Boulder County Diffuse Knapweed Mapping and Biocontrol Agent Report

Research From 2008 and 2009

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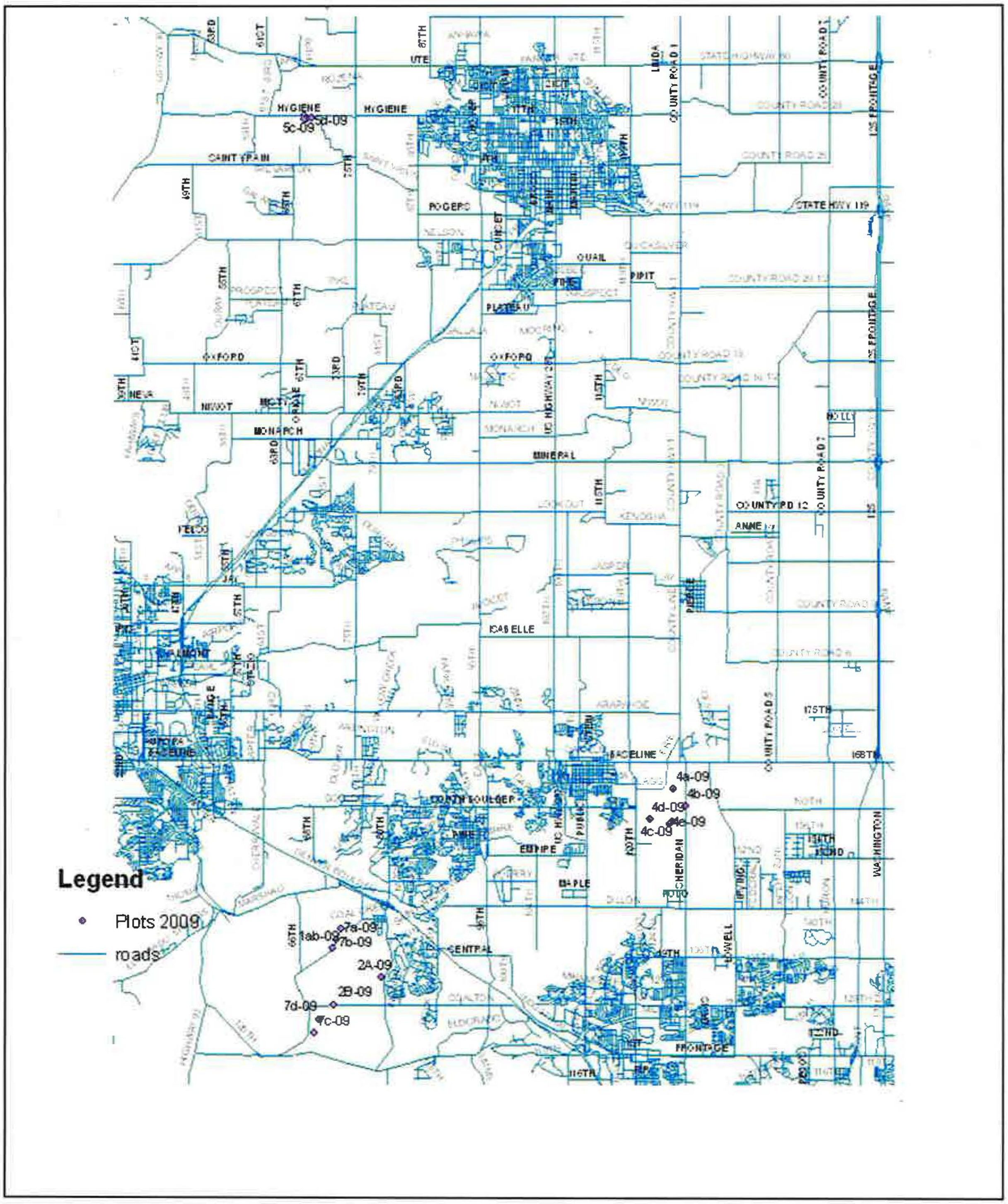
Executive Summary.

Diffuse Knapweed Mapping. A comparison of diffuse knapweed patch size changes between 2008 and 2009 showed that in this 1 year span, there was a huge increase in the amount of diffuse knapweed mapped with precise GPS units. The overall increase for 3 selected areas that were mapped in 2009 showed an increase of 91 acres. It is likely that this major rebound in diffuse knapweed at these sites was due to the excellent moisture and weather patterns that occurred in 2009. These data demonstrate the resilience of this invasive weed and the critical importance of the timely use of management tools, including herbicides, to keep the infestations at manageable levels. In general, diffuse knapweed was well controlled on Boulder Open Space land. Generally, infestations appeared greater and more problematic on private land.

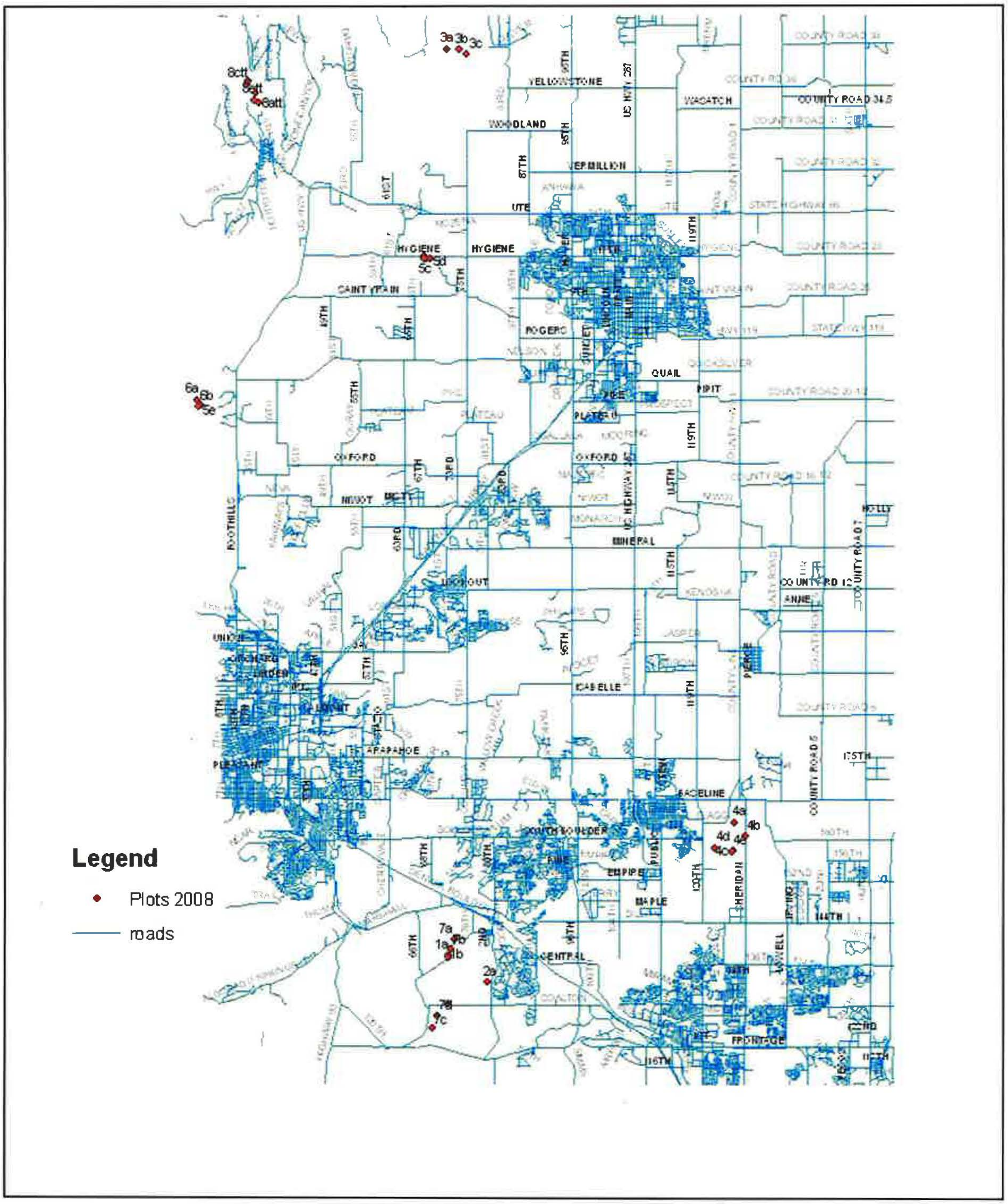
Biocontrol Agent Monitoring. At a static point in time in 2008, our data suggest that presence of biocontrol agents for diffuse knapweed might be related to proximity to streams, although not related in the same way for all evaluated biocontrol insects. Our preliminary data inferred that the trend of the probability of finding *Cyphocleonus* decreased from upland to riparian areas; whereas, the chance of occurrence for the other two species of insects might have a weaker relationship with proximity to streams. Furthermore, overall greater proportions of *Larinus* presence were observed as compared to *Sphenoptera* and *Cyphocleonus* among the patches sampled irrespective of patch location (i.e., upland or riparian).

