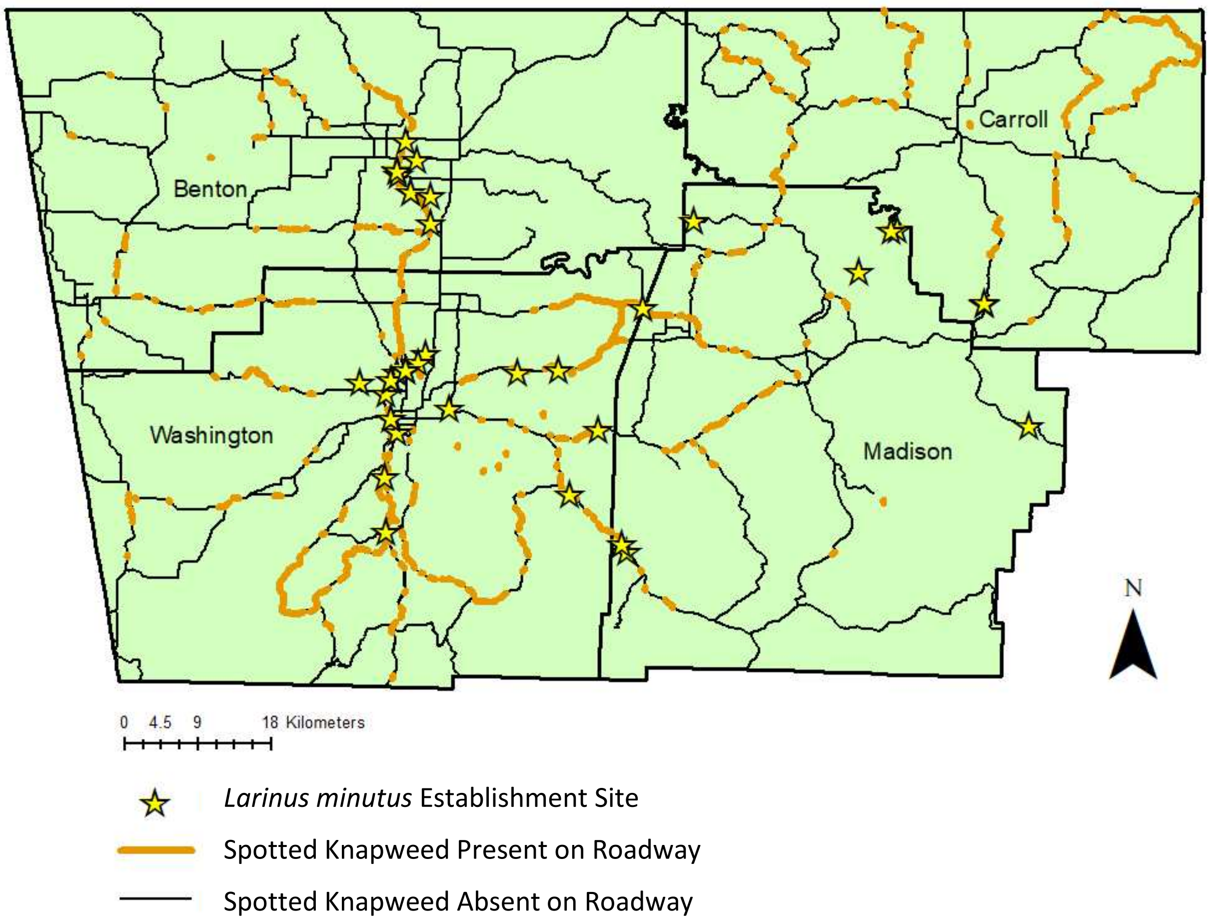

Figure 4.4. Distribution of original *Larinus minutus* release and establishment sites (Minteer et al. 2011).

