DISSERTATION

HYBRIDIZATION BETWEEN SPOTTED KNAPWEED (*CENTAUREA STOEBE*) AND DIFFUSE KNAPWEED (*C. DIFFUSA*): PATTERNS AND IMPLICATIONS FOR INVASION

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY AMY C. BLAIR ENTITLED "HYBRIDIZATION BETWEEN SPOTTED KNAPWEED (*CENTAUREA STOEBE*) AND DIFFUSE KNAPWEED (*C. DIFFUSA*): PATTERNS AND IMPLICATIONS FOR INVASION" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

HYBRIDIZATION BETWEEN SPOTTED KNAPWEED (*CENTAUREA STOEBE*) AND DIFFUSE KNAPWEED (*C. DIFFUSA*): PATTERNS AND IMPLICATIONS FOR INVASION

Hybridization is an evolutionary force that has the potential to alter invasion dynamics. *Centaurea diffusa* Lam. (diffuse knapweed) and *C. stoebe* L. (spotted knapweed) are two problematic invasive weeds in western North America. Anecdotal information suggested these two plants were hybridizing in the introduced range. The overall goal of my dissertation was to examine the patterns of hybridization in the introduced and native ranges in the field and at the molecular level, and determine if hybridization was altering the invasion of either species.

In the field in North America, I detected intermediate hybrid plants in 39 out of 40 diffuse knapweed sites; hybrid-like plants were taller and more often exhibited polycarpy than diffuse-like plants. Hybrid-like plants were not detected in North American spotted knapweed sites. In Europe in most countries surveyed, diffuse and spotted knapweed existed as distinct, non-hybridizing species. However, in the Ukraine, the two species frequently co-existed within a site, resulting in hybrid swarms. It has recently been confirmed that the spotted knapweed in North America is tetraploid while the diffuse knapweed is likely all diploid. Genetic incompatibilities associated with these two cytotypes likely prevents on-going hybridization. Instead, the diffuse knapweed in North America was probably introduced with individuals of hybrid origin.

I used amplified fragment length polymorphisms (AFLPs) to examine hybridization at the molecular level. The Bayesian clustering program, STRUCTURE, found that a majority of the assayed North American diffuse knapweed sites contained individuals with significant introgression from spotted knapweed. Counter to expectation, the hybrid-like plants did not contain more admixture than the plants that looked diffuse-like. A century of back-crossing with diffuse knapweed has likely decoupled the relationship between floral morphology and introgression at the molecular level.

Biological control agents were not strongly influenced by the presence of plants with hybrid morphology. Within North America diffuse-like and hybrid-like plants carried similar herbivore loads. In paired preference tests the seedhead weevil *Larinus minutus* demonstrated a preference for newly created artificial hybrids over North American diffuse knapweed; this preference would likely be short-lived in the field as introgression occurred.

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ABSTRACT

Hybridization is an evolutionary force that has the potential to alter invasion dynamics. Although a number of invasive plant species are of hybrid origin, relatively few studies have focused on the role of hybridization in invasion. Spotted and diffuse knapweed are economically and ecologically destructive weed species introduced from Eurasia to North America around 1900. Anecdotal field observations suggested that these closely related asters were hybridizing in the introduced range. As a first step towards understanding the role of hybridization in these invasions, I conducted field surveys to understand the patterns of hybridization in the introduced and native ranges and measured fitness-related traits among field hybrids and parental species to examine heterosis. In North America I detected intermediate hybrid plants in 39 out of 40 diffuse knapweed sites; hybrid plants were taller and more often exhibited polycarpy than plants with typical diffuse knapweed morphology. Hybrids were not detected in North American spotted knapweed sites. In most European countries, diffuse and spotted knapweed existed as distinct, non-hybridizing species; however, in the Ukraine, the two species frequently co-existed within a site, resulting in hybrid swarms. Hybrids in the Ukraine did not exhibit heterosis for size traits. I suggest that these cross-continental patterns of hybridization are explained by differences in cytology. It has recently been confirmed that spotted knapweed in North America is tetraploid while the diffuse knapweed is likely all diploid. Genetic incompatibilities associated with these two haplotypes likely prevents on-going hybridization. Instead, the diffuse knapweed in North America was probably introduced with hybrid individuals, and even after a century of back-crossing, hybrid traits are still maintained in nearly all diffuse knapweed sites. In the native range, non-overlapping

regions of the diploid species prevents hybridization in much of Europe, while in other locations, they overlap and hybridize. Overall, North American admixed diffuse knapweed individuals are larger than the apparently pure diffuse knapweed in the native range. Invasion is inherently complex, and even more so when hybridization can occur and species have multiple haplotypes.

INTRODUCTION

"There is now no question that hybridization is a frequent and important component of plant evolution and speciation." -L. H. Rieseberg and N.C. Ellstrand, Critical Reviews in Plant Science, 1993.

The role of hybridization in adaptive evolution historically split biologists into those that either viewed hybridization as a reinforcement to species boundaries because hybrids are most often less fit than the parental species (e.g. Dobzhansky 1970; Mayr 1963), and those that viewed hybridization as an important creative evolutionary force (e.g. Anderson and Stebbins 1954). There has generally been more support for hybridization playing a significant role in evolution in plants (i.e. Rieseberg's sunflowers and Arnold's irises) compared to animals; however, examples of hybridization influencing evolutionary change of animals is growing (Dowling and Secor 1997; Fitzpatrick and Shaffer 2007; Nolte et al. 2005).

Intra- and interspecific hybridization are hypothesized to play an important role in plant invasions (Ellstrand and Schierenbeck 2000). Ellstrand and Schierenbeck (2000) and Rieseberg et al. (2007) suggest three mechanisms that may promote the success of hybrids in a new range:

- *Evolutionary novelty and increased genetic variation.* Traits in hybrid individuals may be intermediate to the two parents, or transgress (i.e. be extreme) relative to the parental phenotypes. While some of the novel phenotypes will be less fit than the parental species, some might be *more* fit for a particular environment than either parent species. Additionally, hybridization will result in increased genetic variance, which Ellstrand and Schierenbeck (2000) suggest may in itself be responsible for evolutionary success, as it enables a rapid response to selection.

- *Decreased genetic load.* In the native range, organisms often exist in small and isolated populations; in-breeding and/or genetic drift in such situations can result in homozygosity for deleterious alleles, causing a fitness decline. After hybridization, heterozygosity increases and fewer deleterious alleles are found as homozygotes because it is unlikely that two parent populations will be homozygous for the same deleterious alleles.
- *Heterosis (i.e. hybrid vigor).* The fusion of two distinct genomes in a hybrid can result in increased heterozygosity, which may result in higher fitness (see Reed and Frankham 2003). Heterozygosity and the resulting hybrid vigor can be stabilized if, for example, a plant reproduces clonally or if the hybrid is an allopolyploid.

Ellstrand and Schierenbeck (2000) identified 28 plant species for which there is evidence of hybridization occurring prior to invasiveness. While this suggests that hybridization might confer some advantage to an invasive species, the evidence is not definitive, particularly given that many plant species hybridize naturally within their native ranges. However, even if hybridization occurs in each range, it may be more important in the introduced range, which represents a new habitat and new selection regime. One first step in understanding whether hybridization has influenced an invasion is to quantify the frequency and hybridization patterns in both the introduced and native ranges. For example, if hybrids are encountered more frequently across an organism's introduced distribution compared to its native range, then hybrids might have an advantage within the introduced range. Measuring plant performance in the field can provide initial information to evaluate the consequences of hybridization on fitness. If interesting patterns are discovered (i.e. hybrids are larger than parent plants), further experimental studies can examine the direct relationship between hybridization and fitness (e.g. Lexer et al. 2003).

In western North America, spotted and diffuse knapweed are two of the most costly and ecologically devastating invasive plants (Roché and Roché 1991; Sheley et al. 1999; Watson and Renney 1974). Field observations of morphologically intermediate plants in North America suggested that these two closely related asters were hybridizing. I wanted to determine if hybridization contributed to one or both of these species' spectacular success. I surveyed spotted and diffuse knapweed across the USA and Europe to answer the following questions:

1) Do floral and seed traits provide a consistent way to distinguish among hybrid and parental species?

2) How common are hybrid plants in the native and introduced ranges? Is the pattern of hybridization in North America similar to that found in the native range?

3) Do hybrid plants in the field exhibit heterosis? Are heterotic patterns similar between North America and Europe?

4) Do offspring retain maternal morphological characters?

MATERIALS AND METHODS

Study Species

The genus *Centaurea* L. (Asteraceae) contains approximately 300 species (Garcia-Jacas et al. 2006), a number of which have been introduced globally and become invasive. In North America, at least 34 *Centaurea* species are reported to be introduced, 14 of which are defined as noxious weeds in one or more states (http://plants.usda.gov/). The taxonomy of the genus is complicated: sections within the genus are still being revised, and relationships within sections are not well-resolved (Garcia-Jacas et al. 2006). Thus, there is some uncertainty regarding which taxa are actually present in North America, and the nomenclature is complex.

My research focused on members of the *Centaurea* genus within the section Acrolophus-Phaelolepsis (Garcia-Jacas et al. 2006). More specifically, I focused on members of the *C. stoebe* (sensu latto) species group, which encompasses approximately 33 named taxa (Ochsmann 2000). I worked with *C. stoebe* (sensu stricto) and *C. diffusa* and their hybrids. It is reported that both species have diploid (2n = 18) and tetraploid (4n = 36) cytotypes (Ochsmann 2000). Both cytotypes of diffuse knapweed are referred to simply as *C. diffusa* Lam. The tetraploid seems to be rare, as it has only been reported twice in the literature from one specimen in Bulgaria (Löve 1979) and one in the former Yugoslavia (Löve 1978). The diffuse knapweed plants we worked with are likely to be diploid (A.C. Blair, unpublished data). The two cytotypes of spotted knapweed are both under *C. stoebe* L., a name that takes precedence over the commonly used *C. maculosa* (Ochsmann 2000). The monocarpic diploid is designated *C. stoebe* subsp. *stoebe* L., and the polycarpic tetraploid is designated *C. stoebe* subsp. *micranthos* (Gugler) Hayek (for which *C. biebersteinii* DC. is a synonym). Ploidy number is the only way to unambiguously distinguish the spotted knapweed sub-species (Ochsmann 2001). In the literature the few North American spotted knapweed plants that have been assayed for chromosome number are tetraploids (Moore and Frankton 1954; Müller 1989; Ochsmann

2000). Thus, when I refer to spotted knapweed of North American origin, it is likely to be the tetraploid *C. stoebe* subsp. *micranthos*, while spotted knapweed from Europe may be either cytotype. When ploidy level is known, it is clearly specified. Tetraploid spotted knapweed is a perennial (Ochsmann 2000), while diploid spotted knapweed is reported to be a biennial (Ochsmann 2000), and diffuse knapweed is an annual to short-lived perennial (Watson and Renney 1974). In the greenhouse both species will bolt and flower within 6 months (A.C. Blair, personal observation). Both plants are self-incompatible (Harrod & Taylor 1995; Blair, personal observation) and can produce up to 40,000 seeds/m^2 (Watson and Renney 1974). These knapweeds were accidentally introduced from Eurasia in the late 1800s or early 1900s, and have become a major threat to rangeland productivity and quality across western North America (Roché and Roché 1991; Sheley et al. 1999; Watson and Renney 1974). These plants increase soil erosion (Lacey et al. 1989; Sheley et al. 1997), can alter plant community composition (Tyser and Key 1988), negatively impact biodiversity (Ortega et al. 2006), and are thought to have allelopathic effects on other plants (Callaway and Aschehoug 2000; Fletcher and Renney 1963; but see Blair et al. 2005, 2006; Locken and Kelsey 1987).

Based on intermediate morphology, hybrids between spotted and diffuse knapweed were first identified in the native range in 1909 (Gáyer 1909), and were given the name *C. xpsammogena* Gáyer. It is thought that they only occur in two narrow zones of overlap in Romania and the Ukraine (U. Schaffner, personal communication). More recent studies have found that the hybrids are diploid and result from crosses between diploid spotted and diffuse knapweed (Ochsmann 1998, 1999). The diploid parental species and their hybrids are similar in that they are usually monocarpic and require dry, warm open habitats to grow (J. Ochsmann, personal communication). The plants are often single stemmed. Because of their similarities, floral morphology has been used to delineate the species (Ochsmann 2000; Watson and Renney 1974; Fig. 1.1; Table 1.1). In this study after verifying that floral characteristics consistently separate individuals into three groups (diffuse, spotted, and hybrid), I evaluate how various aspects of those groups differ. As the designation of a plant as a hybrid or a parental species is based on floral morphological characters rather than molecular markers, I call the groups hybrid-like, diffuse-like, or spotted-like to highlight that the classifications contain some uncertainty. Molecular work has corroborated that the presence of intermediate individuals within a region correctly predicts interspecific admixture (Chapter 2).

Spotted and Diffuse Knapweed Field Surveys

Field surveys were conducted in North America and Europe during summers 2005 and 2006. Below I describe the surveys in detail.

Introduced Range - North America 2005 and 2006

During summer 2005, I surveyed a total of 50 knapweed sites (22 spotted knapweed, 27 diffuse knapweed, and one site with both species) in seven states (CO, ID, MT, NY, OR, WA, WY) and Ontario, Canada (Figure 1.2; Table 1.2). The focus of this research was on the western USA where the species are most problematic; only three of the surveys (all spotted knapweed) were conducted in eastern North America. To find regions with spotted and/or diffuse knapweed, I contacted county weed supervisors prior to the collection trip, and initially picked a set of counties haphazardly to visit in each state. In 2005, flowering occurred later than normal across western North America due to cool wet weather. Flower color was a trait that I had interest in surveying, so counties surveyed within states were based largely on floral phenology rather than haphazardly as initially planned. Within a county, sites were selected either by driving until encountering a site or from directions from a county weed supervisor. Prior knowledge of the presence or absence of hybrid-like individuals was generally unknown. At each location, I documented extensive site information (Appendices 1.1 and 1.2).