Some interesting trends to follow in subsequent samples are the proportions and abundance of each insect species in relation to diffuse knapweed cover and phenology. During the time of our survey, we found decreasing proportions of *Sphenoptera* with increasing percent cover and later life stages (flowering vs. budding plants) of diffuse knapweed patches (Figs. 9 and 10). Sampling over the course of the growing season could offer information indicating when diffuse knapweed populations might be more vulnerable to insect herbivory. Moreover, a further investigation of the role of disturbance as it might affect biocontrol agent abundance could be of critical importance when devising a prioritization scheme for diffuse knapweed control. Less human intervention might be required in locations where biocontrol insects are abundant.

Boulder County Knapweed Population Changes 2008 to 2009				
Location	ID	2008 M^2	2009 M^2	+ / - Square Meters Changed
Superior	1a	50	1a+1b*	1a+1b*
Superior	1b	78.5	31400	+ 31272
Superior	2a	700	282600	+ 281900
Superior	2b	153.86	4500	+ 4346
Superior	7a	105	5000	+ 4895
Superior	7b	1725	4200	+ 2475
Superior	7c	706.5	1625	+ 919
Superior	7d	350	40250	+ 39900
Superior Total Change 2008 to 2009				**365,707**
Lafayette	4a	314	4050	+ 3736
Lafayette	4b	153.86	150	- 4
Lafayette	4c	150	314	+ 164
Lafayette	4d	314	400	+ 86
Lafayette	4e	1256	153.86	- 1102
Lafayette Total Change 2008 to 2009				**2,880**
Crane Hollow	5a	105	200	+ 95
Crane Hollow	5b	314	153.86	- 160.1
Crane Hollow	5c	314	314	0
Crane Hollow	5d	153.86	105	- 49
Crane Hollow Total Change 2008 to 2009				**-114.1**
*Patches grew together - assessed as solid patch				
91 Acre increase in infestation from 2008 to 2009				



Research sites in 2008.



Research sites in 2009.

Boulder County 2008 Diffuse Knapweed Mapping and Biocontrol Research Project

Introduction

Diffuse knapweed (*Centaurea diffusa*) is a biennial or short-lived perennial forb originating from southeastern Europe and Asia Minor (Sheley et al., 1998). Diffuse knapweed reproduces exclusively by seed and is a semelparous species that dies after its first reproduction; furthermore, this weed is able to regulate the time it spends in the rosette stage of its life cycle based on environmental stresses and plant density (Powell, 1990) (Fig. 1). Diffuse knapweed and spotted knapweed (*Centaurea maculosa, Centaurea stoebe*) were thought to have been introduced to and spread throughout North America as a contaminant of alfalfa seed (reviewed in Sheley et al., 1998). Both knapweed species are recognized as noxious weeds in several states of the western United States (USDA Plants Database, plants.usda.gov). Diffuse knapweed is currently a List B noxious weed species on the Colorado Noxious Weed List (Colorado Dept. of Agriculture, www.colorado.gov/ag). Because of its legal designation of as a noxious weed, public land managers are required by Colorado State law to have management plans targeted at stopping the spread of diffuse knapweed populations (Colorado Dept. of Agriculture, www.colorado.gov/ag).

A crucial component of any weed management plan should include a method for assessing the success of the management tools employed (Carson et al., 2008). Measuring success or failure of a weed management plan is, first, contingent upon defining overall land management objectives and tailoring the weed management goals to meet those objectives (Rew et al., 2007). Weed population surveys should be administered regularly to monitor the progress of the management plan and to record how weed populations are impacted across different habitat types under different management treatments (e.g., biocontrol, chemical or mechanical control, or some combination of tools) (Muller-Scharer and Schroeder, 1993; Sheley et al., 1998; Carson et al., 2008).

As science and technology have advanced, weed managers have more choices in combinations of management tools for invasive weeds. Management methods for diffuse knapweed include cultural, mechanical, chemical, and biological control (Sheley et al., 1998, Muller-Scharer and Schroeder, 1993). Combinations of these techniques have been recommended as critical components of an integrated and ecologically-based management program aimed at containing, suppressing, or eradicating weed populations while promoting a plant community that meets land management objectives (Sheley et al., 1998; Muller-Scharer and Schroeder, 1993; Carson et al., 2008).

The use of classical biocontrol methods as part of an integrated approach to weed management has become increasing popular as it often allows for control of weed populations in environmentally sensitive or inaccessible areas where other methods are infeasible (McFadyen, 1998; Muller-Scharer and Schroeder, 1993). The goals of most weed biological control programs are not to eradicate the target weed species (as reviewed in DiTomaso, 2000 and McFadyen, 1998) Because of the challenges associated with establishing a biocontrol program, this management method is typically only considered for weed species whose abundance is so great that widespread eradication is not reasonable under any management program (McFayden, 1998). Most biocontrol programs are aimed at containing weed populations and/or reducing population fitness such that existing vegetation will re-establish or other management methods can be employed with greater success of control (Muller-Scharer and Schroeder, 1993; Corn et al., 2006).

The biological control program in North America for both diffuse and spotted knapweed was first launched in Canada in 1970 (Muller-Scharer and Schroeder, 1993). To date there have been 13 insect species released to control diffuse and spotted knapweed (Corn et al., 2006). In Boulder County, Colorado, there has been the release of four insect biocontrol agents: 1) the lesser knapweed flower weevil (*Larinus minutes*), 2) bronze knapweed root borer (*Sphenoptera jugoslavica*), 3) knapweed root weevil (*Cyphocleonus achates*), and 4) the spotted knapweed seedhead moth (*Metzneria paucipunctella*) (Seastedt et al., 2003). Three of these species were recorded at both release and non-release sites in Colorado during a 2006 survey (Hardin and Norton, 2006).

Boulder County is positioned in the north central part of the state and can be characterized as possessing diverse terrain giving way to environmental and ecological diversity. Consequently, characterizing the effectiveness of any weed management strategy has its challenges. Collecting baseline data pertaining to the presence and abundance of diffuse knapweed and its associated biocontrol agents can be a useful start to a longer term monitoring effort.

The objectives of this 2008 survey were to:

i) geo-reference known diffuse knapweed patches,
ii) quantify the presence of three biocontrol agents, lesser knapweed flower weevil (*Larinus minutes*), bronze knapweed root borer (*Sphenoptera jugoslavica*), and knapweed root weevil (*Cyphocleonus achates*) at known diffuse knapweed sites,
iii) identify statistical and environmental trends explaining the presence of those biocontrol agents,
iv) perform a county-wide survey of diffuse knapweed presence on Boulder County Open Space properties, and
v) produce a habitat suitability model for diffuse knapweed as a tool for future monitoring and management efforts.

Methods

Evaluation of Known Diffuse Knapweed Patches

Areas within Boulder County containing known populations of diffuse knapweed were identified by the county's Weed Coordinator, Steve Sauer (Fig. 2).

Between July 25 and August 5, 2008, Colorado State University Weed Research Laboratory mapping crews visited known weed sites and geo-referenced the centers of "natural" diffuse knapweed patches using Trimble Nomad GPS receivers. "Natural" patches were subjective in that they were defined by the mapping crews' interpretation of what constituted a patch by evaluating the connectivity and the environment(s) of the patch. A total of 34 diffuse knapweed patches within the eastern part of Boulder County were geo-referenced and evaluated for diffuse knapweed biocontrol agents (Fig. 2).

Attributes for each diffuse knapweed patch were recorded using the personal digital assistant (PDA) functionality available with the Trimble Nomad GPS receivers. Patch attributes included patch area, percent diffuse knapweed cover, dominant phenology of the diffuse knapweed plants within the patch, general vegetation characteristics, putative disturbance, hydrology of the site (i.e., upland or riparian area), and presence or absence of each biocontrol agent (Table 1). Percent diffuse knapweed cover was evaluated by a member of the mapping crew standing in the center of the patch and estimating the percent area of the patch composed of diffuse knapweed. All attributes for each patch were recorded as a choice from a dropdown menu list (Table 1).

Within each of the diffuse knapweed patches, 50 plants were evaluated for the presence and count of the lesser knapweed flower weevil (*Larinus*), bronze knapweed root borer (*Sphenoptera*), and knapweed root weevil (*Cyphocleonus*). The root boring insects, *Sphenoptera and Cyphocleonus*, were evaluated by uprooting plants and counting insects in the roots. Insect counts were recorded as categories in groups of ten (Table 2). For example, if a plant had 9 weevils present, then that plant would be placed into a count group of 1-10 for that species of biocontrol agent.

Elevation, riparian, and upland attributes of diffuse knapweed patches were identified using GIS layers developed from a USGS 10-meter resolution digital elevation model (DEM) (seamless.usgs.gov).

