Assessing Genetic Diversity and Phenotypic Plasticity in *Cirsium arvense*: Evaluation with Greenhouse Trials and ISSRs

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STATE OF COLORADO

Locations of sample sites

Colorado State University

Knowledge to Go Places

Introduction

➤ The question of whether plant invaders are successful because of phenotypic plasticity or genetic adaptation is unresolved

➤ PLASTICITY versus ADAPTATION
- Many invasive weeds are successful due to phenotypic plasticity and the resulting ability to thrive in different environments
- Evolution in response to different selection pressures in diverse habitats, such as high nutrient levels and annual disturbance in crop habitats and lower nutrient levels and increased competition in natural habitats, may lead to local genetic adaptations
- Gene flow can transfer adaptations to other habitats, which may improve the success of invasive weeds

➤ Several invasive weeds in North America are found in both agricultural and natural habitats

➤ Understanding the ecology of an invasive weed in different habitats will assist land managers in designing integrated weed management strategies

➤ Canada thistle (*Cirsium arvense*) is a particularly invasive weed species that serves as a model system to investigate these ecological and genetic questions due to its wide distribution, success in different habitats, dioecious reproduction, and vegetative growth

Objectives

➤ To evaluate whether phenotypic plasticity or genetic adaptation has a greater influence on the growth of *C. arvense* plants from different geographic regions and habitat types using greenhouse experiments

➤ To assess the extent of genetic differentiation in *C. arvense* populations from different geographic regions and habitat types using molecular markers

Hypotheses

➤ If local genetic adaptations to habitat selection pressures have a greater influence on the competitive ability of *C. arvense* than phenotypic plasticity, then individuals from crop habitats will perform better under high nutrient conditions and individuals from non-crop habitats will perform better under limited nutrient conditions

➤ If genetic differentiation in *C. arvense* is influenced by habitat selection pressures, then population structures will show higher relatedness among individuals from similar habitats and geographic regions

Larimer non-crop (left) and Larimer crop (right) sample sites

Methods

➤ We collected rhizomes from isolated clones in crop and non-crop habitats in northern Colorado (Larimer County) and southeastern Colorado (Prowers County) for the greenhouse trials. Replicate clones from each sample area were subjected to one of two fertility treatments (100 mg/L NO_3-N once a week or no supplemental nitrogen) and one of two water treatments (field capacity or 40% field capacity) in a complete factorial. The trials lasted eight weeks. Measurements of weekly and final height, final root and shoot biomass, and total leaf area were taken. Data were analyzed using Procedure Mixed in SAS, version 8.02 (SAS Institute, Cary, NC). Results with p-values less than 0.05 were considered significant.

➤ We sampled leaf tissue from individuals in crop and non-crop habitats in northern Colorado, southeastern Colorado, and northeastern Colorado (Sterling). Analysis of intersimple sequence repeat (ISSR) markers was performed using TFPGA, version 1.3 (Miller, 1997). The ISSR procedure of Wolfe et al., (1998) was used. One primer in two replications, (CAC)5AG, gave five scoreable loci.

Greenhouse Trials Results

➤ Higher levels of nitrogen and water increased total biomass production and decreased root to shoot ratio (Table 1).

➤ The interaction of nitrogen and water was significant; growth was maximized by high levels of both water and nitrogen (Figure 1) and allocation to roots relative to shoots was maximized by low levels of both water and nitrogen (Figure 2).

➤ Habitat and geographic region did not significantly affect total growth or biomass allocation.

Table 1. Main effects of Nitrogen and Water on total biomass and root to shoot ratio. LS Means across all samples, +/- standard error. Number DF=1 for all effects. F-statistics from analyses of natural log transformation of data.

