"The role of soil nitrogen availability of the defensive chemistry of an invasive plant, Linaria dalmatica (Scrophulariaceae)."

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***2006 Progress report for research conducted on Boulder County Open space***

## I. Introduction and Background

The invasion of non-native plant species has affected ecosystems throughout the world and consequently has become a global conservation concern. Invasive plants pose an important threat to native biodiversity and the aggressiveness of these species to invade a habitat may be facilitated by environmental change factors, such as increased sources of plant available nitrogen. Numerous empirical and theoretical studies suggest that non-native plant species are most likely to invade environments where resource availability exceeds resource demand by resident species. Invasive species may respond more positively to soil nitrogen enrichment in comparison to related native species for various reasons. For example, non-native plants often demonstrate greater phenotypic plasticity when compared to co-occurring native species and thus may demonstrate a greater increase in productivity with increased nitrogen availability. Also, non-native plants may experience less population regulation compared to native species if their specialist enemies (e.g. pathogens and herbivores) are not present in the introduced range. Our research examines whether soil nitrogen enrichment influences trophic interactions as well as the dominance of the invasive plant species, *Linaria dalmatica*. Here I present the results of research conducted on Boulder County Open Space. This research represents a portion of my dissertation research and serves as the foundation for a series of projects that will focus on the role of nitrogen availability on plant-insect interactions as mediated by changes in plant chemistry. *Linaria dalmatica* provides a useful model system for studying the effects of nitrogen enrichment on plant chemical defenses because it produces both carbon-based iridoid glycosides (primarily antirrhinoside) and nitrogen-based quinazoline alkaloids. This research is being conducted in collaboration with Timothy Seastedt and Deane Bowers.

## II. Surveys of *Mecinus janthinus*

During June-August 2006, we surveyed three Boulder County Open Space properties for the presence of *Mecinus janthinus*, an insect biological control agent used to manage the invasive plant species *Linaria dalmatica*. The three properties surveyed were Hall Ranch, Rabbit Mountain, and Heil Ranch. *Mecinus janthinus* adults were released at each property during the spring of 2004 by Boulder County personnel. We surveyed *Linaria dalmatica* plants for evidence of *M. janthinus* establishment. Evidence of establishment included foliar and stem damage characteristic of *M. janthinus* as well as the presence of adults or larvae. At each release site, we randomly collected 100 dead *L. dalmatica* stems (from previous growing seasons). We then measured stem length in the laboratory and counted the number of oviposition scars and emergence holes made by *M. janthinus* with the aid of a dissecting scope. Oviposition scars provide an estimate of the number of eggs laid per stem and emergence holes provide an estimate of the number of adult *M. janthinus* that survived through the final developmental stage.

Results indicate that *M. janthinus* has successfully established at all three release sites. At each site, we found both adult and larval stages of *M. janthinus*, as well as, evidence of leaf and stem damage by *M. janthinus*. Oviposition activity was greatest at Hall Ranch (Tables 1 and 2); however, emergence and mean survivorship was highest at Rabbit Mountain (Table 3). While there is clear evidence of *M. janthinus* oviposition activity on *L. dalmatica* stems at these sites (Table 1 and 2), our results suggest that larval or adult survivorship may be quite low (Table 3).

**Table 1.** Percentage of *Linaria dalmatica* stems with oviposition scars and emergence holes made by *Mecinus janthinus* (a total of 100 stems were examine for each site).

| Release Site | % of stems w/ oviposition scars | % of stems w/ emergence holes |
|---|---|---|
| Hall Ranch | 60 | 25 |
| Rabbit Mountain | 39 | 14 |
| Heil Ranch | 46 | 12 |

**Table 2.** Summary results. At each site, 100 dead *L. dalmatica* stems were collected and examined for *M. janthinus* oviposition scars which provide an estimate of the # eggs laid per stem and emergence holes which provide an estimate of the # of surviving adults that emerge from each stem (mean ± 1SD).

| Release site | stem length | # of oviposition scars | # emergence holes |
|---|---|---|---|
| Hall Ranch | 50.6 cm | 18.5 ± 20.6 | 1.1 ± 1.9 |
| Rabbit Mountain | 42.6 cm | 11.7 ± 6.7 | 1.9 ± 3.8 |
| Heil Ranch | 40.8 cm | 10.4 ± 10.1 | 0.8 ± 2.1 |

**Table 3.** Survivorship percentages for *Mecinus janthinus*. Survivorship was calculated by taking the # of oviposition scars divided by the # of emergence holes per *Linaria dalmatica* stem.

| Release Site | Mean | maximum | minimum |
|---|---|---|---|
| Hall Ranch | 5.2 ± 10.4 | 50 | 0 |
| Rabbit Mountain | 11.7 ± 20.6 | 75 | 0 |
| Heil Ranch | 6.0 ± 13.1 | 47 | 0 |

**III. Surveys of *Linaria dalmatica***

To establish the range of variation in anti-herbivore defense compounds found in *L. dalmatica* populations as well as the role of soil nitrogen availability on such variation, soil and plant samples were collected from Hall Ranch and Rabbit Mountain during the summer of 2006. Soil nitrogen availability was measured at each population using ion exchange resin bags (method in Binkley and Vitousek 1989), which provided an index of the flux of plant-available inorganic nitrogen over the course of the growing season. Twenty resin bags were buried in pairs at each study site (10 microsites per location) at a depth of 5-10cm. Upon collection, resin beads were air-dried, weighed, and samples were analyzed for KCl-extractable ammonium and nitrate at the Natural Resource Ecology Laboratory (Colorado State University, Fort Collins, Colorado). Plant samples were collected near the location of resin bags (10 plants per population) on two dates during the growing season. Plant samples were oven dried at 50° C for 36 hrs. Iridoid glycosides concentrations were quantified using gas chromatography (methods described in Gardner and Stermitz 1988 and Bowers and Stamp 1993).