County-Wide Public Lands Diffuse Knapweed Survey

The CSU Weed Research Laboratory received a research permit to survey and sample diffuse knapweed populations on Boulder County properties that were deemed accessible by County officials (Fig. 3). The open space properties covered by the research permit were divided into three different elevation zones. Within each elevation zone, open space properties were divided into either riparian or upland locations. All stratification of the open space properties were performed using ESRI Spatial Analyst tools in ArcGIS 9.2. Sample locations were randomly located within each strata defined by elevation and hydrological designation (Fig. 4). CSU Weed Lab mapping crews composed of at least two individuals navigated to the randomly located sample sites during June and July of 2008. At each site, a circular plot (Fig. 5) was assessed for the presence of diffuse knapweed and, if diffuse knapweed was present, the presence and abundance of biocontrol insects as detailed previously in this Methods section.

Data Analysis:

Data were analyzed using a second-order generalized linear model with a logit link function (i.e., logistic regression; Eq. 1) where the response variable was presence of any biocontrol agent or the absence of all agents. Explanatory variables used included the biocontrol agent, location (i.e., either riparian or upland), and the two-way interaction between these terms. All statistical analyses were performed using R statistical software (www.r-project.org).

$$\text{est}\{\pi_i\} = [\exp(\beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{i,p-1})] / [1 + \exp(\beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{i,p-1})] \qquad \textbf{Eq. 1}$$

Logit link function

$$\ln(\pi_i / 1 - \pi_i) = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{i,p-1}$$

Where...

$\text{est}\{\pi_i\}$ is the estimated probability of success or occurrence

The relationships between proportions of a specific biocontrol agent and different patch attributes were graphically depicted. The county-wide survey data of diffuse knapweed abundance was evaluated qualitatively as no diffuse knapweed was found within surveyed sites.

Results and Discussion

<u>Characterization of Known Diffuse Knapweed Patches</u>

The probability of a presence of any biocontrol agent was dependent on the specific insect agent and proximity to riparian areas as defined by quantitative GIS methods (Table 3). Elevation was not included in the modeling process because there was not sufficient variation in elevation among the subjectively selected sampling sites (Fig. 1). Likewise, insect density or count data were not included in the model because the vast majority of these counts fell into either a count category of 0 or 1 (Table1; Appendix A). Overall, *Larinus* species insects were most commonly observed in greater proportions at most evaluated diffuse knapweed patches; whereas, *Cyphocleonus* species insects were observed at lower proportions as compared to the other biocontrol agents (Appendix A). These results were in agreement with observations recorded by Hardin and Norton in their 2006 survey of biocontrol programs in Colorado. They found greater proportions of *Larinus* and lower proportions of *Cyphocleonus* in release and non-release sites relative to other diffuse and spotted knapweed biocontrol insects surveyed. The authors of that survey suggested that the dispersal capabilities of the root-boring agent limited its abundance at non-release sites.

The coefficient estimates resulting from a multiple logistic regression model (Eq. 1) summarized in Table 2 can be used to construct the mean probability of occurrence estimates for each species within each environmental location (Table 4). Overlapping 95% confidence intervals for mean probability estimates indicates that there is insufficient evidence of a statistical difference between those probabilities. For instance, there was only a 6% probability of *Cyphocleonus* presence in riparian areas; whereas, there was a 42% probability of its presence in upland areas (Table 4). Although these probabilities appear to be very different, they are not statistically different because their respective confidence intervals include some of the same probabilities. Conversely, the probability of a presence of *Cyphocleonus* in a riparian area compared to the presence of *Larinus* or *Sphenoptera* could be interpreted as statistically different (Table 4; Fig. 6); however, with a low sample size for *Cyphocleonus* (n=6), these results should be interpreted with extreme caution. Ninety-five percent confidence intervals for this study were interpreted as the following: if this study were repeated 100 times, mean probability estimates would fall within that confidence interval's range of values 95 times.

It is important to bring to one's attention the range of the confidence intervals in Table 3. All confidence intervals have large ranges meaning that although diffuse knapweed patch location (i.e., either riparian or upland areas) was significant in explaining the presence of biocontrol agents, there was considerable variability in these presence data. This variability, given that these data represent only one year and timing of observation, has led to inconclusive results. It would be interesting to see if these trends continue to exist from year to year, if other variables contribute to explaining biocontrol insect presence, and how timing of insect sampling affects observed trends. Because of variation in the life cycles of these agents, time of sampling for presence and density would likely influence our results.

Those estimated probabilities of occurrence with corresponding 95% confidence intervals described in Table 3 are more clearly illustrated in Fig. 7. In Fig. 7, each graph displays the relationship between location (i.e., upland=0 or riparian=1) and a particular species' probability of occurrence. For instance, there was not much difference in the probability of *Larinus* or *Sphenoptera* being present in a riparian area compared to an upland location. However, there was a decrease in the probability of *Cyphocleonus* in riparian areas as compared to upland areas, although this difference was not statistically significant (Fig. 7).

Attributes of diffuse knapweed patches (Fig. 2) were collected (see Table 1) in the field, and proportions of biocontrol insects were plotted versus each diffuse knapweed patch attribute to qualitatively evaluate trends. As diffuse knapweed patch area increased, there was a slight increase in the proportion of each insect observed; however, this trend was likely not significant for any agent due to the variability in the proportion data for each agent, a low sample size for *Cyphocleonus* (n=6), and a possible outlier patch size (Fig. 8). From this limited survey, we detected a trend of decreasing variability in the proportions of *Larinus* present as patches became larger (Fig. 8). Likewise, the variability in proportions of each biocontrol agent across diffuse knapweed percent cover prevented detecting differences in insect abundance (Fig. 9). Sphenoptera did exhibit more abundance (i.e., greater proportion of presence on sampled plants) within patches of low diffuse knapweed cover (Fig. 9).

During the time of sampling (July 25 – Aug. 5, 2008) the sampled diffuse knapweed patches exhibited either budding or flowering plants (Fig. 10). Larger proportions of *Larinus* were present in patches that were flowering versus those patches whose dominant phenology was budding (Fig. 10). The opposite trend was observed with *Sphenoptera* where greater proportions of those insects were observed on diffuse knapweed patches where the plants were flowering (Fig. 10). These observations must be qualified with the fact that this survey was conducted when diffuse knapweed patches were in the more mature stages of its life cycle. We do not know how insect abundance relates to weed patches in the earlier part of the growing season.

Both *Larinus* and *Sphenoptera* species abundance differed with the dominant vegetation at the locations of diffuse knapweed patches where lower proportions of insects were found in shrubland areas as compared to grassland areas (Fig. 11). Likewise, there were some interesting trends observed when the proportions of each insect species were plotted against the putative disturbances at the diffuse knapweed sites (Figs. 12-14). Greater proportions of both *Larinus* and *Sphenoptera* were observed in areas deemed as not disturbed. To qualify these observations, disturbance was visually assessed by the mapping crews. It was very likely, given the proximity of many of these patches to roads and urban areas, that all sites had some degree of disturbance.

<u>Characterization of County-Wide Diffuse Knapweed Survey</u>

Accessible Boulder County public lands under the management of the Boulder County Parks and Open Space department were surveyed in a stratified random sample for diffuse knapweed (Fig. 4); however, of the 30 plots surveyed, no diffuse knapweed was found.

Conclusions

At a static point in time, our data suggest that presence of biocontrol agents for diffuse knapweed might be related to proximity to streams, although not related in the same way for all evaluated biocontrol insects. Our preliminary data inferred that the trend of the probability of finding *Cyphocleonus* decreased from upland to riparian areas; whereas, the chance of occurrence for the other two species of insects might have a weaker relationship with proximity to streams. Furthermore, overall greater proportions of *Larinus* presence were observed as compared to *Sphenoptera* and *Cyphocleonus* among the patches sampled irrespective of patch location (i.e., upland or riparian).

Some interesting trends to follow in subsequent samples are the proportions and abundance of each insect species in relation to diffuse knapweed cover and phenology. During the time of our survey, we found decreasing proportions of *Sphenoptera* with increasing percent cover and later life stages (flowering vs. budding plants) of diffuse knapweed patches (Figs. 9 and 10). Sampling over the course of the growing season could offer information indicating when diffuse knapweed populations might be more vulnerable to insect herbivory. Moreover, a further investigation of the role of disturbance as it might affect biocontrol agent abundance could be of critical importance when devising a prioritization scheme for diffuse knapweed control. Less human intervention might be required in locations where biocontrol insects are abundant.