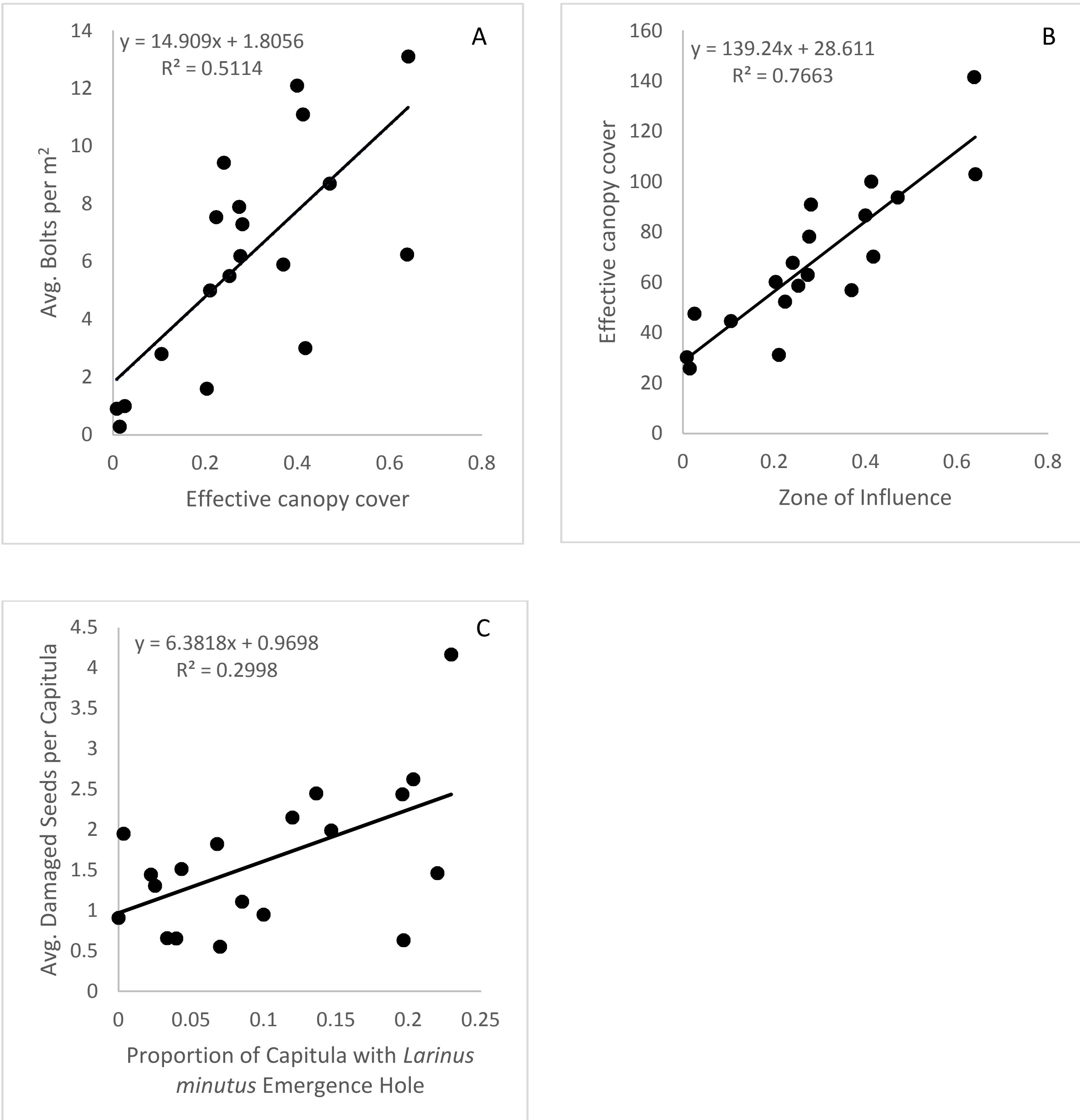

Figure 4.4. 2017 regression equations for bolt and effective canopy cover (A), canopy height and effective canopy cover (B), and damaged seeds and emergence hole (C).