I visually inspected each site for the presence of hybrid-like individuals, and if present, I counted the number of hybrid-like and parental-like plants. If a site was too large to exhaustively count all individuals, I haphazardly ran transects through the site and counted plants along the transects. The number of transects depended on the site size and plant density. At 28 of the sites (13 spotted knapweed, 14 diffuse knapweed, 1 diffuse + spotted knapweed), I conducted more intensive measurements on 30 individual plants. I haphazardly ran a 50 m transect through the population and surveyed plants on one side of the tape measure every 1-meter (or more if plants were spaced further apart). I measured the following traits:

- Height from the ground to the highest point of the plant
- Diameter across the widest part of the plant
- Polycarpy – evidence of flowering stalk(s) from previous seasons
- Inflorescence color – classified as 1 (white ray and disc flowers), 2 (light lavender ray and/or disc flowers + white ray and/or disc flowers), 3 (light purple ray flowers, white disc flowers), 4 (light purple ray and disc flowers), 5 (deep purple ray and disc flowers)

- Bract pigmentation – classified as 0 (golden) and 1-3 for light to dark bract pigmentation
- Apical spine length – classified as 0 mm if absent, if present, 3 spines were measured from 3 different seedheads mid-capitula
- Capitula – width and length of one capitula from a freshly opened flower
- Seed characters – seed length averaged from three seeds selected haphazardly, and the presence or absence of a seed pappus (when seeds were available for collection)

Hybrid-like plants were found only in North American diffuse knapweed sites; therefore, this research focused largely on that species (see below). Because fruits were not mature at most sites visited in 2005, in summer 2006, I visited an additional 13 diffuse knapweed sites throughout western North America (Table 1.2) to measure the seed characters noted above. Hybrid-like plants were found at all 13 sites, and when possible, I collected seedheads from 20 hybrid-like and 10 diffuse-like plants within each site.

Native Range - Europe 2005 and 2006

To compare patterns of hybridization in North America to those in the native range, I conducted field surveys in Europe. In the native range, there are regions where diploid spotted and diffuse knapweed do not overlap and hybridization should be absent, while in other regions the diploid variants do overlap and hybridization is possible (U. Schaffner, personal communication). I selected site locations to attempt to encompass both region types. Regarding the former region type, I surveyed spotted knapweed sites in Austria and Hungary in 2005 and Switzerland in 2006 and diffuse knapweed sites in Turkey in 2006 (Fig. 1.3; Table 1.2). Regarding the latter region type, I surveyed both species in Romania in 2005 and the Ukraine in 2006. While I had general predictions whether or not I would detect hybridization within a region, individual sites were surveyed without prior knowledge of the presence or absence of hybrids. Either local botanists identified sites for me, or I found sites while driving through the countryside. The basic procedure for sampling and obtaining morphological measures described above for North America in 2005 were followed, except for in Turkey, where a local botanist simply inspected the sites exhaustively for hybrid-like individuals.

Because of another unexpectedly cool spring in 2006, plants were just starting to flower during the Ukraine survey, so I could not record the flower color for most plants. Additionally, capitulum measurements and spine length were measured mostly on immature seedheads, so the numbers for floral traits are not directly comparable to those from North America and Central Europe. At seven of the Ukraine sites, plants were marked with metal tags, and Ukrainian colleagues returned later in the summer to collect seed. To obtain a better understanding of spotted and diffuse knapweed in the Ukraine, I surveyed herbarium specimens in the Kiev N.G. Kholodny Institute of Botany to look for hybrid-like plants within the spotted and diffuse knapweed collections.

<u>Relationship of Field Collected Parents to Offspring Phenotypes</u>

To determine whether offspring retain the morphology of their maternal parent, I grew field-collected North American seed in a common garden. As I almost always found hybrid-like plants within diffuse knapweed sites and never within spotted knapweed sites in North America, this experiment focused mainly on seed from hybrid-like and diffuse-like plants. In late spring 2006, approximately 75 seeds were planted from the following four sample locations: Hood River, Oregon; Estes Park, Colorado; Pendleton, Oregon; and Natrona, Wyoming. The Hood River site contained hybrid-like, diffuse-like, and spotted-like plants. The other three locations were diffuse knapweed sites, with hybrid-like individuals comprising less than 10% of the plants at each site. Seeds were selected from maternal spotted-like plants (only from the Hood River site), and hybrid-like and diffuse-like plants from all of the sites in approximately equal numbers of maternal plants. By April 2007, 54 of the plants had flowered. The experiment was terminated at this point because of an infestation of armored scale insects.

Statistical Analyses

Floral Traits

In the field, a plant was subjectively classified as spotted-like, diffuse-like, or hybrid-like based on floral characters. To more rigorously classify plants prior to further analyses, I utilized hierarchical clustering to identify natural groupings based on the floral data. Hierarchical clustering is a multivariate technique that groups individuals that share similar values across multiple variables. In short, each individual starts as its own point, and at each progressive step, the two individuals closest together are combined in to one cluster (JMP Statistics and Graphics Guide, 2002, pg. 383). I used this clustering approach for the surveyed plants in North America with the following five floral characters: flower color, bract pigmentation, capitula width, capitula length, and spine length. I recorded these data for 538 of the 822 plants surveyed in North America 2005. I used a similar approach to classify the Ukrainian plants (the only location in Europe I surveyed with hybrids), except that many plants did not have open flowers, so I could not include flower color. I had data on the remaining floral traits for 208 out of 419 plants surveyed in the Ukraine in 2006. Prior to analysis, data were standardized by the variable mean and standard deviation. I selected 'Ward's minimum variance' clustering method, as it has been employed frequently in the literature and has been found to outperform other hierarchical methods (Khattree and Naik 2000, pg. 442). I also compared this method to 'average linkage' and the 'centroid method;' all methods found very similar patterns. For these analyses, I used JMP ver. 6.0 (SAS Institute Inc., USA).

All other statistical analyses were conducted in SAS v. 9.1 (SAS Institute 2002). I used canonical discriminant analysis (Proc CANDISC in SAS) to determine which of the following six floral morphological traits most strongly distinguished among North American spotted-like, diffuse-like, and hybrid-like plants: flower color, bract pigmentation, capitula width, capitula length, spine length, and the presence or absence of vertical veins on bracts.

Global Patterns of Hybridization

I compared the frequency of hybridization in the introduced and native ranges with two different analyses. (1) To obtain an overall estimate of which region contained more diffuse knapweed sites with hybrid-like plants, I used contingency table analysis (i.e. G-tests). Sites with hybrids were denoted as 'yes' and those without as 'no.' (2) To determine if the proportion of hybrid-like plants within diffuse knapweed sites differed between regions, I used Oneway ANOVA with region as a fixed effect and the proportion of hybrid-like plants within a site as the response variable.

Performance Comparisons

To determine if performance differed between diffuse-like and hybrid-like individuals, I compared the following traits from plants within the 15 North American diffuse knapweed field sites sampled in 2005: plant diameter, plant height, number of stems, and seed length from the 2006 North American collections. Residuals were inspected for normality and heteroscedasticity, and diameter, number of stems +1, and height were all log-transformed prior to analysis. The presented values have been back-transformed. I analyzed these traits with a mixed model in SAS (PROC MIXED) with population as a random effect and classification as a fixed effect (i.e. diffuse-like or hybrid-like). I used a mixed model in SAS (PROC GLIMMIX) with the same main effects to compare the proportion of diffuse-like and hybrid-like individuals that (1) exhibited polycarpy (i.e. presence of the previous year's flowering stalks) and (2) had a seed pappus.

On-going hybridization was detected in some of the 2006 Ukraine sites (see below). Based on the groups found by the cluster analysis (see above), I examined plant size (plant diameter, plant height, stem number) and seed length among the groups with a mixed model in SAS (PROC MIXED) with population as a random effect and plant type as fixed effect (i.e. spotted-like, diffuse-like, or hybrid-like). If the model was significant, I used Tukey-Kramer post-hoc tests to determine which groups differed. Again, plant height, diameter, and stem number + 1 were log transformed prior to analysis. I compared the proportion of individuals from the three groups that (1) exhibited polycarpy and (2) had a seed pappus with a model with the same main effects (PROC GLIMMIX), and as above, if the model was significant, I used Tukey-Kramer post-hoc tests to determine which classes differed. Finally, I calculated the coefficient of variation (CV) to discern whether hybrid-like plants in the Ukraine are inherently more variable for quantitative traits than the parental-like plants (CV = standard deviation/mean X 100).

As hybrid-like individuals were not detected in the other countries surveyed in Europe in 2005 and 2006, similar analyses were not conducted for those sites.

To determine if diffuse knapweed performance differed between the native and introduced ranges, I compared measures of size (plant diameter, plant height, and stem number) and seed length between diffuse knapweed sites in Europe lacking hybrid individuals (n = 7 sites, 5 in Romania, 2 in the Ukraine) to the assayed admixed North American diffuse knapweed sites sampled in 2005 (n = 15 sites). For these analyses I combined diffuse-like and hybrid-like individuals from North America in one group, as I was interested in the overall differences at the continent scale instead of among morphological variants within a site. All of the size measures were log-transformed, and as above, the reported means have been back-transformed. I analyzed these traits with a mixed model in SAS (PROC MIXED) with population nested within continent as a random effect and continent as a fixed effect (i.e. North America or Europe). I used a model with the same main effects (PROC GLIMMIX) to compare the proportion of North American vs. European individuals that (1) exhibited polycarpy and (2) had a seed pappus.

RESULTS

Field Surveys of Spotted and Diffuse Knapweed

1) Do floral and seed traits provide a consistent way to distinguish among hybrid and parental species?

For the North American floral data, the Ward's phenogram revealed three major clusters (Fig. 1.4). Cluster 1 was comprised largely of what I classified as hybrid-like in the field (73.8% hybrid), cluster 2 contained only individuals I classified as diffuse-like in the field (100%), and cluster 3 was comprised mainly of individuals I classified as spotted-like (98.8%). I used these three clusters to categorize individuals prior to further analyses. I lacked a complete set of floral data on 284 individuals, so I grouped them based on the observational field classification. This approach was validated since I correctly grouped individuals 92% of the time when compared to the cluster groups (i.e. a plant I grouped as spotted was placed in the cluster dominated by spotted).

The canonical discriminant analysis with six floral traits from North American plants proved instructive for discerning which traits contributed the most in distinguishing amongst the three plant types. Canonical dimension 1 described 92.05% of the variation in the data set (eigen-value = 93.01), while canonical dimension 2 described 7.9% of the variation (eigen-value = 8.0). For both canonical dimension 1 and 2, bract shading and flower color contributed the most weight towards describing the variation in the data set (Fig. 1.5). However, certain flower color values (1-5) and bract shading (0-3) contributed differentially to each canonical dimension.

Seeds from diffuse-like versus hybrid-like plants measured in 14 sites across western North America in 2006 did not differ in length ($F_{1,232} = 0.69$, $P = 0.41$; diffuse-like = 2.58 ± 0.03 mm vs. hybrid-like 2.60 ± 0.03 mm). Additionally, a similar proportion of the two plant types had pappae ($F_{1,243} = 0.10$, $P = 0.75$; diffuse-like = 0.29 vs. hybrid-like = 0.30).

For the Ukrainian floral data, the hierarchical cluster analysis also revealed three major clusters (Fig. 1.6). Cluster 1 contained individuals that I classified as diffuse-like in the field (74%), cluster 2 was comprised only of hybrid-like plants (100%), and cluster 3 contained a majority of plants classified as spotted-like (98%). My field classification matched the groups from the cluster analysis 81% of the time. The 211 individuals not included in the analysis because of missing data were therefore grouped based on observational field classification.

Seed length did not differ significantly among the three Ukranian plant types ($F_{2,219} = 0.99$, $P = 0.37$). The presence of pappea did differ among the classes ($F_{2,222} = 3.98$; $P = 0.02$). Individuals in the diffuse-like cluster had significantly fewer seeds with pappae than individuals in the spotted-like cluster (Fig. 1.7).

A comparison of the floral and seed traits between the North American admixed sites and the European diffuse knapweed sites lacking hybrids provided additional support for introgression from spotted knapweed in North American plants. All of the quantitative floral traits differed between continents. Capitula width ($F_{1,17} = 35.93$, $P<0.0001$), capitula length ($F_{1,17} = 8.76$, $P = 0.009$), and spine length ($F_{1,17} = 12.25$, $P = 0.003$) were all significantly larger for North American plants (Fig. 1.8), as predicted with introgression from spotted knapweed. When hybrid-like plants were removed from these analyses, the results did not change (data not shown). Seeds from the 13 diffuse knapweed sites collected across western North America in 2006 did not differ in length from the seeds collected from the morphologically typical diffuse knapweed sites in Europe ($F_{1,18} = 0.90$, $P = 0.36$; North America = 2.59 ± 0.14 mm vs. Europe 2.82 ± 0.20 mm); however, a significantly smaller proportion of European seeds had pappae ($F_{1,20} = 9.62$, $P = 0.0056$) (Fig. 1.9), also expected if North American diffuse knapweed plants contain introgression from spotted knapweed.

2) How common are hybrid plants in the native and introduced ranges? Is the pattern of hybridization in North America similar to that found in the native range?

In North America in 2005 and 2006, 39 of the 40 surveyed diffuse knapweed sites contained at least some hybrid-like individuals (Table 1.3). Four of the 22 spotted knapweed sites contained a handful of aberrant individuals that had white flowers and golden bracts, which seemed like variants of spotted knapweed rather than hybrids. The prevalence of hybrid-like plants in the diffuse knapweed sites led me to focus on that species. The percentage of hybrid-like plants in a diffuse knapweed site ranged from 1 to 95% (Fig. 1.10), with most sites containing less than 20% hybrid-like individuals (median value = 13.5%, mean ± SE = 24 ± 5%).