This study performed two *snapshot in time* surveys of the biocontrol agent presence at known diffuse knapweed sites and the abundance of diffuse knapweed across Boulder County open space properties. This study, however, could not be used to determine the effect of biocontrol insects on the abundance of diffuse knapweed or to assess the effectiveness of the current suite of diffuse knapweed management methods employed by Boulder County Parks and Open Space. A recent study administered over a 5-year time period in Boulder County suggested that the three biocontrol agents evaluated in our study did decrease the abundance of diffuse knapweed populations on their non-grazed study site (Seastedt et al., 2003). What the Seastedt et al. (2003) study did not employ was a controlled experimental design with complete replication across space nor could it explain the effects of other unmeasured variables (e.g., effect of grazing) on diffuse knapweed populations. Therefore, cause and effect relationships between presence and abundance of biocontrol insects and changes in the population dynamics of diffuse knapweed were ambiguous. The only way to infer any cause-effect relationships would be to administer an experiment with manipulated treatments. Seastedt et al. (2003) did, however, provide 5 years of consistent monitoring and compared diffuse knapweed abundance and seed production every year to base line data collected prior to the release of additional biocontrol insects. In short, their study illustrated the potential of a combination of biocontrol insects to reduce diffuse knapweed populations. Furthermore, other studies conducted in Montana reported declines in spotted knapweed abundance after the introduction of *Cyphocleonus* (Corn et al., 2006; Story et al., 2006).

Future Management and Research Recommendations

Weed management is just a piece of the land management puzzle. Clearly defining weed management goals should be a product of how weeds affect the overall system being targeted for management. Naturally, different levels of tolerance for the presence or abundance of weed populations will result from different land management goals. For instance, a farmer might have less tolerance for weed populations in his wheat field; whereas, a rancher might have a greater tolerance for weed populations in her pasture. In one case, weeds might be competing with crop yields; in the other case, weeds could be consumed as forage for livestock. Likewise, not all weed populations are equal. In other words, some populations are more aggressive than others exhibiting varying levels of invasion potential or threat to an ecosystem of interest (Sheley et al., 1998, Rew et al., 2007). Land use, management practices, and environmental/habitat conditions have been shown to affect the viability of weed populations and the efficacy of the biocontrol agents that prey on them (Seastedt et al., 2003; Beck and Rittenhouse, 2001). Furthermore, varying compensatory behavior of the target weeds could affect the performance of biological control management (Garren and Strauss, 2009).

The first step in instituting a diffuse knapweed management program is clearly defining land use/management goals. Second, it is important to critically ask how weed management fits into the overall land use objectives and how those objectives might enhance or suppress existing or future weed populations. For instance, grazing management could either suppress diffuse knapweed populations or open up niches for continued weed colonization (Sheley et al., 1998). Third, clearly define how weed management objectives will be measured. A monitoring program designed to answer questions within and among multiple growing seasons is vital to assessing the success of weed management goals (Muller-Scharer and Schroeder, 1993; Sheley et al., 1998; Rew et al., 2007; Carson et al., 2008). These key decisions are necessary to evaluate weed management efficacy. It is difficult to measure whether a management practice or program is effective when effectiveness is not defined. Finally, because plants are living creatures their response to management is not always predictable or consistent. Therefore, realistic goals should be identified that allow for variability in different weed populations' responses to management processes.

Although this survey provided some limited insight into the abundance of both biocontrol agents and diffuse knapweed, it cannot infer the true status of the diffuse knapweed in Boulder County. Sound management decisions should be derived from sound observational (i.e., continued monitoring) and experimental research. The effectiveness of biocontrol as a tool in Boulder County's integrated pest management toolbox should continue to be evaluated using a program that focuses on spatial and temporal monitoring and a suite of manipulated experiments.

Weed management, or just as importantly, overall ecosystem management should be a dynamic and integrated process that utilizes multiple management tools (e.g., pesticides, grazing, mowing, biocontrol) and adapts to changing environmental conditions (both spatial and temporal), disturbance events (e.g., wildfire, flooding, etc), evolution of plants and insects, and land management goals. There is rarely one solution or a *one-size-fits-all* answer when it comes to controlling or eradicating an invasive plant species.

References

Beck, K.G. and L.R. Rittenhouse. 2001. The influence of cattle grazing on diffuse knapweed. Boulder, CO: Report to City of Boulder Open Space.

Carson, W.P., S.M. Hovick, A.J. Baumert, D.E. Bunker, and T.H. Pendergast IV. 2008. Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. Arthropod-Plant Interactions. 2:77-86.

Corn, J.G., J.M. Story, and L.J. White. 2006. Impacts of the biological control agent *Cyphocleonus achates* on spotted knapweed, *Centaurea masculosa*, in experimental plots. Biological Control. 37:75-81.

Hardin, J.G. and A.P. Norton. 2007. Evaluation of Colorado weed biological control programs—survey and monitoring, 2006. Report to Environmental Protection Agency, Region 8. Denver, CO.

Garren, J.M. and S.Y. Strauss. 2009. Population-level compensation by an invasive thistle thwarts biological control from seed predators. Ecological Applications. 19:709-721.

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

Muller-Scharer, H. and D. Schroeder. 1993. The biological control of *Centaurea* spp in North America—Do insects solve the problem. Pesticide Science. 37:343-353.

Powell, R.D. 1990. The role of spatial pattern in the population biology of *Centaurea diffusa*. Journal of Ecology. 78:374-388.

Rew, L.J., E.A. Lehnhoff, and B.D. Maxwell. 2007. Non-indigenous species management using a population prioritization framework. Canadian Journal of Plant Science. 87:1029-1036.

Seastedt, T.R., N. Gregory, and D. Buckner. 2003. Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland. Weed Science. 51:237-245.

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 Science. 12:353-362.

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 within large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biological Control. 38:227-232.

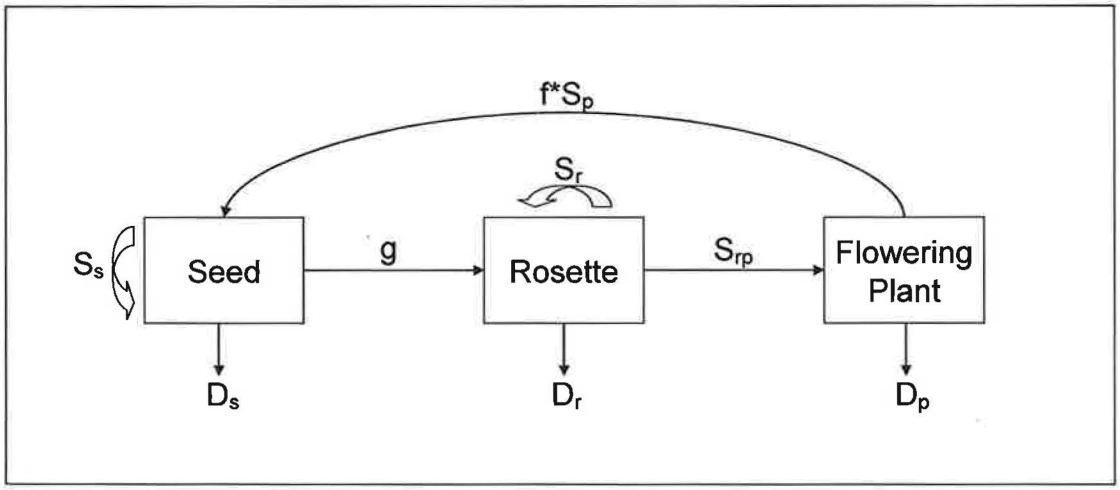


Fig. 1. Diffuse knapweed life cycle (adapted from Powell, 1990 and Sheley et al., 1998). Abbreviations: Ss, seed survival in the seed life stage; Ds, seed mortality; g, seed germination rate; Sr, rosette survival in the rosette life stage; Dr, rosette mortality; Srp, rosette survival to flowering plant life stage; Dp, post-reproductive mortality of the adult plant (100%); f, fecundity of reproductive plant, Sp, survival rate of flowering plant to successful reproduction.

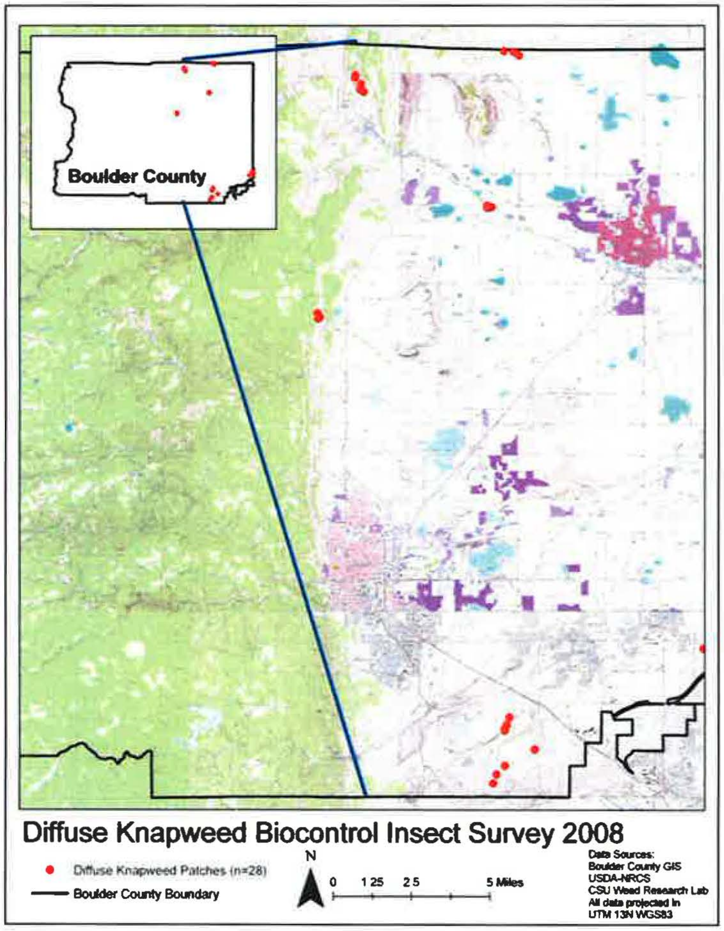


Fig. 2. Location of diffuse knapweed patches evaluated for presence of biocontrol insects.