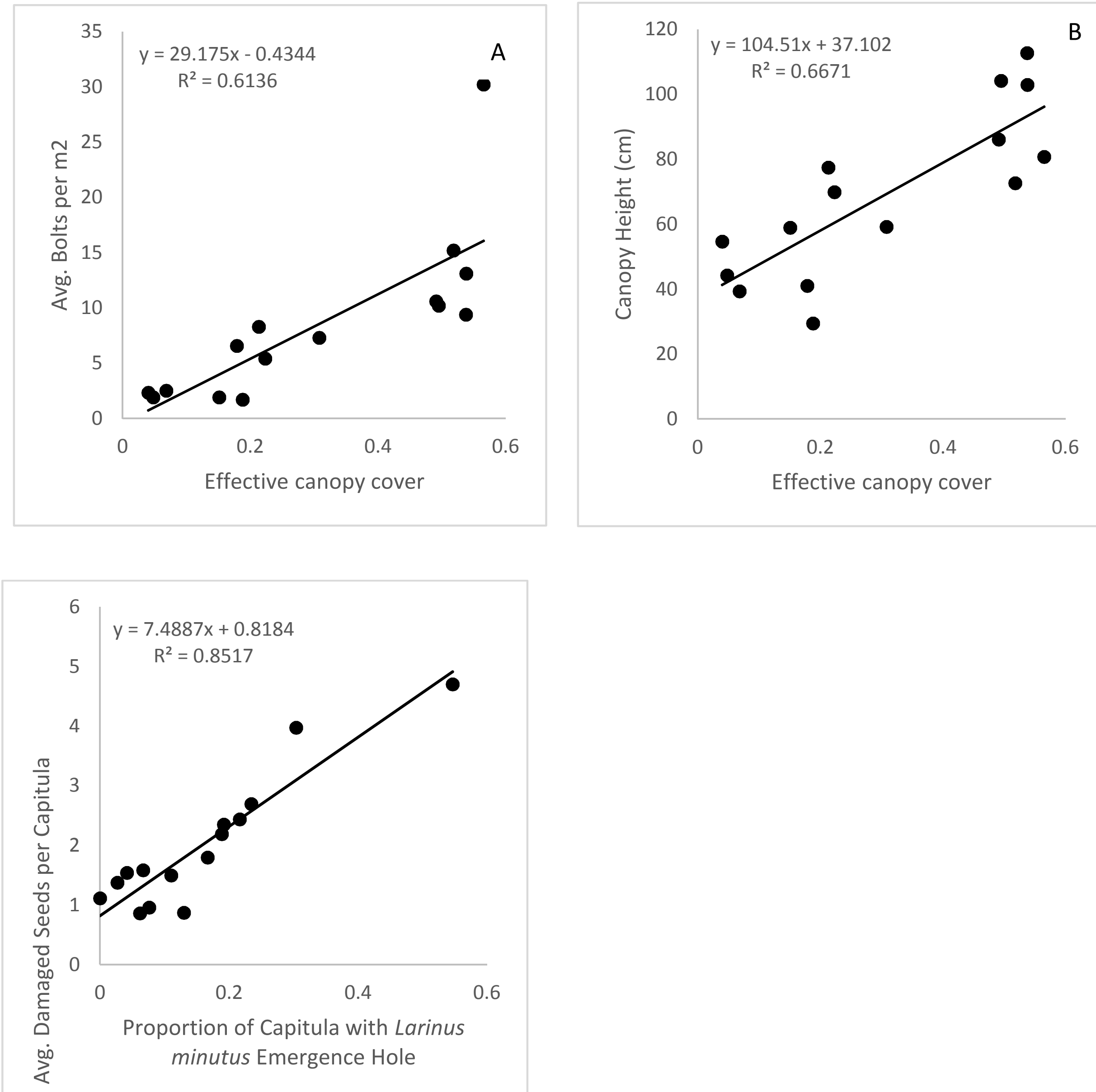

Figure 4.5. 2018 regression equations for bolt and effective canopy cover (A), canopy height and effective canopy cover (B), and damaged seeds and emergence hole (C).