As predicted, hybridization was not detected in the regions where diploid spotted and diffuse knapweed were not thought to overlap (Austria, Hungary, Switzerland, and Turkey). While I initially expected to encounter hybridization in Romania, none of the spotted knapweed sites contained hybrids and only one of the five diffuse knapweed sites contained hybrid-like individuals (< 5% of the site). If hybridization is on-going in Romania, my surveys did not detect it.

As anticipated, the surveys in the Ukraine in 2006 revealed that spotted and diffuse knapweed overlapped more extensively, with four out of fourteen sites exhibiting a gradient from pure spotted-like to pure diffuse-like (Table 1.3). Such hybrid swarms were not encountered in North America or elsewhere in Europe. Six out of eight of the diffuse knapweed sites lacking spotted knapweed individuals in the Ukraine still contained hybrid-like plants, as seen in North America.

Overall, more North American diffuse knapweed sites contained hybrid-like plants than all of Europe ($G = 11.2$, $P < 0.001$; North America = 0.98; Europe = 0.58). However, when North America was compared to just the Ukraine, where hybridization appeared to be on-going, a similar proportion of sites contained hybrid-like plants ($G = 2.84$, $P = 0.09$; North America = 0.98; Ukraine = 0.83). Interestingly, the proportion of hybrid-like individuals within diffuse knapweed sites was similar in North America and Europe ($F_{1,39} = 0.95$, $P = 0.34$; North America = 0.23 ± 0.07; Europe = 0.34 ± 0.08). Dividing Europe into Central Europe and the Ukraine produced a different outcome. North American diffuse knapweed sites contained a greater proportion of hybrid-like plants than Central Europe ($F_{1,27} = 3.66$, $P = 0.06$), while a smaller proportion of hybrid-like plants was found in North American sites when compared to the Ukraine ($F_{1,34} = 4.54$, $P = 0.04$) (Fig. 1.11). Clearly, the outcome is dependent upon the region of comparison within Europe.

In the survey of the spotted and diffuse knapweed collections in the Kiev N.G. Kholodny Institute of Botany herbarium in 2006, there were 353 collections labeled as *C. diffusa*. Of these collections, 13.9% were plants I would classify as hybrid-like. A majority of these hybrid-like plants were labeled as *C. diffusa*, while several were labeled as *C. diffusa x micranthos*. There were 78 *C. micranthos* (= *stoebe, maculosa, biebersteinni*) specimens, and of those, three (3.8%) exhibited hybrid-like morphology.

3) Do hybrid plants in the field exhibit heterosis? Are heterotic patterns similar between North America and Europe?

I wanted to know if hybrid-like plants within diffuse knapweed sites in North America were larger, or more vigorous, than diffuse-like plants, which might suggest a reason for being maintained. Plant diameter ($F_{1,419} = 1.85$, $P = 0.16$) and number of stems ($F_{1,421} = 1.63$, $P = 0.20$) did not differ between diffuse-like and hybrid-like individuals, while plant height was significantly different ($F_{1,421} = 6.09$, $P = 0.014$); hybrid-like plants were taller than diffuse-like plants (Fig. 1.12). Additionally, more hybrid-like plants exhibited evidence of polycarpy (i.e. flowering across multiple seasons) than diffuse-like plants ($F_{1,420} = 9.45$, $P = 0.002$) (Fig. 1.13).

Vegetative morphological traits were similar among spotted-like, diffuse-like and hybrid-like plants in the Ukraine. Neither plant height ($F_{2,403} = 0.24$, $P = 0.79$), plant diameter ($F_{2,403} = 0.33$, $P = 0.72$), or number of stems ($F_{2,403} = 1.72$, $P = 0.18$) significantly differed among the plant groups. The coefficient of variation (CV) across the three plant types was relatively similar for plant height and diameter, while the spotted-like group had a CV approximately 23% higher for number of stems than the other two groups (Table 1.4). Plant groups differed in the proportion of polycarpy ($F_{2,398} = 5.53$, $P = 0.0043$) (Fig. 1.14). Diffuse-like and hybrid-like plants were less often polycarpic than spotted-like plants.

I compared vegetative data from North American diffuse knapweed sites to the Central European diffuse knapweed sites lacking hybrids to see if the plants from the admixed North American sites were larger. The number of stems did not differ between continents ($F_{1,20} = 3.35$, $P = 0.0823$) (Fig. 1.15A). North American plants were larger than European plants in both plant diameter ($F_{1,20} = 12.4$, $P = 0.0021$) (Fig. 1.15B) and height ($F_{1,20} = 10.50$, $P = 0.0041$) (Fig. 1.15C). As hybrid-like plants were significantly taller than diffuse-like plants in North American sites (Fig. 1.11), I wanted to determine if the presence of hybrid-like individuals resulted in the pattern of North American plants being taller than European plants. I removed all hybrid-like plants from the North American sites and re-ran the analyses. Even with the absence of such individuals, North American plants were still significantly taller than European plants ($F_{1,20} = 9.49$, $P = 0.0059$). More North American plants exhibited polycarpy than European plants, but this trend was not significant ($F_{1,20} = 2.24$, $P = 0.15$; North American = 0.099, European = 0.024).

4) Do offspring retain maternal morphological characters?

All of the seed from the spotted-like plants generated offspring that were morphologically spotted (6/6). When looking at the diffuse-like plants pooled across the four sites (Hood River, Oregon; Estes Park, Colorado; Pendleton, Oregon, and Natrona, Wyoming), 79% of the plants bred true (15/19); the remaining plants exhibited hybrid-like morphology. Hybrid-like plants pooled across the four sites bred true 55% of the time (17/31). The other 45% of the offspring exhibited diffuse-like morphology.

DISCUSSION

Delineation of plant groups by floral characters

The first step in examining hybridization between spotted and diffuse knapweed was to confirm that floral characters could be used to consistently place hybrid-like and parental-like individuals in to non-overlapping clusters. Based on cluster analyses, the combination of four to five floral characters reliably grouped individuals as parent-like or hybrid-like in North America and the Ukraine. Canonical discriminant analysis revealed that flower color and bract shading were the most important characters used in separating the plant types. Thus, floral traits worked well to group plants, and I could then confidently proceed to make comparisons among the various plant groups.

Global patterns of hybridization

Based on the European and North American field surveys of spotted and diffuse knapweed, a complicated story emerges. In their native range, it is clear that in some regions (i.e. the Ukraine), hybridization is currently ongoing and likely results from the distributional overlap of diploid spotted and diffuse knapweed. In other places, hybridization is not detected (i.e. Central Europe and Turkey), and this likely results from regions of non-overlapping distributions of the diploid species. Indeed, in Romania where spotted and diffuse knapweed were both found but hybridization was largely undetected, two of the spotted knapweed sites assayed for ploidy contained tetraploid individuals (H. Müller-Schärer, unpublished data). In the introduced range, hybrid-like plants were not detected within spotted knapweed sites, but instead were found almost ubiquitously at varying frequencies in diffuse knapweed sites. Hybrid-like individuals were not restricted to certain areas of North America, but instead were detected in 39 out of 40 diffuse knapweed sites across western North America. Thus, it seems two scenarios in the introduced range are plausible: 1) hybridization is frequent and on-going, but back-crossing occurs only in the direction of diffuse knapweed, or 2) diffuse knapweed was introduced as a hybrid swarm, and the initial variation included with pure and mixed genotypes has been maintained since introduction at the turn of the century. I will argue this latter scenario matches the evidence most parsimoniously.

It has long been thought that the spotted knapweed in North America is tetraploid, but until recently this statement was based on a small number of samples (Moore and Frankton 1954; Müller 1989). Moore and Frankton (1954) analyzed two spotted knapweed individuals from western North America (British Columbia, Canada and Washington, USA), and both individuals were tetraploid. Chromosome number is the only unambiguous character to discern between diploid subsp. *stoebe* and tetraploid subsp. *micranthos* (Ochsmann 2001). Recently, extensive surveys of spotted knapweed ploidy across North America corroborate that only the tetraploid *C. stoebe* subsp. *micranthos* is present in North America (H. Müller-Schärer, personal communication). Additionally, it has recently been found that North American individuals have up to four alleles at microsatellite loci, which strongly suggests they are tetraploid (R.A. Marrs, unpublished data). As the diffuse knapweed in North America is likely all diploid (A.C. Blair, unpublished data; Marrs et al. 2008; Moore and Frankton 1954), and triploids have never been encountered (Moore and Frankton 1954; H. Müller-Schärer, personal communication), on-going hybridization seems unlikely. Also, I tried to create artificial hybrids through hand-pollinations between spotted and diffuse knapweed individuals from across North America. Although I successfully made North American spotted X spotted, diffuse X diffuse crosses, and European diploid spotted X North American diffuse, I never successfully created an F1 triploid individual (unpublished data), which fits with genetic incompatibilities resulting from spotted being tetraploid and diffuse diploid. Finally, the distributions of spotted and diffuse knapweed rarely overlapped in North America, and when they did co-occur, I did not detect different patterns of hybridization than when I surveyed just diffuse knapweed sites (personal observation). Further, overlapping spotted and diffuse knapweed sites did not resemble the mixed sites in the Ukraine that contained a gradient of morphologically intermediate plants. It therefore seems unlikely that the hybridization detected in the field stems from recent events, and instead, it seems probable that diffuse knapweed was introduced one or multiple times (Hufbauer & Sforza 2008; Marrs et al. 2008) with introgressed individuals. The molecular data support that at least some individuals within most diffuse knapweed sites have a detectable portion of their genome derived from diploid spotted knapweed (Chapter 2).

Moore and Frankton (1954) also noticed these hybrid plants in North American diffuse knapweed sites, but concluded they were not hybrids based on the fact that they were not triploid (i.e. the tetraploid spotted knapweed x the diploid diffuse knapweed), and instead had the same diploid chromosome number as diffuse knapweed. My findings agree that the hybrids in the field are not F1 hybrids between tetraploid spotted and diploid diffuse knapweed, but are instead introgressed individuals that resulted from crosses between diploid spotted and diffuse knapweed prior to introduction. Typically, diffuse knapweed seeds lack a pappus, while spotted knapweed seeds have a 1-2.5 mm pappus (Ochsmann 2000; Watson and Renney 1974). Moore and Frankton (1954) concluded again that hybrid-like plants are not actually hybrid, because there was no consistency with whether or not a hybrid-like plant or a diffuse-like plant bore seeds with pappea. My research again agrees with this finding; hybrid-like individuals were no more likely to have seeds with a pappus than diffuse-like individuals. Cross-continent comparisons however, reveal the presence of hybridization in North America. Plants from the diffuse knapweed sites in Europe lacking hybrids had significantly fewer seeds with pappea than those from the North American diffuse knapweed sites (7.7% versus 30% respectively). Additionally, the presence of diffuse knapweed sites lacking hybrid-like individuals in the native range strongly suggests that the intermediate traits in North America do not simply result from within-species variation, but instead indicate genetic admixture with diploid spotted knapweed.

Patterns of heterosis

The North American plants that clustered as hybrid-like were significantly taller and exhibited polycarpy more often than plants that clustered as diffuse-like. While the surveyed hybrid-like plants were not necessarily more introgressed than those diffuse-like in North America because of the century of backcrossing into diffuse knapweed (Chapter 2), there appears to be some advantages to those that display hybrid floral characters. While not intuitively obvious, it seems plausible that increased height and polycarpy may be linked to the intermediate floral traits. This linkage may explain part of the reason why these traits have been maintained in nearly all diffuse knapweed sites across western North America.

Overall, plants from the North American admixed diffuse knapweed sites were more robust than plants from the pure European diffuse knapweed sites. North American plants had both greater diameter and height than European plants. Whether this enhanced vigor stems from the presence of introgression in North America is speculative, as other factors, including environmental conditions, could influence these traits. On-going experiments are looking at whether hybridization, *per se*, confers a fitness advantage. This pattern does seem to fit a growing trend, however, that introduced organisms are larger than their conspecifics in the native range (Blair and Wolfe 2004; Groholz and Ruiz 2003; Jakobs et al. 2004; Leger and Rice 2003; Siemann and Rogers 2001; but see Thébaud and Simberloff 2001; Willis et al. 2000). A popular idea in the literature to explain this enhanced vigor is the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzold 1995). This hypothesis proposes that when plants are introduced in to a new region, they are freed from their natural predators and parasites; natural selection then favors individuals that invest more energy into growth and reproduction, and less energy towards defense. Blumenthal and Hufbauer (2007) included diffuse knapweed in a multi-species test of the EICA hypothesis and found no evidence of increased growth of introduced compared to native plants in a common garden in the absence of competition. While these results are based on only four populations, it suggests that in my study, diffuse knapweed may be larger in North America because of differential herbivory or other environmental factors, rather than genetically based differences.

In the Ukraine I found that plants from the parental-like and hybrid-like classes did not differ for size-related vegetative morphological traits. Additionally, the coefficients of variation for vegetative traits were not obviously larger for the hybrid-like class compared to the parental-like classes. This finding makes sense in light of the molecular data; even individuals that appeared to be typical diffuse or spotted knapweed in hybrid swarms had significant interspecific admixture (Chapter 2). Thus, all of the classes were comprised at least partly of hybrids and would be expected to be similarly variable. The presence or absence of pappea followed the trend that spotted-like plants made significantly more seed with pappea than diffuse-like plants. As would be expected, the hybrid-like plants made seed with pappea at a frequency between the two parent groups.