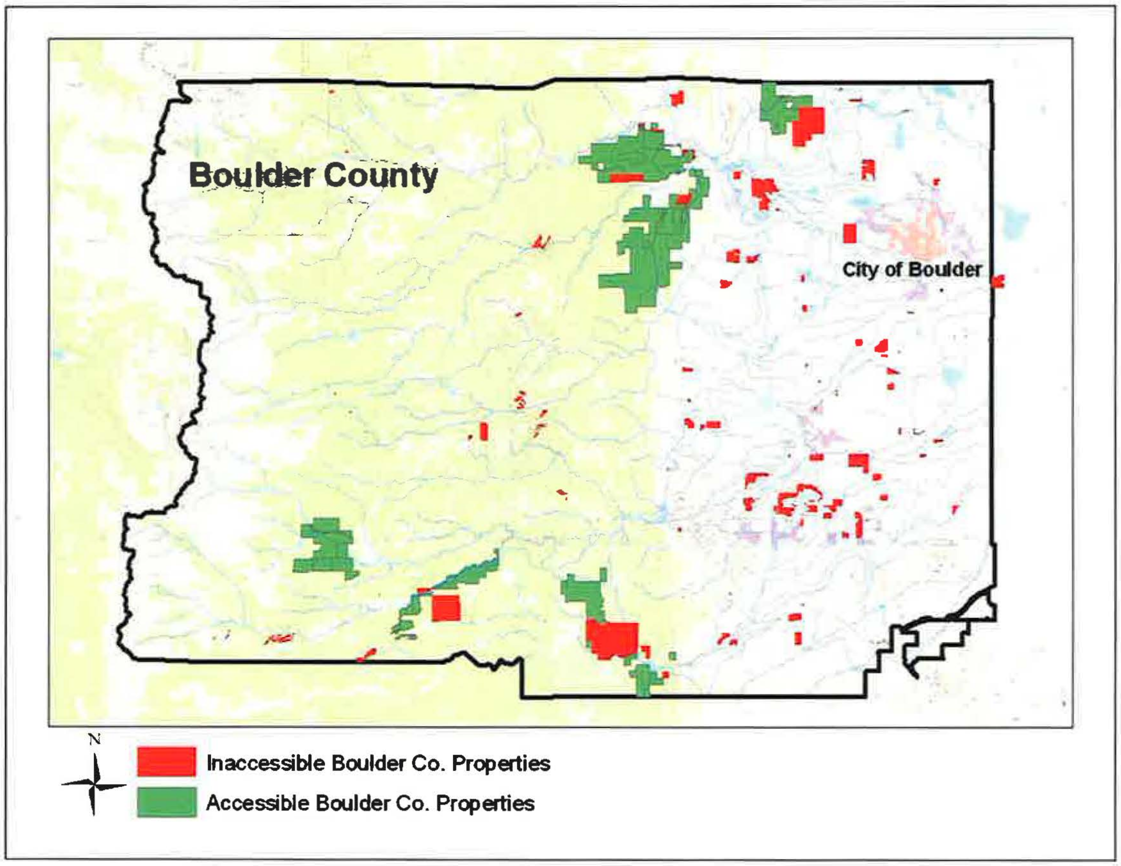


Fig. 3. Accessible and inaccessible Boulder County open space properties as covered by the CSU Weed Research Laboratory/Boulder County Parks and Open Space research permit for the summer of 2008.

Fig. 4. Diffuse knapweed survey locations during June and July of 2008 as stratified by elevation and hydrological zones.

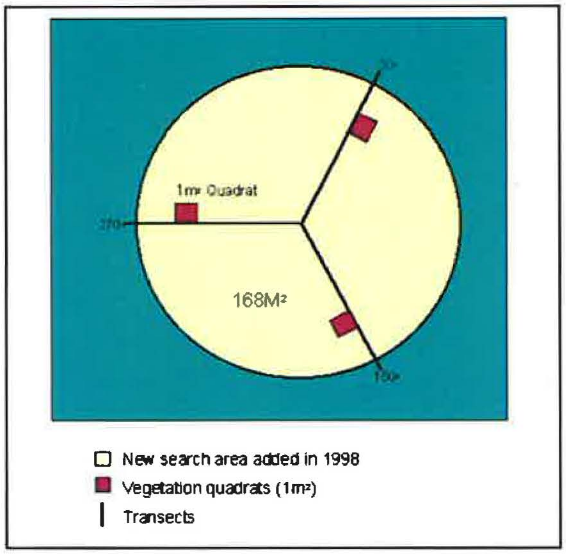


Fig. 5. Example of the modified Forest Health Monitoring plots used in the diffuse knapweed survey to characterize d. knapweed cover, density, and biocontrol agent abundance.

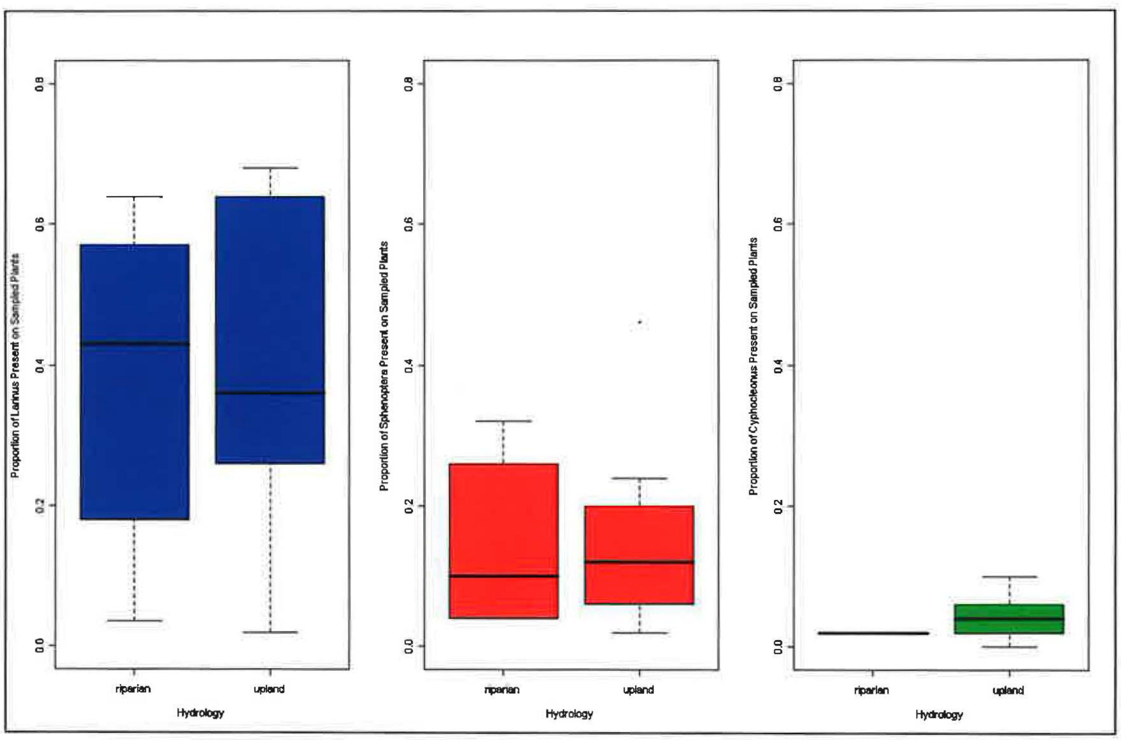


Fig. 6. Proportions of each biocontrol agent present on diffuse knapweed patches versus the hydrological characteristics of where the patches were located.