Chapter 5

Summary

The interaction of spotted knapweed (*Centaurea stoebe* Gugler), *Larinus minutus* (Gyllenhal), and roadside management is complex. Improving our understanding of these interactions is crucial to improving management of spotted knapweed. This study focused primarily on how roadside mowing potentially reduces the effectiveness of *L. minutus* at different stages of reproduction and development. Secondarily, impacts on the plant due to mowing and if *L. minutus* was reducing seeds was examined. Finally, a large-scale survey to better understand where spotted knapweed can be found, and its severity was undertaken so any management recommendations could be applied in areas of highest concern.

While spotted knapweed has been the target of a biological control program in Arkansas, the impact of *L. minutus* on spotted knapweed has been less successful than expected. It is important to remember that weed control program success must often be measured in terms of years and decades. Nonetheless, some reduction in infestations have been observed, but little reduction of spotted knapweed has been observed at the landscape level.

The frequent mowing of spotted knapweed may have disrupted the biology of *L. minutus*, but mowing has not reduced seeds or occurrence of spotted knapweed to the point that knapweed patches begin to decline. Rather, there has been a spread of spotted knapweed as the seeds are moved on mowing equipment.

Chapters 2 and 3 were focused on aspects of the biology of *L. minutus* and how those may be altered by disturbance and removal of resources. This research revealed the importance of not only having access to floral resources, but also that the timing of those resources and duration was important to maximizing egg production. Removal of flowers at the beginning of the season likely delayed the maturation of reproduction organs and thus reduced egg deposition. This is important due to the dual role of spotted knapweed as a nutrient source and oviposition site for *L. minutus* as well as the site of larval development.

Even though it appears that the second mowing event has the most deleterious effect on *L. minutus,* the impact is due to the cumulative effects of the first and second mowing events. Spotted knapweed in these mowed areas are phenologically behind by 2-3 weeks as compared to spotted knapweed that is un-mowed. Mowed spotted knapweed may not be capable of supporting larval development through to adulthood because larvae have fewer seeds available. In contrast, those plants that were un-mowed had more seeds present providing more resources to larvae to complete development. It is assumed that further seed development is arrested after the capitulum is removed from the plant.

A reduction of oviposition sites and increased larval loss is observed in mowed areas. The result is far fewer weevils in the system to comprise the next generation occurring in spotted knapweed.

We need to consider the negative effects of leaving spotted knapweed undisturbed. Un-mowed knapweed plants were capable of producing more seeds, and specifically more mature seeds, that may contribute to the seedbank. The occurrence of *L. minutus* within patches should increase with more of a necessary resource available however, more plants and seeds will be present in earlier years, but there is a possibility of reduction long term.

Current roadside management strategies may need to be altered to reduce spotted knapweed densities and area covered and speed the impact of the biological control program. Cessation of mowing is unrealistic to consider and would have an economic impact to mowing contractors. The current number of mowing events could be retained by adjusting the impact on *L. minutus.* An adjustment would involve making the May mowing event earlier as spotted knapweed has just begun to produce bolts but has not begun to produce buds. Other plants along the roadsides are also putting on new vegetation and undergoing growth, so the purpose of this mowing event would still be maintained. Delaying the second mowing event, for approximately two weeks to the beginning of August until most spotted knapweed is in senescence with few developing flowers present could improve the likelihood that larvae present in the capitulum would survive to adulthood even if removed from the plant. However, this may be in conflict with the standards for aesthetics and safety.

Changes to management would need to be monitored for multiple years. Monitoring over several years would provide data that would not be reflecting seasonal variation. A monitoring program was initiated in 2017 to measure spotted knapweed infestations and *L. minutus.* While variability of spotted knapweed vegetation indices was high, the differences between the two seasons was low. Because this was only two years, I would be hesitant to make predictions about long-term patterns, but the similarity within patches demonstrates some stability of *L. minutus* and spotted knapweed. It is my hope that data collected from the four counties surveyed will allow targeting of infestations for aggressive management.