Hybrid-like plants do not breed true in North America

Hybrid-like plants in North American diffuse knapweed sites are not acting as a distinct entity. Almost half of the seed collected from hybrid-like plants from four field sites gave rise to plants with diffuse-like morphology, while the other half maintained hybrid characters. Even some of the seed from diffuse-like plants produced offspring with hybrid morphology (21%). While I am uncertain as to the proportion of hybrid-like plants at the Hood River site, such individuals comprised less than ≈ 10% of the site at the other three locations. In sites with such low percentages of hybrid-like individuals, one could assume that diffuse-like pollen would outnumber that of hybrid-like pollen. This is evidenced by the fact that a majority of the seed from diffuse-like plants bred true. Thus, it is quite interesting that hybrid-like plants were still able to produce hybrid-like offspring over half of the time, especially because these plants are self-incompatible (A.C. Blair personal observation; Harrod and Taylor 1995). It seems plausible that certain pollinators might specifically target plants with similar flower color, discriminating against other colors (Waser and Price 1981). For example, Stanton (1987) found that honeybees preferred yellow or white flowers of wild radish, while discriminating against bronze color. Similarly, in that study syphrids preferred pink to other flower colors. Such a pollinator response might result in hybrid-like plants, which are generally purple, being pollinated by other hybrid-like individuals, creating more hybrid-like seed than expected by chance alone. Alternately, pollen from hybrid-like plants might perform superiorly on similar hybrid-like stigmas. These possible mechanisms need further examination before a conclusive answer can be reached. As all of these sites were viewed in a snapshot in time, it would also be enlightening to sample across seasons to see how the number of hybrid-like individuals fluctuates through time.

Conclusions

Hybridization has recently been posited as a mechanism that may stimulate or aid invasiveness (Ellstrand and Schierenbeck 2000). Indeed, some of the most problematic invaders have turned out to be hybrids. Gaskin and Schaal (2002) recently discovered that the invasive *Tamarix* in North America is a hybrid undetected in the native range. The authors suggest that multiple introductions brought together historically isolated genotypes from the native range. *Reynoutria* species are invasive in the Czech Republic, and it was found that an inter-specific hybrid (*R.* x*bohemica*) grew faster than the parental species (Katerina et al. 2004). A final interesting example of hybridization influencing invasion is between a native salamander in California, USA and an introduced salamander (Fitzpatrick and Shaffer 2007). Hybrids showed increased vigor relative to parental species, and it seems possible that the hybrid may eventually replace the threatened California Tiger Salamander.

Based on the examples provided here and elsewhere (e.g. Ellstrand and Schierenbeck 2000), hybridization has the potential to alter invasion dynamics. Diffuse knapweed is larger in North America than Europe, and has attained densities that are ecologically and environmentally damaging in the introduced range (Watson and Renney 1974). Introduced diffuse knapweed individuals may have, by chance, initially had the correct suite of traits to flourish in the new range, including sufficient plasticity, or enough variation for adaptation to occur through natural selection. Pre-introduction hybridization could have influenced both processes by creating more vigorous individuals and/or novel or enhanced variation. Future studies are focusing on comparisons of fitness-related traits between hybrids and parental species in common garden studies to make a more direct causal link between hybridization and invasion by diffuse knapweed.

REFERENCES

Anderson, E. and G. L. Stebbins. 1954. Hybridization as an evolutionary stimulus. Evolution 8:378-388.

Blair, A. C. and L. M. Wolfe. 2004. The evolution of an invasive plant: An experimental study with *Silene latifolia*. Ecology 85:3035-3042.

Blair, A. C., B. D. Hanson, G. R. Brunk, R. A. Marrs, P. Westra, S. J. Nissen, and R. A. Hufbauer. 2005. New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecol. Lett. 8:1039-1047.

Blair, A. C., S. J. Nissen, G. R. Brunk, and R. A. Hufbauer. 2006. A lack of evidence for a role of the putative allelochemical (±)-catechin in spotted knapweed invasion success. J. Chem. Ecol. 32:2327-2331.

Blossey, B. and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. J. Ecol. 83:887-889.

Blumenthal, D. M. and R. A. Hufbauer. 2007. Increased plant size in exotic populations: a common-garden test with 14 invasive species. Ecology 88:2758-2765.

Callaway, R. M. and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290:521-523.

Dobzhansky, T. 1970. Genetics of the Evolutionary Process. Columbia University Press.

Dowling, T. E. and C. L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annu. Rev. Ecol. Syst. 28:593-619.

Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. U.S.A. 97:7043-7050.

Fitzpatrick, B. M. and H. B. Shaffer. 2007. Hybrid vigor between native and introduced salamanders raises new challenges for conservation. Proc. Natl. Acad. Sci. U.S.A. 104:15793-15798.

Fletcher, R. A. and A. J. Renney. 1963. A growth inhibitor found in *Centaurea* spp. Can. J. Plant Sci. 43:475-481.

Garcia-Jacas, J., T. Uysal, K. Romashchenko, V. N. Suarez-Santiago, K. Ertugrul, and A. Susanna. 2006. *Centaurea* revisited: a molecular survey of the *Jacea* group. Ann. Bot. 98:741-753.

Gaskin, J. F. and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proc. Natl. Acad. Sci. U.S.A. 99: 11256-11259.

Gáyer, G. 1909. Vier neue Centaureen der Flora Botanikai von Ungarn. Magyar Botanikai Lapok 8:58-61.

Grosholz, E. D. and G. M. Ruiz. 2003. Biological invasions drive size increases in marine and estuarine invertebrates. Ecol. Lett. 6:705-710.

Harrod, R. J., R. J. Taylor. 1995. Reproduction and pollination biology of *Centaurea* and *Acroptilon* species, with emphasis on *C. diffusa*. NW. Sci. 69:97-105.

Hufbauer, R. A. and R. Sforza. 2008. Multiple introductions of two invasive *Centaurea* taxa inferred from cpDNA haplotypes. Diversity and Distributions. DOI: 10.1111/j.1472-4642.2007.00424.x.

Jakobs, G., E. Weber, and P. J. Edwards. 2004. Introduced plants of the invasive *Solidago gigantean* (Asteraceae) are larger and grow denser than conspecifics in the native range. Diversity and Distributions 10:11-19.

JMP Statistics and Graphing Guide, Version 5. (2002) SAS Institute Inc., Cary, NC, USA.

Katerina, B., B. Mandak, and I. Kasparova. 2004. How does *Reynoutria* invasion fit the various theories of invasibility? J. Veg. Sci. 15:495-504.

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

Leger, E. A. and K. J. Rice. 2003. Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced compeition. Ecol. Lett. 6:257-264.

Lexer, C., M. E. Welch, O. Raymond, and L. H. Rieseberg. 2003. The origin of ecological divergence in *Helianthus paradoxus* (Asteracea): selection on transgressive characters in a novel hybrid habitat. Evolution 57:1989-2000.

Locken, L. J. and R. G. Kelsey. 1987. Cnicin concentrations in *Centaurea maculosa*, spotted knapweed. Biochem. Systemat. Ecol. 15:313-320.

Löve, A., 1978. IOPB Chromosome Number Reports LIX. Taxon 27, 53-61.

Löve, A., 1979. IOPB Chromosome Number Reports LXIV. Taxon 28, 391-408.

Marrs, R. A., R. Sforza, and R. A. Hufbauer. 2008. When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biological Invasions. DOI 10.1007/s10530-007-9153-6.

Mayr, E. 1963. *Animal Species and Evolution.* Harvard University Press.

Moore, R. J., and C. Frankton. 1954. Cytotaxonomy of three species of *Centaurea* adventive in Canada. Can. J. Bot. 32:182-186.

Müller, H. 1989. Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.:Cochylidae). Weed Res. 29:103-111.

Nolte, A. W., J. Freyhof, K. C. Stemshorn, and D. Tautz. 2005. An invasive lineage of sculpins, *Cottus* sp (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. Proc. R. Soc. London Ser. B 272:2379-2387.

Ochsmann, J. 1998. Ein bestand von *Cenaurea* x*psammogena* Gáyer (*Centaurea diffusa* Lam. x *Centaurea stoebe* L.) am NSG Sonnenstein (Thüringen). Florist. Rundbr. 31:118-125.

Ochsmann, J. 1999. Chromosomenzahlen einiger europäischer *Centaurea*-Sippen. Haussknechtia 7:59-65.

Ochsmann, J. 2000. Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. - Diss. Bot. 324 (Ph.D. Dissertation).

Ochsmann, J. 2001. On the taxonomy of spotted knapweed (*Centaurea stoebe* L.). Pages 33-41 in L. Smith, ed. The First International Knapweed Symposium of the Twenty-First Century. Couer d'Alene, ID.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17:230-237.

Rieseberg, L. H. and N. C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization? Crit. Rev. Plant Sci. 12:213-241.

Rieseberg, L. H., S. C. Kim, R. A. Randell, K. D. Whitney, B. L. Gross, C. Lexer , and K. Clay. 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129:149-165.

Roché, B. F. and C. T. Roché. 1991. Identification, introduction, distribution, and economics of *Centaurea* species. Pages 274-291 in L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child, eds. Noxious Range Weeds. Boulder, CO: Westview Press.

Rosenthal, S. S., G. Campobasso, L. Fornasari, R. Sobhian, C. E. Turner. 1991.

Biological control of *Centaurea* spp. Pages 292-302 in L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child, eds. Noxious Range Weeds. Boulder, CO: Westview Press.

Sheley, R. L., B. E. Olson, and L. L. Larson. 1997. Effect of weed seed rate and grass defoliation level on diffuse knapweed seedlings. J. Range Manage. 50:39-43.

Sheley, R. L., J. S. Jacobs, and M. L. Carpinelli. 1999. Spotted knapweed. Pages 350-361 in R. L. Sheley and J. K. Petroff, eds. Biology and Management of Noxious Rangeland Weeds. Oregon State University Press, Corvallis.

Siemann, E. and W. E. Rogers. 2001. Genetic differences in growth of an invasive tree. Ecol. Lett. 4:514-518.

Stanton, M. L. 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: I. Pollinator response to color morphs. Am. J. Bot. 74:178-187.

Thebaud, C. and D. Simberloff. 2001. Are plants really larger in their introduced ranges? Am. Nat. 157:231-236.

Thompson, J. D. 1991. The biology of an invasive plant. BioScience 41:393-401.

Tyser, R. W. and C. H. Key. 1988. Spotted knapweed in natural areas fescue grasslands – an ecological assessment. NW. Sci. 62:151-160.

Waser, N. M. and M. V. Price. 1981. Pollinator choice and stabilizing selection for flower color *Delphinium nelsonii*. Evolution 35:376-390.

Watson A. K. and A. J. Renney. 1974. The biology of Canadian weeds: *Centaurea diffusa* and *C. maculosa*. Can. J. Plant Sci. 54:687-701.

Willis, A. J., J. Memnot, and R. I. Forrester. 2000. Is there evidence for the post-invasvion evolution of increased size among invasive plant species? Ecol. Lett. 3:275-283.

Table 1.1 Floral characters used to delineate diffuse knapweed (*Centaurea diffusa*), spotted knapweed (*C. stoebe*), and diffuse *x* spotted knapweed hybrids (*C.* x*psammogena*) in North America (Watson and Renney 1974; Ochsmann 2000). Spotted knapweed plants are likely tetraploid (subsp. *micranthos*), diffuse knapweed diploid, and hybrids diploid. Bold phrasing and numbers in the table represent findings from this study.

Species	Flower Color	Phyllary Appendages	Bract Shading	Capitula Width and Length	Achene Size Pappus
Diffuse knapweed	white, rarely pink	distinct terminal spine, ≈ 3 mm, **range 2.2 to 4.3 mm**	pale yellowish-green, **golden**	**W – 3.0 to 6.8 mm** **L – 5.5 to 12.0 mm**	small (2-3 mm) **(2-3.2 mm)** pappus absent to rudimentary
Spotted knapweed†	purple, rarely white	4-7 fringes per side, terminal spine absent	black triangular pointy tip	**W – 3.0 to 8.8 mm** **L – 6.2 to 12.6 mm**	large (3-3.5 mm) **(2.4-3.6 mm)** pappus persistent, 1-2.5 mm
Spotted x Diffuse hybrids	whitish, purple, **lavender**, or mixed	distinct terminal spine, ≈ **3 mm, range 1.7 to 4.3 mm**	light to dark tip, variable	**W – 2.6 to 6.8 mm** **L – 5.9 to 10.0 mm**	intermediate (2.5-3.5 mm) **(2.0-3.0 mm)** pappus absent to rudimentary

† While these trait descriptions refer specifically to the North American tetraploid spotted knapweed, diploid European spotted knapweed traits overlap extensively (see Ochsmann 2001 for a comparison of floral morphology between the sub-species). Chromosome counts are the only way to definitively separate the two sub-species (Ochsmann 2001).

Table 1.2. The spotted and diffuse knapweed sites surveyed in Europe and North America in 2005 and 2006. Species overlap refers to whether or not I anticipated diploid spotted and diploid diffuse knapweed distributional overlap (yes = expected overlap, no = expected disjunct distributions, and ? = unknown prior to surveys). Surveys were either (1) comprehensive with a visual survey of the entire site for hybrid-like plants, in addition to taking detailed morphological measurements on a subset of plants, or (2) simply a visual survey of the site for hybrid-like plants. DK = diffuse knapweed, SK = spotted knapweed, H = plants with hybrid-like morphology.