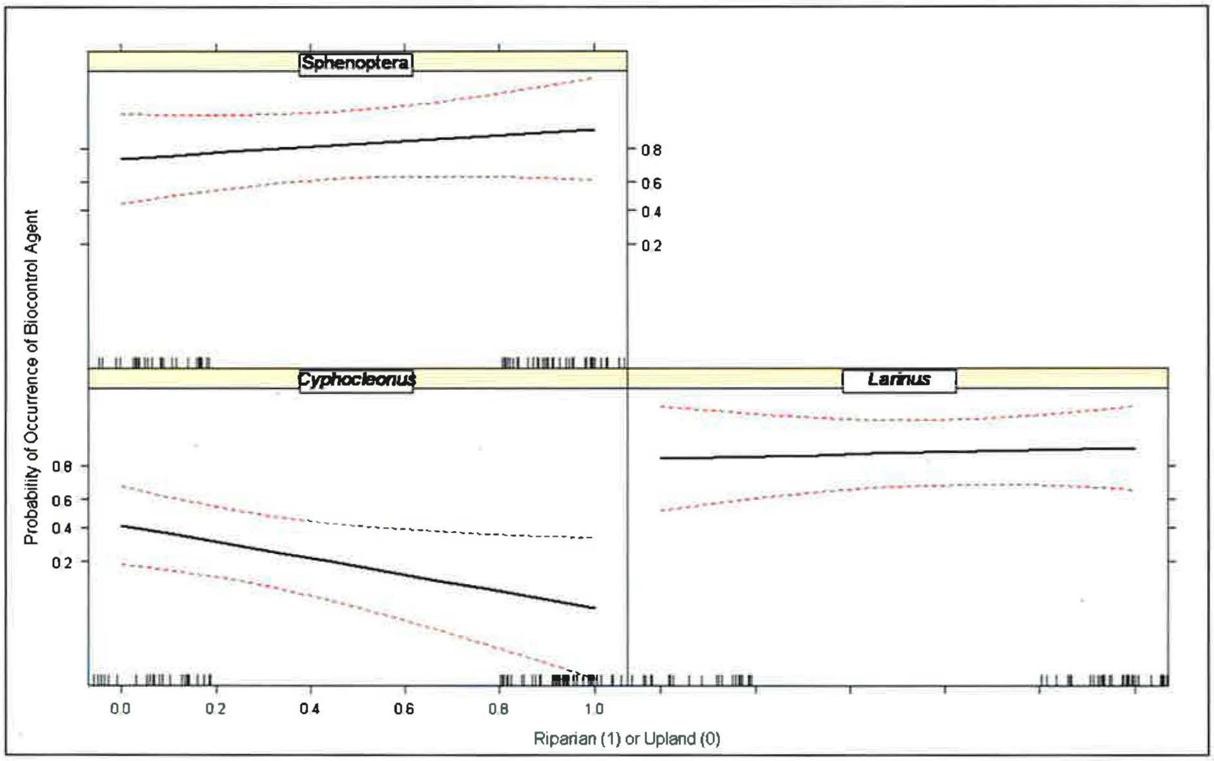


Fig. 7. Graphical representation of the effects of the presence of biocontrol agents described in Table 3. The black line represents the mean effect and the red dashed lines are the upper and lower 95% confidence limits for the estimated mean probabilities of occurrence. Note: response variable is plotted as the $\ln(\pi_i/1-\pi_i)$ and the y-axis is scaled and labeled as the π_i.

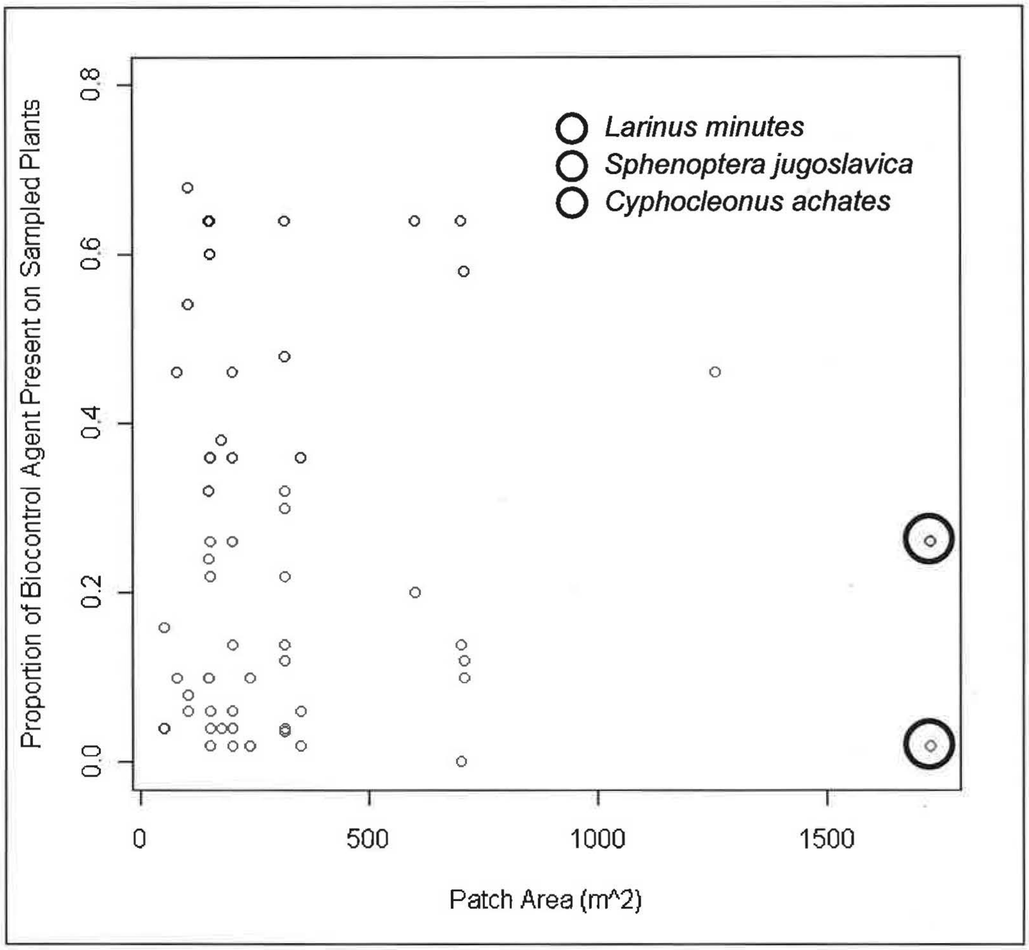


Fig. 8. Graphical representation of the proportions of each biocontrol agent present in each diffuse knapweed patch versus diffuse knapweed patch area. Those data points circled in black were likely outliers (i.e., there was likely not a patch that was nearly 2000 m^2 in area).

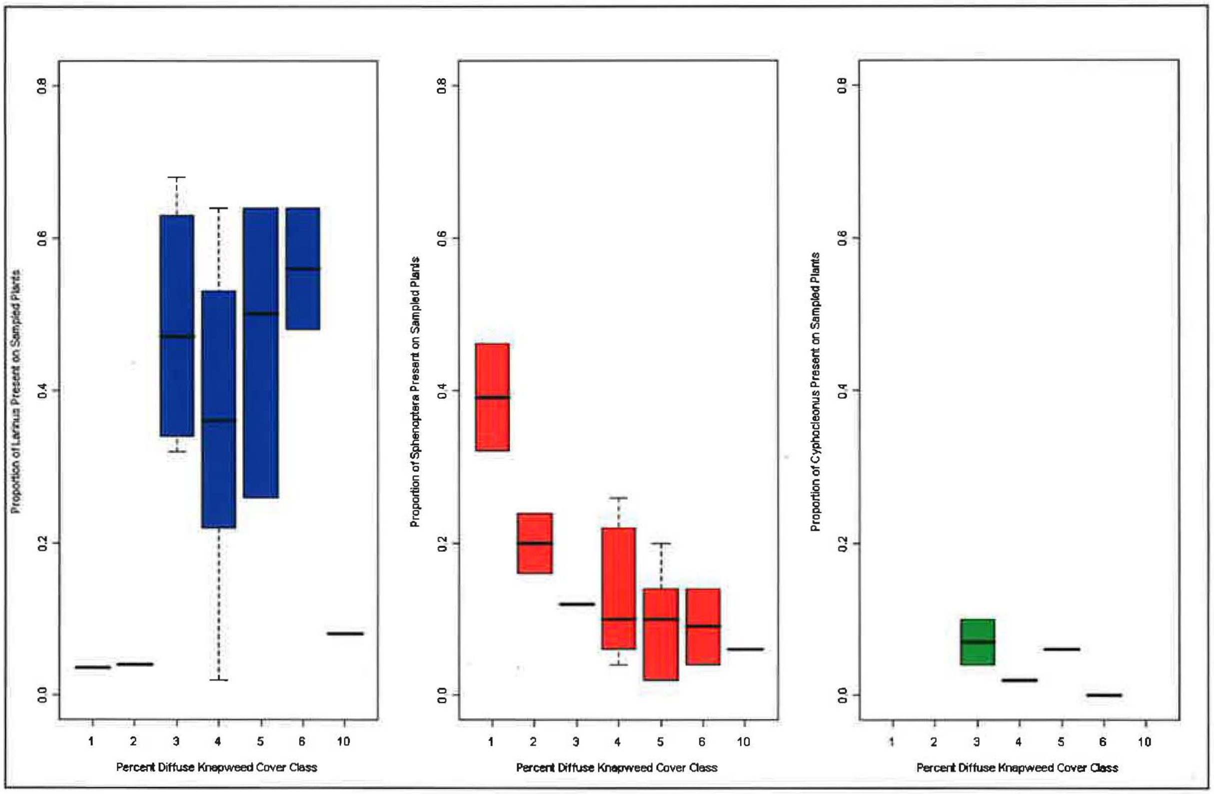


Fig. 9. Proportion of each biocontrol agent present in each diffuse knapweed patch versus the percent cover class of the diffuse knapweed patch (see Table 1 for Cover Class Code). **Box and whiskers plots**, such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