Results from data collected during the 2006 growing season indicate no significant difference in the flux of plant available nitrogen between the two sites ($p = 0.08$; Figure 1). Additionally there was no significant difference in iridoid glycoside (antirrhinoside) concentration between sites ($p = 0.236$); however, there was a significant difference in concentrations across dates ($p < 0.001$). Antirrhinoside concentrations ranged from 19.1 to 1.75 percent dry weight, with the highest levels observed early in the growing season (Figure 2).

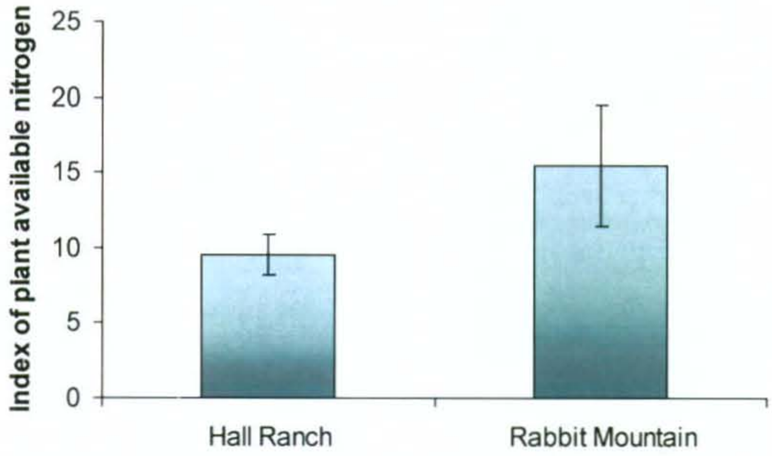


**Figure 1.** Index of plant available nitrogen for field sites ($n = 20$ samples per site). The index of plant-available N represents a flux of $NO_3$ and $NH_4$ (ppm/g resin) measured over the course of the growing season using ion exchange resin bags.

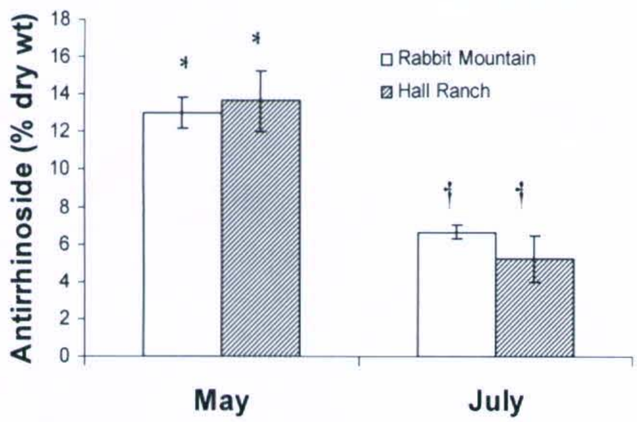


**Figure 2.** Variation in *L. dalmatica* antirrhinoside concentration in relation to date and location of plant samples (significant differences at $\alpha = 0.05$ are indicated by different symbols). Plants were sampled at two Boulder County Open Space locations (Rabbit Mountain and Hall Ranch).

**III. Significance of research**

While nitrogen deposition is thought to be a problem occurring primarily in the eastern United States, recent concerns about nitrogen deposition in the western United States have arisen due to rapidly increasing population growth. Currently, extensive areas of the western US are being exposed to chronic low level nitrogen deposition and a number of nitrogen hotspots have been identified downwind of large metropolitan and/or agricultural areas. The eastern slope of Colorado's Front Range represents one of these N deposition hotspots and is experiencing a particularly high degree of fertilization effects. In addition to nitrogen deposition, there are a number of additional environmental change factors occurring throughout the West that may contribute to soil nitrogen enrichment, including fire suppression, increased grazing pressure, and exotic plant invasions.

*Mecinus janthinus* surveys suggest that survivorship of this biological control agent may vary across release sites. While we have not detected significant differences in plant or soil chemistry across our surveyed field sites, related research suggests that soil nitrogen availability can play an important role in the production of plant anti-herbivore defenses and potentially insect survivorship. In a greenhouse study conducted in the summer of 2004, we found that soil nitrogen availability had a significant positive effect on plant productivity and a significant negative effect on plant defensive chemistry for *Linaria dalmatica*. Plants grown under the high nitrogen treatment had approximately twice the aboveground biomass as plants in the control group; whereas, increased nitrogen availability decreased anti-herbivore defenses, yielding an approximate 30% reduction in antirrhinoside concentration for the high nitrogen treatment compared to the control group.

Soil nitrogen enrichment may influence the efficacy of insect biological control of invasive plants through a number of mechanisms, including through changes in plant productivity, nutritional quality, and defensive chemistry. Our research incorporates greenhouse, common garden, and field studies that address the role of soil nitrogen availability on plant chemistry and plant-insect interactions using *Linaria dalmatica* as a model study system.