Location	Species	GPS	Species overlap	Survey type
2005 Europe				
Romania 3	DK	N43°54'8.76" E28°34'26.1"	?	Comprehensive
Romania 4	DK	N44°23'22.8" E28°31'35.9"	?	Comprehensive
Romania 5	DK	N44°94'34.3" E28°91'4.9"	?	Comprehensive
Romania 6	DK	N45°11'8.8" E28°47'8.3"	?	Comprehensive
Romania 7	DK	N45°29'52.3" E27°54'42.5"	?	Comprehensive
Austria 1	SK	N47°53'3" E16°16'40.9"	No	Comprehensive
Hungary 1	SK	N46°48'1.7" E17°12'20.0"	No	Comprehensive
Romania 2	SK	N47°13'59.9" E26°30'57.4"	?	Comprehensive
Romania 8	SK	N47°28'30.3" E26°16'6.0"	?	Comprehensive
Romania 9	SK	N46°21'33.1" E25°47'38.7"	?	Comprehensive
2005 North America				
CO 1	DK + H	N39°40'17.0" W102°33'1.3"	?	Comprehensive
CO 2	DK + H	N39°39'21.0" W102°33'18.6"	?	Visual
CO 3	DK + H	N39°40'30.5" W102°33'22.3"	?	Comprehensive
CO 4	DK + H	N40°06'44.8" W103°11'27.6"	?	Comprehensive
CO 43	DK + H	N39°42'10.2" W106°40'32.8"	?	Comprehensive
CO 46	DK + H	N39°37'18.0" W106°28'17.9"	?	Visual
CO 47	DK + H	N39°41'48.5" W105°11'32.7"	?	Comprehensive
CO 44	SK	N39°39'27.0" W106°38'13.3"	?	Visual
CO 45	SK	N39°39'22.3" W106°35'59.9"	?	Comprehensive
ID 29	DK + H	N43°24'52.5" W114°52'17.3"	?	Visual

ID 30	SK	N43°18'16.6" W114°48'6.4"	?	Comprehensive
ID 27	SK	N43°38'35.5" W116°15'19.7"	?	Comprehensive
ID 28	SK	N43°32'55.0" 116°09'38.3"	?	Visual
ID 31	SK	N46°02'37.1" 112°48'52.2"	?	Visual
MT 32	SK	N45°46'23.7" W109°47'56.8"	?	Comprehensive
MT 33	SK	N46°04'29.3" W109°56'8.7"	?	Comprehensive
MT 34	SK	N45°41'21.8" W108°46'17.2"	?	Comprehensive
MT 35	SK	N45°44'26.9" W108°31'56.9"	?	Comprehensive
NY 48	SK	N44°16'42" W73°31'52.6"	?	Comprehensive
NY 49	SK	N42°17'33.9" W76°42'49.0"	?	Comprehensive
Ontario 50	SK	N43°20'58.5" W80°06'43.6"	?	Comprehensive
OR 21	DK + H	N45°54'58.8" W119°33'31.8"	?	Comprehensive
OR 22	DK	N45°40'26.0" W118°49'15.5"	?	Visual
OR 24	DK + H	N45°14'14.6" W120°11'6.5"	?	Visual
OR 25	DK + H	N45°36'17.1" W121°11'2.3"	?	Comprehensive
OR 26	DK + H	N45°35'53.3" W121°08'19.5"	?	Visual
OR 23	SK	N45°18'6.8" W120°11'7.4"	?	Visual
WA 5	DK + H	N46°34'58.9" W102°28'15.8"	?	Visual
WA 6	DK + H	N46°35'6.7" W120°27'33.0"	?	Comprehensive
WA 7	DK + H	N46°24'0.34" W120°16'46.3"	?	Visual
WA 8	DK + H	N46°35'6.4" W120°28'7.0"	?	Visual
WA 9	DK + H	N46°32'32.5" W120°27'39.6"	?	Comprehensive
WA 11	DK + H	N47°33'40.4" W120°16'11.3"	?	Comprehensive
WA 12	DK + H	N47°22'32.6" W120°14'59.0"	?	Visual
WA 13	DK + H	N47°28'14.4" W120°20'11.5"	?	Comprehensive
WA 15	DK + H	N47°35'56.2" W120°39'4.5"	?	Visual
WA 16	DK + H	N47°29'6.0" W120°18'0.2"	?	Visual
WA 20	DK + H	N46°43'16.9" W117°09'50.5"	?	Comprehensive

WA 10	SK + DK + H	N46°32'7.4" W120°52'2.6"	?	Visual
WA 14	SK	N47°36'31.3" W120°52'46.5"	?	Visual
WA 17	SK	N46°44'47.9" W117°10'30.3"	?	Comprehensive
WA 18	SK	N46°44'14.4" W117°03'4.0"	?	Visual
WA 19	SK	N46°44'27.3" W117°11'42.4"	?	Visual
WY 39	DK + H	N43°50'13.7" W106°52'28.6"	?	Comprehensive
WY 40	DK + H	N42°53'11.5" W106°26'24.8"	?	Visual
WY 41	DK + H	N43°23'7.9" W107°03'45.6"	?	Comprehensive
WY 36	SK	N44°30'3.7" W109°11'27.9"	?	Visual
WY 37	SK	N44°29'59.9" W109°12'44.3"	?	Comprehensive
WY 38	SK	N44°22'38.7" W106°42'37.2"	?	Comprehensive
WY 42	SK	N42°54'14.6" W106°27'9.7"	?	Comprehensive
2006 Europe				
UA 2	DK + H	N48°38'45.7" E30°46'30.3"	Yes	Comprehensive
UA 3	DK + H	N48°52'18.2" E30°42'40.3"	Yes	Comprehensive
UA 5	DK	N48°48'22.8" E30°33'54.9"	Yes	Comprehensive
UA 7	DK + H	N48°30'42.7" E37°44'10.3"	Yes	Comprehensive
UA 9	DK	N47°51'43.2" E38°27'38.5"	Yes	Comprehensive
UA 10	DK + H	N48°06'02.4" E37°48'58.0"	Yes	Comprehensive
UA 11	DK + H	N48°09'8.4" E37°50'26.1"	Yes	Comprehensive
UA 12	DK + H	N48°00'43.0" E37°47'16.5"	Yes	Comprehensive
UA 4	SK + DK + H	N48°53'31.2" E30°40'33.2"	Yes	Comprehensive
UA 6	SK + DK + H	N48°34'51.9" E37°54'36.9"	Yes	Comprehensive
UA 13	SK + DK + H	N50°29'1.2" E30°30'25.4"	Yes	Comprehensive
UA 14	SK + DK + H	N50°28'50.7" E30°29'10.7"	Yes	Comprehensive
UA 1	SK	N48°38'45.7" E30°46'30.3"	Yes	Comprehensive
UA 8	SK	N47°48'24.7" E38°33'10.0"	Yes	Comprehensive
Switzerland	SK	N47°16'58.5" E8°08'51.9"	Yes	Comprehensive

Istanbul, Turkey 1	DK	N/A	No	Visual
Istanbul, Turkey 2	DK	N/A	No	Visual
2006 North America				
CO 1	DK + H	N39°56'31.1" W104°51'43.1"	?	
CO 2	DK + H	N39°52'02.5" W104°55'30.8"	?	Visual
CO 3	DK + H	N39°24'36.7" W104°52'12.0"	?	Visual
CO 4	DK + H	N39°20'23.8" W104°49'53.3"	?	Visual
CO 5	DK + H	N40°22'09.5" W105°31'54.2"	?	Visual
OR 1	DK + H	N44°50'34.6" W120°54'01.7"	?	Visual
OR 2	DK + H	N45°14'35.7" W120°10'55.5"	?	Visual
OR 3	DK + H	N45°20'34.4" W119°32'58.1"	?	Visual
OR 4	DK + H	N45°47'28.2" W120°01'51.8"	?	Visual
OR 5	DK + H	N45°41'01.9" W121°24'08.3"	?	Visual
OR 6	DK + H	N45°38'08.8" W122°45'24.4"	?	Visual
OR 7	DK + H	N45°15'14.9" W121°09'05.8"	?	Visual
WY 1	DK + H	N41°04'55.1" W105°29'06.2"	?	Visual

Table 1.3. Patterns of hybridization across the global distribution of spotted and diffuse knapweed.

Pattern of Hybridization	North America	Central Europe + Turkey	Ukraine
Spotted Knapweed	No hybrids detected. Surveyed sites likely tetraploid. **(0 out of 22 sites, 0%)**	No hybrids detected. **(0 out of 6 sites, 0%)**	Hybrid swarms with diffuse knapweed - many intermediate individuals. **(4 out of 6 sites, 67%)**
Diffuse Knapweed	Hybrid individuals detected in nearly all sites. Surveyed sites likely diploid. **(39 out of 40 sites, 98%)**	< 5% hybrid-like detected in one out of seven sites. **(1 out of 7 sites, 20%)**	Hybrid swarms with spotted knapweed - many intermediate individuals. **(10 out of 12 sites, 83%)**

Table 1.4. A comparison of the coefficient of variation (CV) between diffuse-like, hybrid-like, and spotted-like plants within Ukrainian field sites for vegetative and morphological traits. The highest CV is bold.

Vegetative Trait	**Diffuse-like**	**Hybrid-like**	**Spotted-like**
Diameter	16.6%	20.3%	**22.4%**
Number of stems	43.9%	45.0%	**67.9%**
Height	9.1%	**10.8%**	9.6%
Floral Trait			
Capitula width	17.8%	**23.5%**	10.6%
Capitula length	16.8%	**20.7%**	13.7%
Spine length	17.6%	**49.8%**	0

Figure 1.1. Floral morphology of A) spotted knapweed, B) spotted x diffuse hybrid, and C) diffuse knapweed. Spotted bracts are diagnostic for spotted knapweed, while apical spines and golden bracts are diagnostic for diffuse knapweed. Note the spotted bracts with terminal spines in the hybrid. Flower color is a less reliable indicator of species status, but hybrids generally have both purple and white flowers.

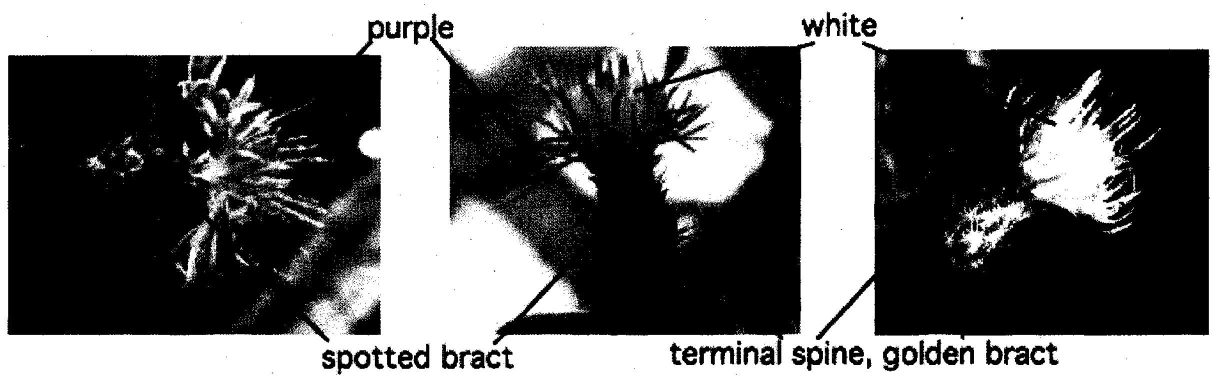


Figure 1.2. Map of sites for the field survey of spotted (SK) and diffuse knapweed (DK) conducted across North America in 2005 and 2006.

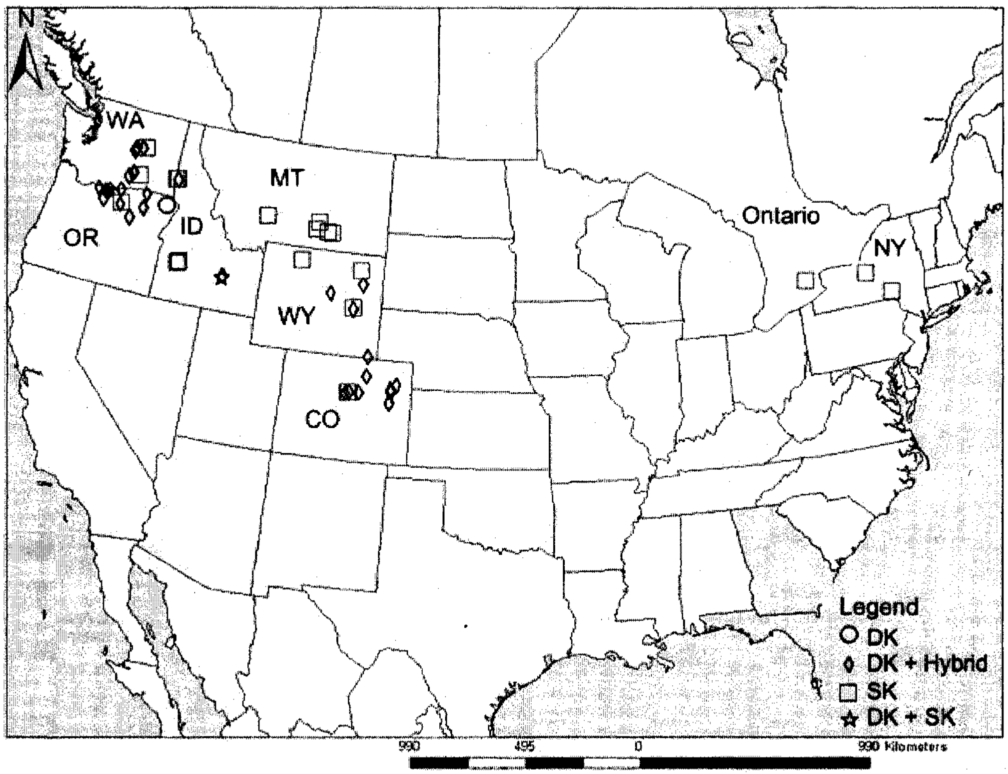


Figure 1.3. Map of sites for the field survey of spotted (SK) and diffuse knapweed (DK) conducted across Europe in 2005 and 2006.