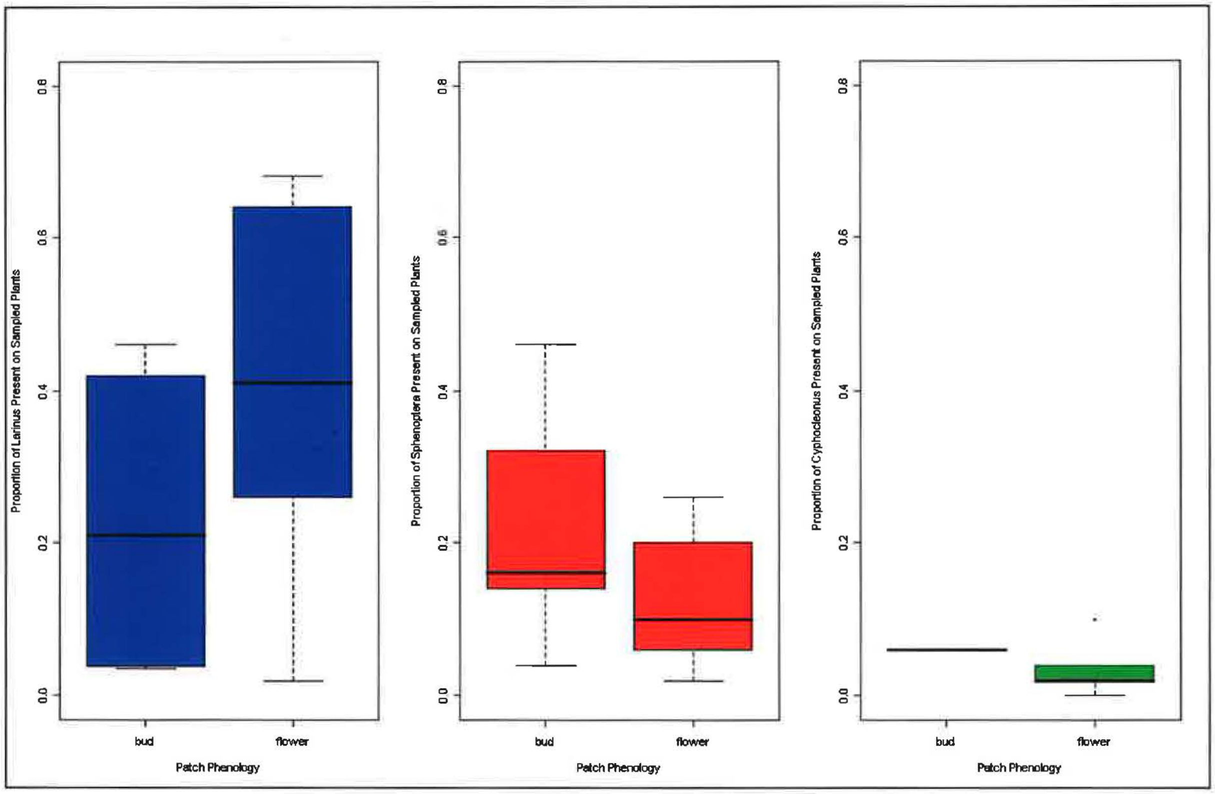


Fig. 10. Proportion of each biocontrol agent present in each diffuse knapweed patch versus the dominant plant phenology of the diffuse knapweed patch. **Box and whiskers plots**, such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

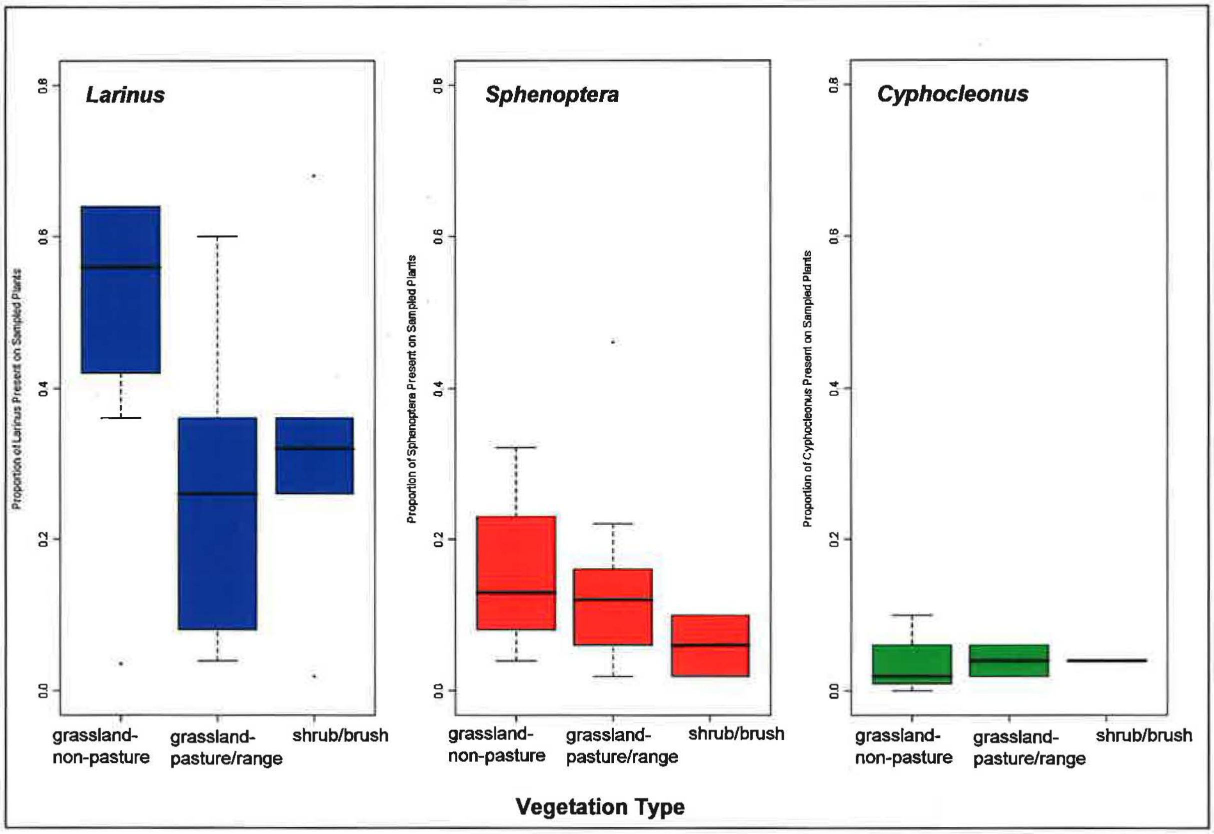


Fig. 11. Proportion of each biocontrol agent present in each diffuse knapweed patch versus the dominant vegetation type at the location of the diffuse knapweed patch. **Box and whiskers plots**, such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