The measures of spotted knapweed growth varied considerably from those in northern and western states. There are differences in the length of the growing season between the southern and the northern states. In the south knapweed germinates earlier and bloom period is extended as compared to the north. Additionally, spotted knapweed in Arkansas has not died back to the crown with the basal rosette and bolts often remaining green throughout the winter. Knapweed in the northern states is often recorded as growing between 60-120cm, but it was not uncommon for average height within patches to be at the upper end of the range and individual measures of canopy height from 152-183cm. Taller plants likely produced more flowers and therefore more seeds. Instead, the rosette continued to grow and, in some case, produced a third set of bolts and flowers. These flowers lack mature seeds (likely due to absence of any pollinators present in November-January), but show the plant had sufficient resources to create reproductive parts.

It would be challenging for this biological control agent to suppress weed populations that are greater in size and capable of producing more seed even without disturbance. When we also consider the stress on *L. minutus* caused by mowing, it is surprising that we have seen reduction of spotted knapweed infestations at some patches. While it would appear that more spotted knapweed biomass, especially flowerheads, would lead to greater numbers of *L. minutus*, other interference from mowing would inhibit increases in *L. minutus* numbers.

Moving into the future of the spotted knapweed biological control program, it seems that there are two areas of special interest where current management can be approved. The first is facilitating the biological control agent and its top-down suppression of spotted knapweed. The management approach for *L. minutus* will likely differ from those used in northern states to increase the effectiveness of *L. minutus.* We must always consider that these environments are not operating in a vacuum and, if we do have success in reducing spotted knapweed, it is also our responsibility to insure its replacement is not other invasive plants that will continue to have negative impacts on these habitats. Rangelands differ fundamentally from roadsides and can be left undisturbed by grazing animals. The possibility of providing bottom-up pressure by native grasses is also more feasible. Along roadsides, the parties who would be responsible for replanting lack the incentive to do so. Roadsides may also be predisposed to invasion by non-native weeds as disturbance of the habitat occurs with regularity.

It is possible the program is still too new to see landscape-level changes in Arkansas. By continued monitoring of *L. minutus* and spotted knapweed, a more complete picture can be constructed of any changes occurring. It is well within the realm of possibility that spotted knapweed can be reduced in Arkansas.

Appendix

Values for spotted knapweed patches sampled in 2017 and 2018. GPS coordinates listed in decimal degrees as centroid of the patch area.

Number Assigned	Year(s) Sampled	Centroid Coordinate	Width (m)	Length (m)	Area (m^2)
14	2018	-94.311011, 35.831663	4	80	341
18	2017	-94.292391, 36.178067	4	182	666
19	2017	-94.005835, 36.103045	23	485	11087
20	2017, 2018	-94.050345, 36.100442	3	796	2426
21	2018	-94.496644, 36.190796	11	372	4082
33	2018	-94.198690, 35.984383	1	829	758
46	2017, 2018	-93.958467, 35.926888	10	277	2786
47	2017	-93.975776, 35.945032	2	240	585
48	2017, 2018	-94.006154, 36.009933	2	390	594
50	2017	-93.974745, 36.031062	4	548	2004
51	2017	-93.955900, 36.034489	4	161	638
52	2017, 2018	-93.945621, 36.122205	3	917	3074
53	2017	-93.933628, 36.132484	4	399	1703
66	2017, 2018	-93.938860, 36.154463	9	333	2943
67	2017	-93.955900, 36.169033	7	283	2070
69	2017, 2018	-93.981598, 36.026494	9	670	6126
70	2017, 2018	-94.004441, 36.021354	6	268	1552
71	2017	-94.003870, 36.021925	7	116	778
72	2017, 2018	-94.287242, 35.887057	4	78	333
73	2017, 2018	-93.916496, 36.173601	2	257	627
74	2017, 2018	-94.293316, 35.833517	6	277	1689
75	2017, 2018	-94.305003, 35.834423	5	172	839
217	2018	-93.848250, 36.130098	5	134	653