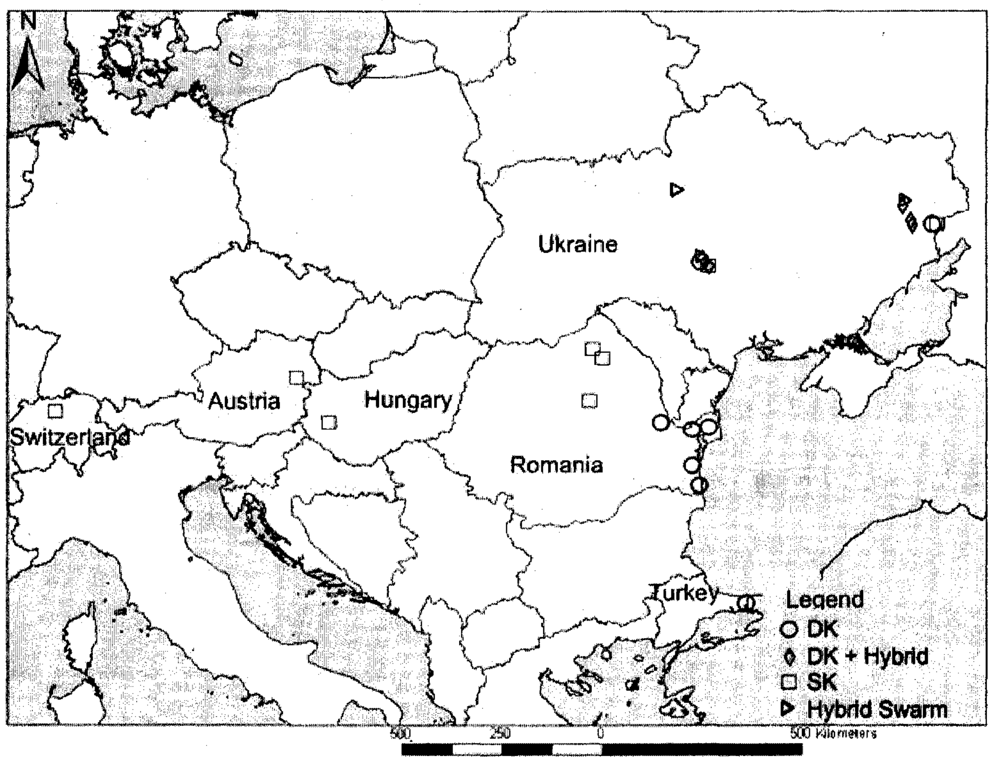


Figure 1.4. A phenogram from hierarchical cluster analysis (Ward's method) of spotted-like, diffuse-like, and hybrid-like individuals from 12 spotted knapweed and 11 diffuse knapweed sites across North America surveyed in 2005. Five morphological floral characters were analyzed. The boxes show how individuals in each cluster were classified based on field observations. See text for details.

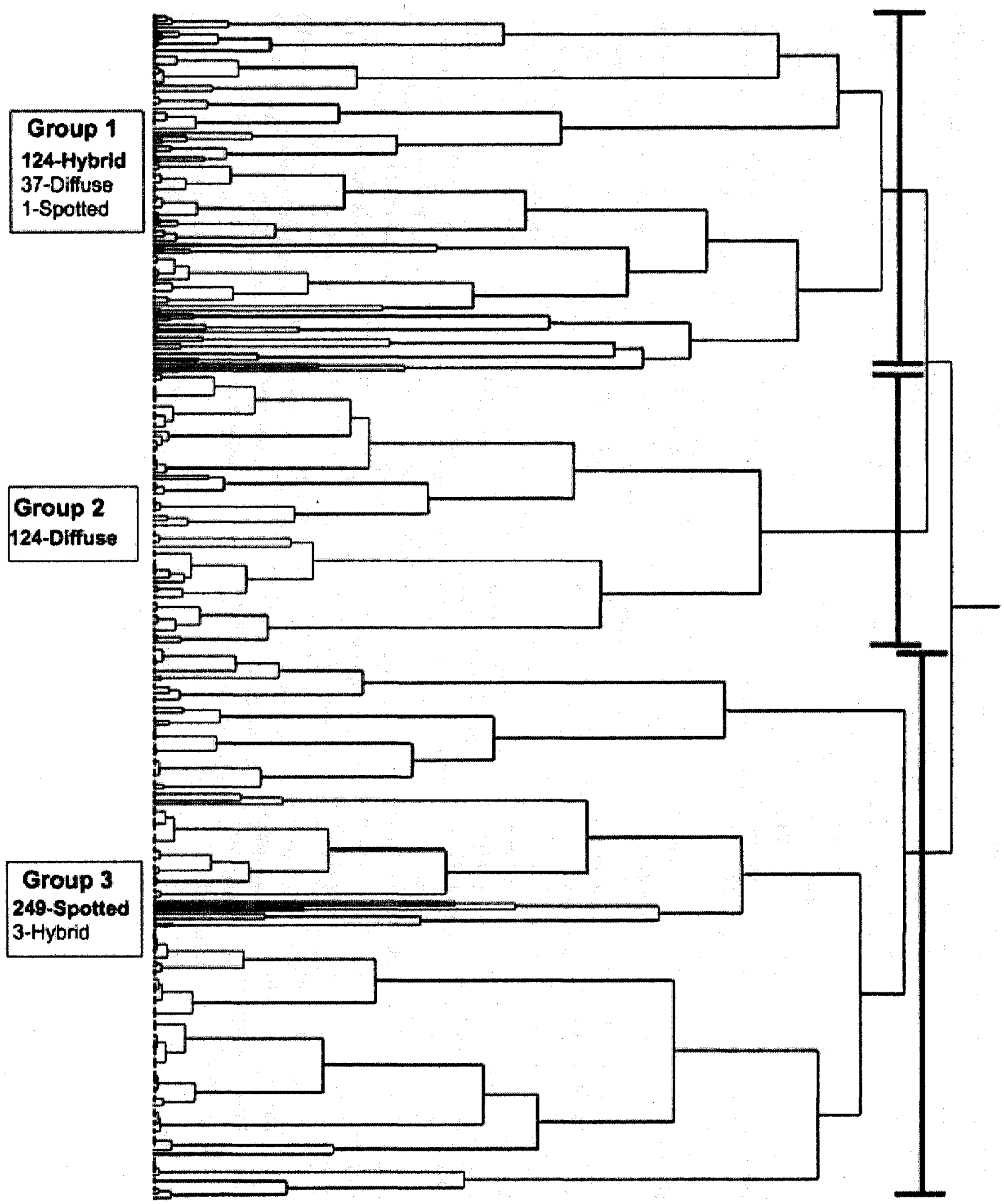

Group 1
124-Hybrid
37-Diffuse
1-Spotted
Group 2
124-Diffuse
Group 3
249-Spotted
3-Hybrid

Figure 1.5. A plot of the first two canonical variables from a canonical discriminant analysis with the following floral characters: flower color, capitula width, capitula length, spine length, presence or absence of vertical veins on bracts, and bract shading (from golden to darkly spotted). Canonical dimension 1 described 92.05% of the variation, while canonical dimension 2 described 7.9% of the variation. Based on field classification, red triangles = diffuse-like, open circles = hybrid-like, and green squares = spotted-like.

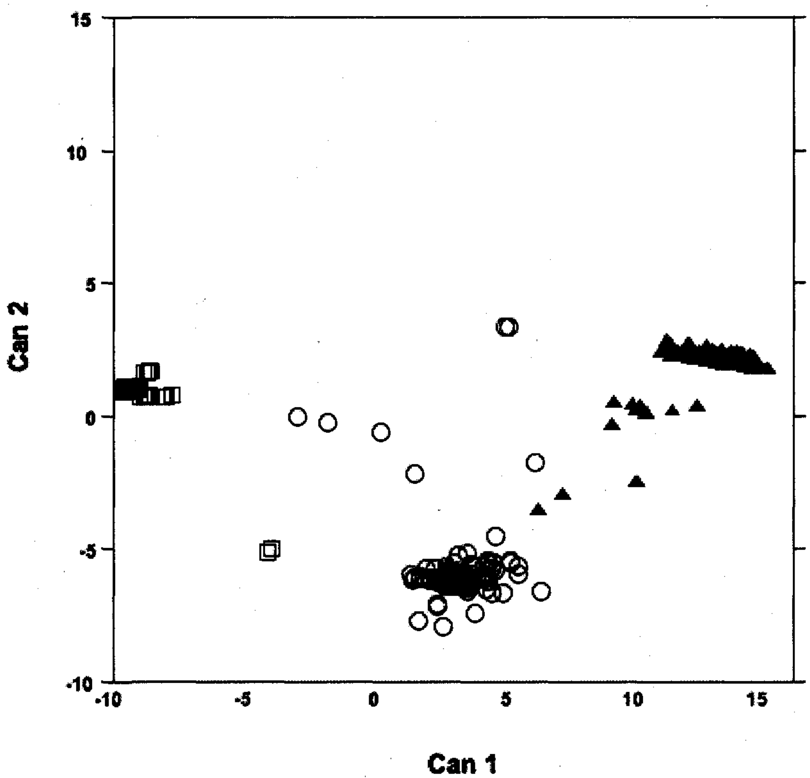

15
10
5
0
-5
-10
Can 2
-10
-5
0
5
10
15
Can 1

Figure 1.6. A phenogram from hierarchical cluster analysis (Ward's method) of spotted-like, diffuse-like, and hybrid-like individuals from 11 sites in the Ukraine surveyed in 2006. Four morphological floral characters were analyzed. The boxes show how individuals in each cluster were classified based on field observations. See text for details.

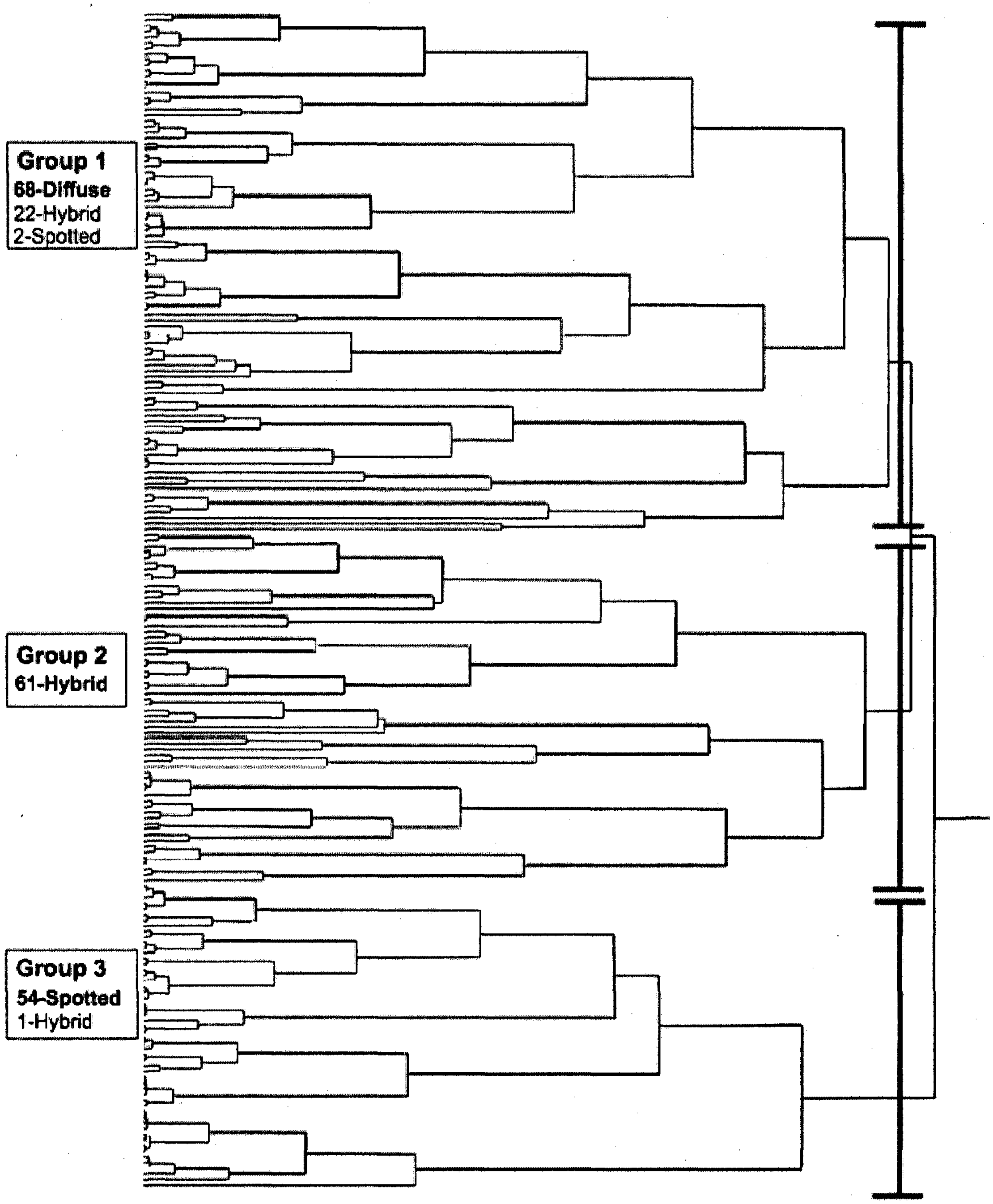

Group 1
68-Diffuse
22-Hybrid
2-Spotted
Group 2
61-Hybrid
Group 3
54-Spotted
1-Hybrid

Figure 1.7. The proportion of diffuse-like, hybrid-like, and spotted-like field-collected seed from the Ukraine with a pappus. Different letters denote significantly different means (Tukey's test P<0.05). †P<0.1

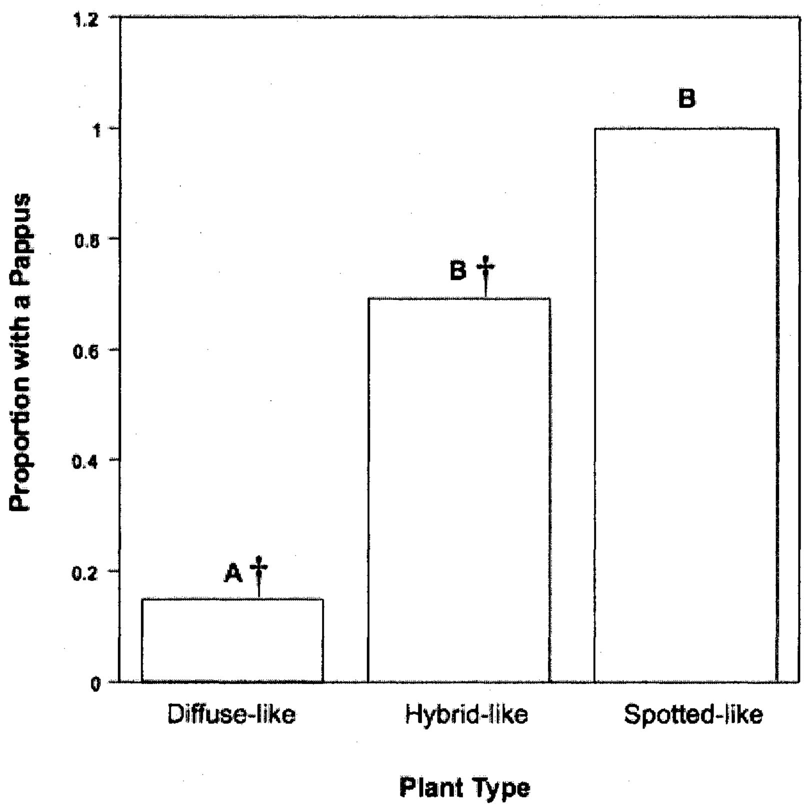


Figure 1.8. Differences between North American and European field-surveyed diffuse knapweed for A) capitula width, B) capitula length, and C) terminal spine length. Values are lsmeans ± SE. *P<0.01; **P<0.0001.