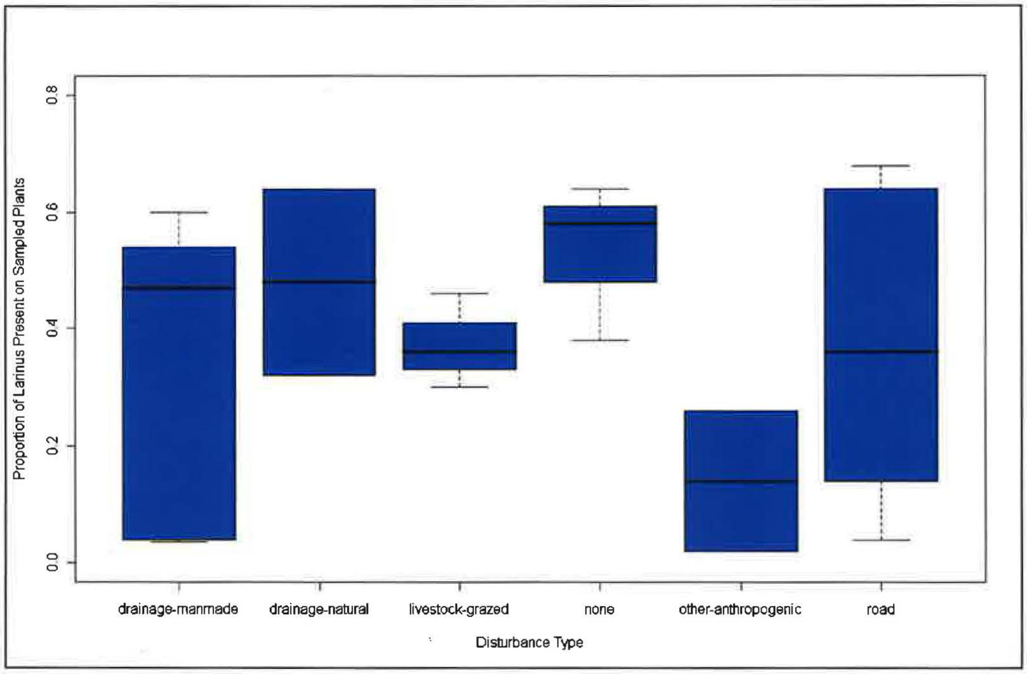


Fig. 12. Proportion of *Larinus* insects present at each sampled diffuse knapweed patch versus the putative disturbance recorded by CSU Weed Lab mapping crews (see Table 1). **Box and whiskers plots,** such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

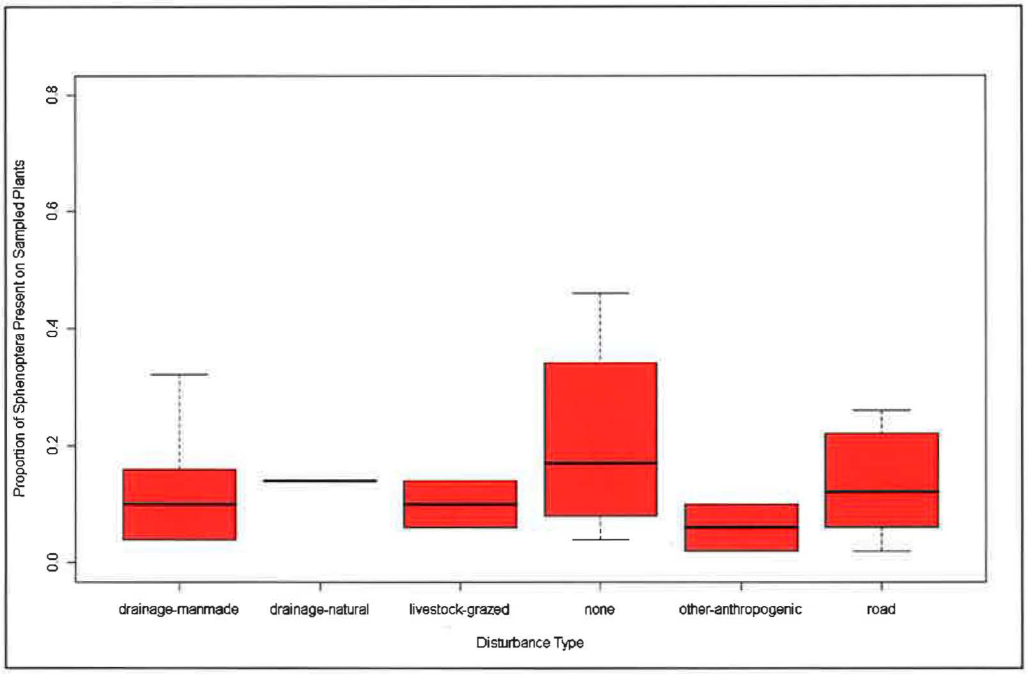


Fig. 13. Proportion of *Sphenoptera* insects present at each sampled diffuse knapweed patch versus the putative disturbance recorded by CSU Weed Lab mapping crews (see Table 1). **Box and whiskers plots**, such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

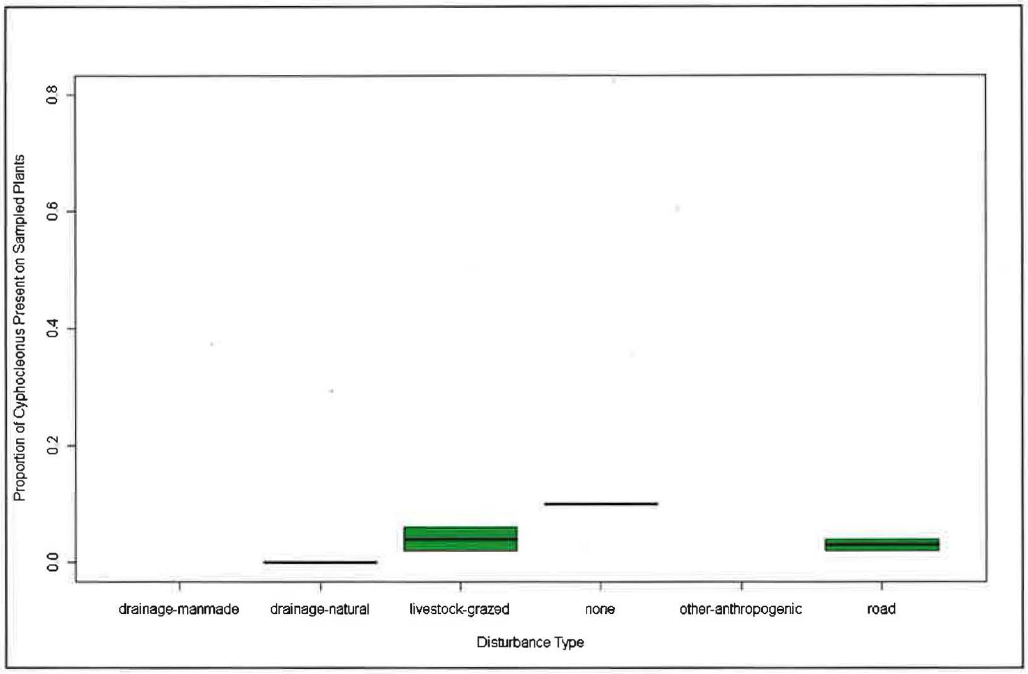


Fig. 14. Proportion of *Cyphocleonus* insects present at each sampled diffuse knapweed patch versus the putative disturbance recorded by CSU Weed Lab mapping crews (see Table 1). **Box and whiskers plots**, such as the graphs presented here, depict the range of the data (i.e., the whiskers), the 25 and 75 percentile quantities (i.e., the hinges or edges of the box), and median of the data (i.e., black line in box). Box and whiskers plots describe the range, variability, and distribution of the data for a particular treatment. The edges or hinges of the box represents the maximum and/or minimum data value when no whiskers are present.

Table 1. GPS menu choices for diffuse knapweed patch attributes.

Cover Class	Cover Class Code	Phenology	Vegetation Type	Disturbance Type	Hydrology
>1	1	Rosette	Aspen Forest	Drainage-natural	Upland
1-5	2	Bud	Conifer Forest	Drainage-manmade	Riparian
6-10	3	Flower	Park/Recreation Area	Livestock-grazed	
11-20	4		Grassland-pasture/range	Fire/Burned	
21-30	5		Grassland-non-pasture	Other-anthropogenic	
31-40	6		Shrub/brush	None	
41-50	7				
51-60	8				
61-70	9				
71-80	10				
81-90	11				
91-100	12				

Table 2. Biocontrol insect count categories.

Count	Category †
0	0
1-10	1
11-20	2
21-30	3
31-40	4
41-50	5
>50	6

†This code is used in within the digital GIS data attribute tables.

Table 3. Summary table of the final logistic regression model.

Variable	Estimate	Std. Error	z-value	p-value†
Intercept	-0.3365	0.5855	-0.575	0.5655
Larinus	1.9459	0.9710	2.004	0.0451 **
Sphenoptera	1.4351	0.8873	1.617	0.1058
Riparian	-2.3716	1.1872	-1.998	0.0458 **
*Larinus**Riparian	2.6593	1.5469	1.719	0.0856 *
*Sphenoptera**Riparian	3.2189	1.5574	2.067	0.0387 **

†Statistical significance codes. *significant at the 0.10 level; **significant at the 0.05 level

Table 4. Mean probability of occurrence and 95% confidence intervals for every combination of biocontrol agent and location (i.e., riparian or upland).

	Riparian	95%Confidence Interval		Upland	95%Confidence Interval	
	Estimate†	Lower	Upper	Estimate†	Lower	Upper
Cyphocleonus	0.06	0.01	0.34	0.42	0.18	0.69
Larinus	0.87	0.66	0.99	0.83	0.52	0.96
Sphenoptera	0.88	0.61	0.97	0.75	0.45	0.92

†The results of a logistic regression are probabilities. These probabilities correspond to the probabilities of occurrence of a particular biocontrol agent at a particular environmental zone (riparian or upland).