	Total Biomass		Root to Shoot Ratio	
	---- N ----	---- H_2O ----	---- N ----	---- H_2O ----
High	4.27 g (+/- 0.36)	4.25 g (+/- 0.34)	0.71 (+/- 0.11)	0.69 (+/- 0.1)
Low	1.78 g (+/- 0.31)	1.79 g (+/- 0.33)	1.35 (+/- 0.08)	1.37 (+/- 0.09)
Pr > F	0.0002	0.0001	0.0013	0.0007
F Value	32.88	34.94	17.44	20.43
Den DF	9.65	10.3	11.8	11.8

Figure 1. Interaction of Nitrogen and Water on total biomass. (p=0.0221, $F_{1, 7.29}$=7.29)
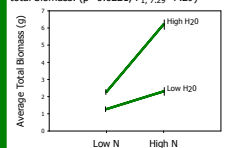
High H2O
Low H2O

Low N   High N

Average Total Biomass (g)

* LS Means across all samples (+/- standard error). F-statistics from natural log transformation of data.

Figure 2. Interaction of Nitrogen and Water on root to shoot ratio. (p=0.0364, $F_{1,11.6}$=5.6)
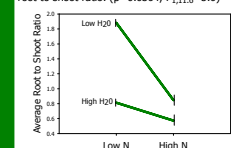
Low H2O
High H2O

Low N   High N

Average Root to Shoot Ratio

* LS Means across all samples (+/- standard error). F-statistics from natural log transformation of data.

ISSR Results

➤ Some genetic differentiation was found among geographic regions and habitats, but the largest differentiation was found among different sample sites within the same habitats (Table 2).

➤ An unweighted pair group method with arithmetic mean analysis (UPGMA) tree shows clustering of populations at the geographic scale (Figure 3).

Table 2. F-statistics with 95% Confidence Interval for subsubpopulations (sites) within subpopulations (habitats) within populations (geographic region). F (theta) ranges from 0 (no differentiation) to 1 (complete differentiation). Values of theta over 0.05 are biologically significant, and over 0.1 are considered high.

Primer	Theta P
(CAC)5AG	0.2362 (0.4217, 0.0248)
Theta S	Theta SS
0.1681 (0.4072, -0.0568)	0.7718 (0.8020, 0.6543)

Figure 3. UPGMA tree; data analyzed as subpopulations (habitats) within populations (geographic region)

Prowers Crop
Prowers Non-Crop
Larimer Crop
Larimer Non-Crop
Sterling Crop
Sterling Non-Crop
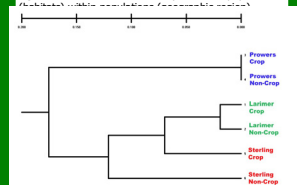
Discussion

➤ Results from greenhouse experiments showed that nutrient levels, rather than habitat of origin, had the greatest influence on the growth of *C. arvense*. These results support the position that phenotypic plasticity (ability to adjust to conditions) has the greatest influence on the productivity of this species. Selection pressures due to differences in habitats, such as nutrient levels, may still influence genetic adaptations, but these results do not demonstrate that significant adaptations have occurred in the sampled populations.

➤ Because of challenges in collecting viable rhizomes and methods used in beginning greenhouse treatments, less than half of the planted clones were available for the experiment (a logistic regression analysis indicated that fertility and water treatments did not adversely affect survival of clones). Resulting small sample sizes decreased statistical power. Further investigation using more sample sites within the habitats and improved experimental methods would allow increased power to detect potential differences resulting from genetic adaptations to habitat.

➤ The UPGMA tree does show higher relatedness between individuals from similar geographic regions, and little evidence that samples from similar habitats (crop or non-crop) in different regions are related.

➤ Theta values for genetic differentiation show higher levels of differentiation among sample sites within similar habitats as compared to differentiation between habitats or geographic regions. This indicates that the ISSR marker used is highly variable for *C. arvense* and habitat selection pressures may not be influencing the observed differentiation.

➤ Some gene flow may be occurring among sites within a geographic region; increasing physical distance between sample sites may allow genetic drift. Variability in the genome is significantly different when viewed between sample sites, but becomes less significant when averaged across many individuals from a larger spatial scale. This variability may be due to multiple founders' effects.

➤ Further investigation with additional ISSR markers or microsatellite markers is warranted to clarify genetic differentiation within *Cirsium arvense*.

References

➤ Miller, M.P. 1997. Tools for Population Genetic Analysis, v.1.3. Software distributed by author.

➤ Wolfe, A.D., Q. Xiang, S.R. Kephart. 1998. Molecular Ecology. 7, 1107-1125.