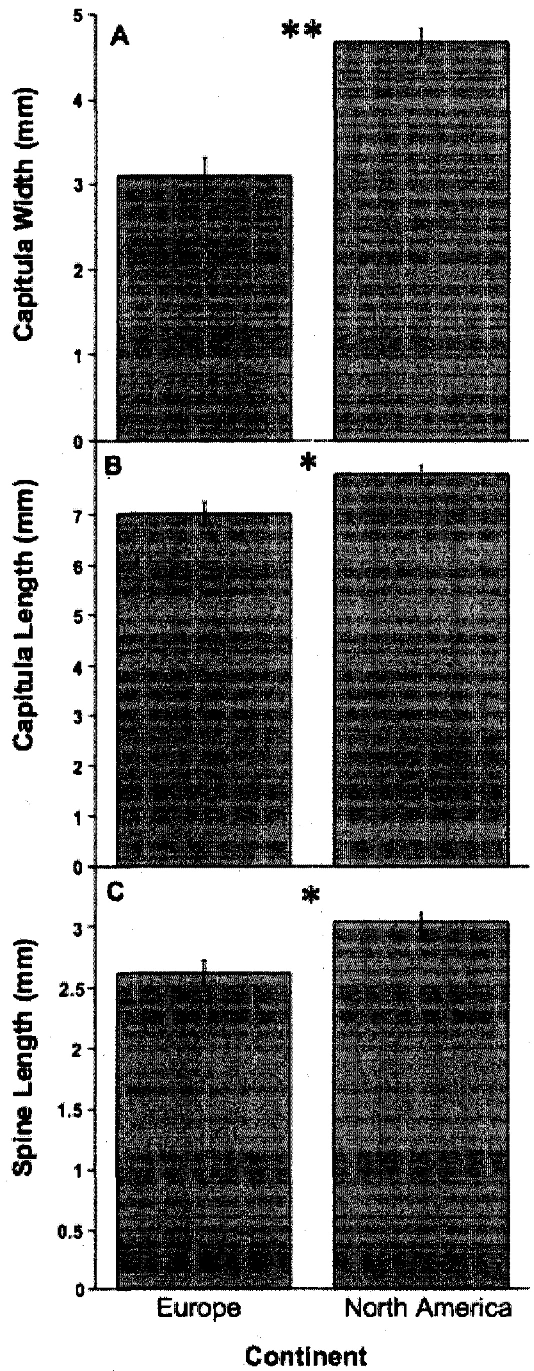


Figure 1.9. The proportion of seeds with a pappus from European versus North American diffuse knapweed sites. *P<0.01.

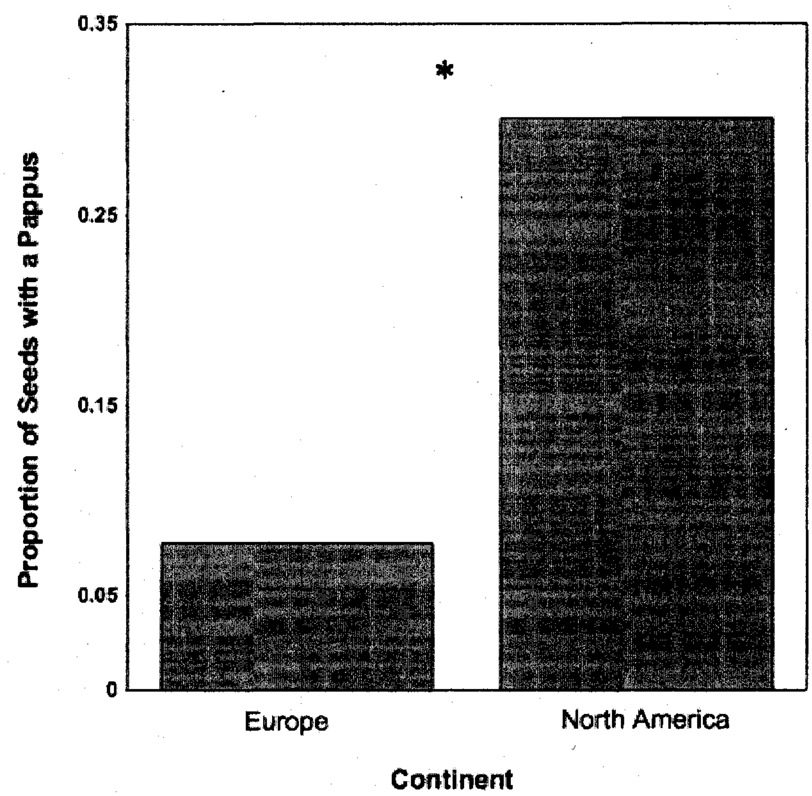


Figure 1.10. The proportion of hybrid-like individuals within diffuse knapweed sites in North America. Sites were surveyed in 2005 and 2006.

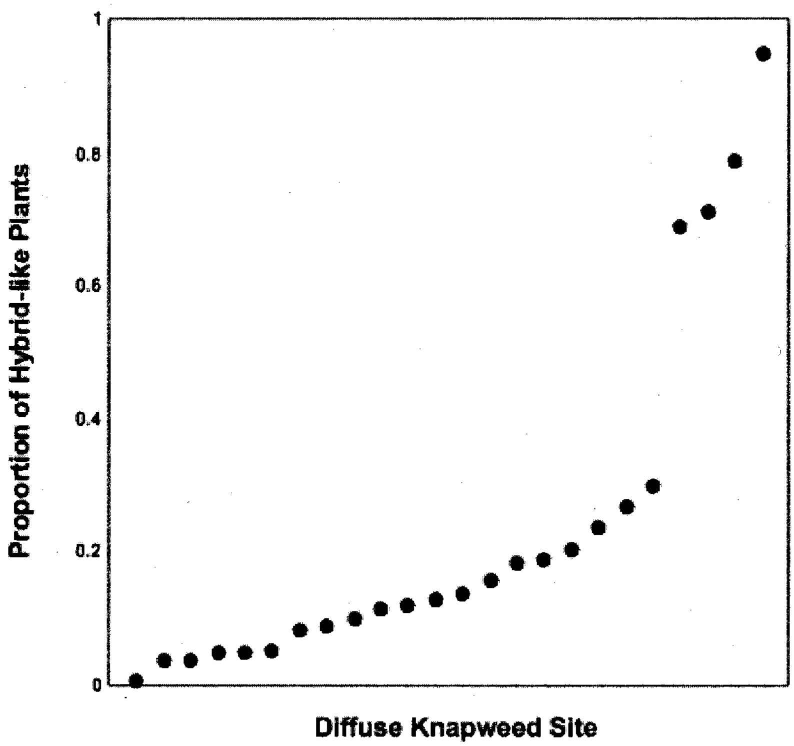


Figure 1.11. The proportion of hybrid-like plants found within diffuse knapweed sites in North America, Central Europe, and the Ukraine. Values are least square mean ± 1SE.

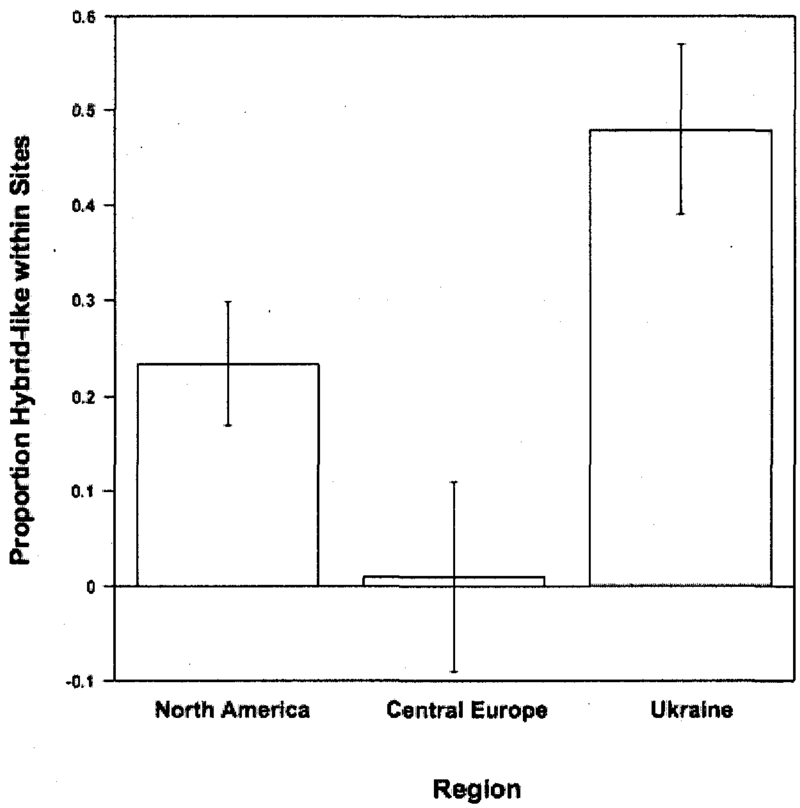


Figure 1.12. Differences in plant height (mean ± SE) between North American field-surveyed diffuse-like and hybrid-like plants. Plant height was ln transformed prior to analysis; reported values are back-transformed. *$P < 0.05$.

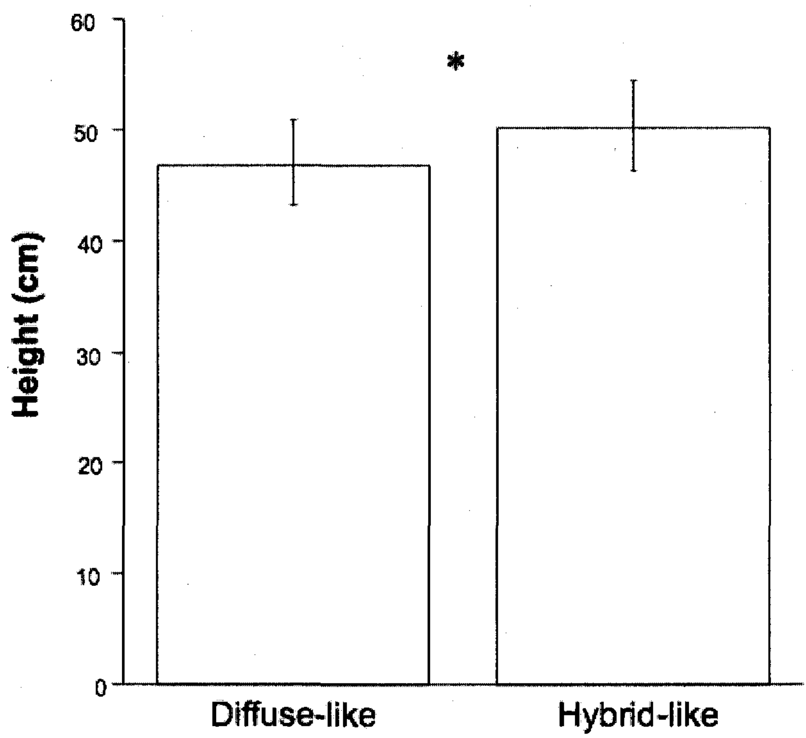


Figure 1.13. The proportion of diffuse-like and hybrid-like plants that exhibited polycarpy. Data were collected from fourteen diffuse knapweed field sites surveyed across western North America in 2005. *P<0.01

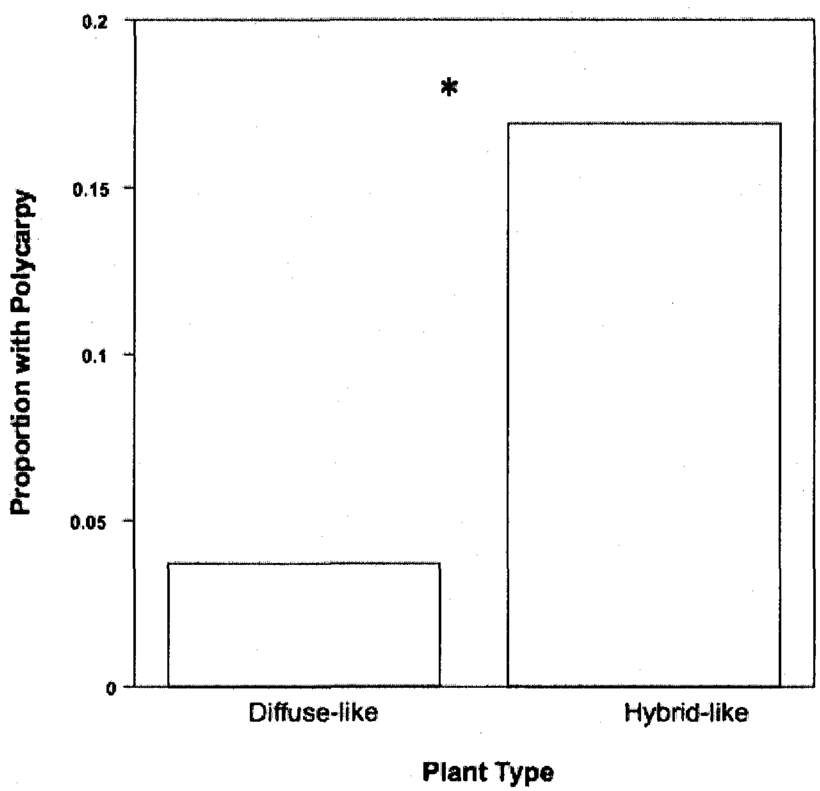


Figure 1.14. The proportion of field surveyed diffuse-like, hybrid-like, and spotted-like plants from the Ukraine that exhibited polycarpy (i.e. evidence of last year's flowering stalks). Different letters denote significantly different means (Tukey's test P<0.05).

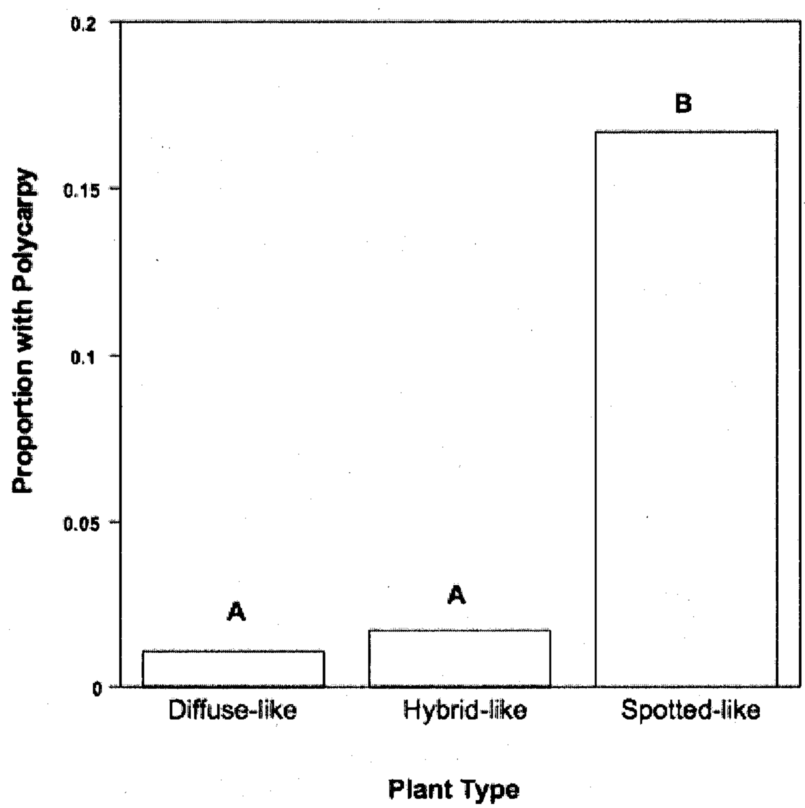
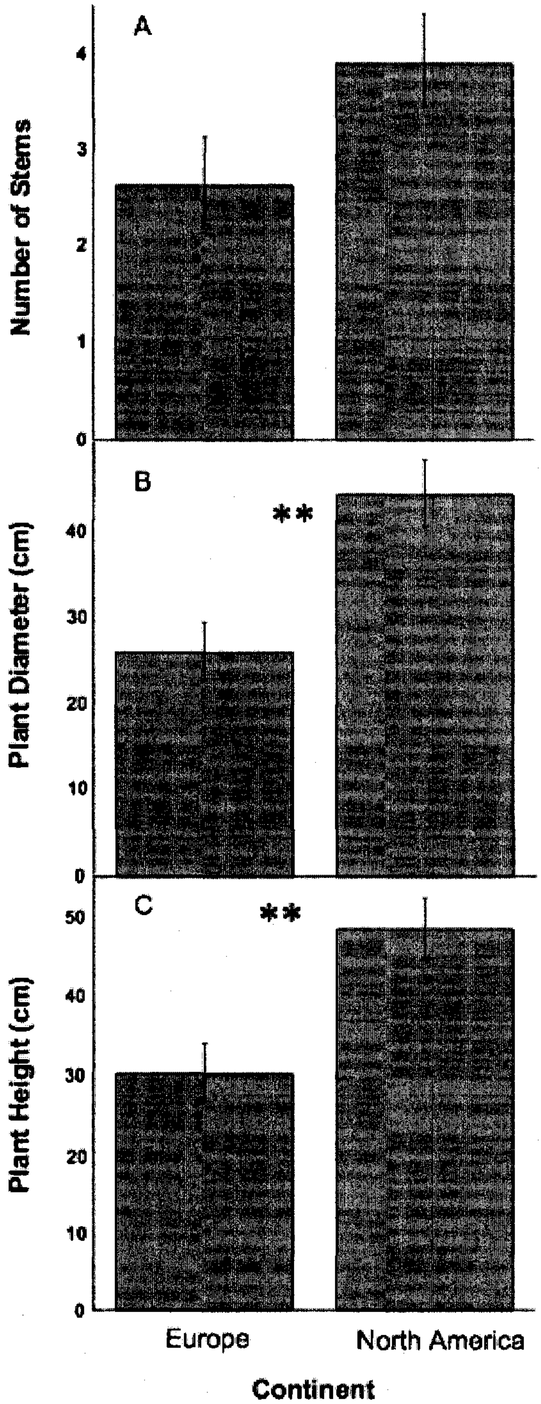


Figure 1.15. Differences among field surveyed diffuse knapweed in Europe versus North America for A) number of stems B) plant diameter, and C) plant height (mean ± SE). The three traits were ln transformed prior to analysis; reported values are back-transformed. ** $P < 0.01$.



A
B
C
Number of Stems
Plant Diameter (cm)
Plant Height (cm)
**
**
0
1
2
3
4
10
20
30
40
50
Europe
North America
Continent

APPENDIX 1 – CHAPTER 1

RECORDED SITE CHARACTERISTICS

At each field site, I noted the following site characters. 1) I measured the extent of the knapweed infestation by pacing out two axes: the greatest length and greatest width. As I was measuring the site, I inspected the plants closely to look for phenotypes that might be indicative of hybridization. I noted dominant plant species, the level of disturbance at the site (i.e. highly disturbed, moderately disturbed, or relatively undisturbed), and the approximate percent cover of the target plant across the site. 2) I looked for the densest area of knapweed and counted the plants in a square meter to estimate the high range of knapweed density. The low range was zero at every site (i.e. there were always patches of ground that did not contain knapweed within a site). 3) If possible, I collected 6" soil cores to obtain basic soil characteristics (i.e. pH and C:N). Some areas were too rocky to collect soil from. Upon collection, the soil bags were left open to air dry (see Appendix 2 for soil analyses). 4) I photographed the site to have a record of the location and pressed one or two voucher specimens for reference. 5) I noted the GPS location, the elevation of the site, and approximated the slope and aspect. 6) If hybrids were present, I randomly selected a patch of ground up to 20 x 30 m to map the plant distribution on grid paper. If the site was smaller than that, I mapped the entire area. I mapped the site to have a record of the spatial distribution of hybrids versus parental phenotypes.

The following pages are the specific notes taken at each site visited in Europe and North America in 2005 and 2006.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Au1

Location and Contact: Eggendorf

Date and Time: Aug 20, 2005, 14:00

GPS Coordinates: N: 47' 53.050 E: 16' 16.682

Elevation: 1719 feet   Slope: Aspect (slope faces):

Dominant Plant Species:
Artemisia absinthium

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
ruderal

Size of knapweed population:
1000 x 5

Density: ranging from ___0,3_________/m^2 to ___1,5_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects in 50m

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Hu. 1

Location and Contact: Heviz

Date and Time: Aug 22, 2005, 9am

GPS Coordinates: N:46° 48.028 E: 17° 12.334

Elevation: 454 feet | Slope: 5° | Aspect (slope faces): W

Dominant Plant Species:
Erigeron (Stenactis) annua, plantago lanceolata, achillea millefolium

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal grassland

Size of knapweed population:
30 x 15m

Density: ranging from ____0,4________/m² to _____2_______/m²

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects in 50m

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Ro2

Location and Contact: Plaesu

Date and Time: Aug 28, 2005, 16:00

GPS Coordinates: N: 47° 13.999 E: 26° 30.956

Elevation: 982ft Slope: Aspect (slope faces):

Dominant Plant Species:
C. maculosa, Daucus carota

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):

Size of knapweed population:

Density: ranging from ___7________/m^2 to ___15________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects 50m

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: Ro. 6

Location and Contact: Tulcea

Date and Time: Aug 28, 2005, 13:00

GPS Coordinates: N:45° 11.146' E: 28° 47.139'

Elevation: 27feet Slope: Aspect (slope faces):

Dominant Plant Species:
Anboosda artemisifolia

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal; along railway tracks

Size of knapweed population:
10 x 150m

Density: ranging from ___0,3________/m^2 to ___3________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects 50m

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: Ro7

Location and Contact: Sendreni (Galati GL county)

Date and Time: Aug 28, 2005, 16:40

GPS Coordinates: N: 45° 29.872' E: 27° 54.709

Elevation: 51feet Slope: 2 Aspect (slope faces): S

Dominant Plant Species:
Achillea millefolium, Centaurea diffusa

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal grassland, grazing?

Size of knapweed population:
100 x 400m

Density: ranging from ___0,8_________/m^2 to ___9_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects 50m
von Noden links vor Ortstafel (Bangelande)

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: Ro3

Location and Contact: Costinesti

Date and Time: Aug 27, 18:30

GPS Coordinates: N:43° 54.146' E: 28° 34.435'

Elevation: 57ft Slope: 2° Aspect (slope faces): SW

Dominant Plant Species:
Erigeron (Stenactis) canadensisi; Artemisia austriaca; Delphinium sp; Xeranthemum annum

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal grassland, grazed

Size of knapweed population:
30 x 40m

Density: ranging from ___0,5_________/m² to ___5_________/m²

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Hybrids present? 3?

4, 25m transects

See map on actual sheet.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: Ro4

Location and Contact: Sibioara

Date and Time: August 28, 2005

GPS Coordinates: N: 44° 23.380 E: 28° 31.599

Elevation: 57ft Slope: Aspect (slope faces):

Dominant Plant Species:
Cichorium istybois, Berforara incana; grass, ruderal grassland

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grazed

Size of knapweed population:
200 x 500m

Density: ranging from ___0,4_________/m^2 to ___3_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
96 km from Tulcea
2 transects 50m

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Ro5

Location and Contact: Baia (Tulcea TL county)

Date and Time: August 28, 2005

GPS Coordinates: N:44° 94.571 E: 28° 91.083

Elevation: 79ft Slope: Aspect (slope faces):

Dominant Plant Species:
Centaurea diffusa, Erigeron canadensis; Stenactis

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal grassland, grazed

Size of knapweed population:
Approx. 50 hectares

Density: ranging from ___1_________/m^2 to ___8_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
2 transects at 50m
km 55 from Tulcea

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Ro8

Location and Contact: Opriseni

Date and Time: Aug 29, 2005, 12:00

GPS Coordinates: N: 47° 28.505 E: 26° 16.101

Elevation: 1203 ft Slope: 10-20° Aspect (slope faces): W

Dominant Plant Species:
No dominant species. Salvia pratense, C. maculosa

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
pasture

Size of knapweed population:
100 x 300m

Density: ranging from ___0,2_________/m^2 to ___3_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
See map on actual worksheet.

2 transects 50m/

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Ro9

Location and Contact: Miercurea Civc (Train Station)

Date and Time: Aug 31, 2005

GPS Coordinates: N: 46° 21.552 E: 25° 47.645'

Elevation: 2182 Slope: Aspect (slope faces):

Dominant Plant Species:
Artemisia absinthium, Tanacetum vulgare

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Ruderal, along rail tracks

Size of knapweed population:
50 x 300m

Density: ranging from ___0,1_________/m^2 to ___1,5_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
1-15 1st transect
16-22 2nd transect
23-30 3rd transect

C. diffusa present at same site, seeds and leaves collected from 3 plants (9.31-9.33).

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 1-H (diffuse-like)

Location and Contact: Fred Raisch, Idalia, CO Hwy 36 W. past MM 194, Right on Road P – Right Rd under power lines

Date and Time: June 21, 2005, 2pm

GPS Coordinates: N:39° 40' 17.0" W: 102° 33' 01.3"

Elevation: 1246m Slope: 0 Aspect (slope faces): N/A

Dominant Plant Species:
Hybrid mix, needle and thread grass

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grassy CPR mix along phone pole line

Size of knapweed population:
320 acres

Density: ranging from __15__________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:

Sprayed field – selected better, healthier plants.
2/3 of the field had fairly dense diffuse/hybrids.

Slightly larger % of diffuse than hybrids. No spotted around.

Soil pH = 7.01

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 2-H (diffuse-like)

Location and Contact: Fred Raish, S. of Road 36 Co. Road P – 1/4 mile off Hwy 36 near Idalia, CO

Date and Time: June 22, 2005, 6am
GPS Coordinates: N: 39° 39' 21.0" W: 102° 33' 18.6"

Elevation: 1236 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
CRP Grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grass, pastureland adjacent to corn field and gravel road

Size of knapweed population:
About 100 plants

Density: ranging from _____10_______/m^2 to _____0_______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken: 0

Notes:

61 diffuse-like plants
26 hybrid-like plants

Triangle 255m x 110m x 210m at corner of Hwy 36 and Road P.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 3H (diffuse-like)

Location and Contact: Fred Raish, Hwy 36 W of Idalia, CO. County Rd P., N 1/2 mile along Road

Date and Time: June 22, 2005 6:30am

GPS Coordinates: N:39° 40' 30.5" W: 102° 33' 22.3"

Elevation: 1260 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Fesque, alfalfa, grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
CRP Grassland

Size of knapweed population:
120m EW x 355m NS

Density: ranging from ____20________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4
Notes:
Clumped distribution

Soil pH = 6.28

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 4H (diffuse-like)

Location and Contact: Washington County, CO. South of Akron. CR DD. Mr. Kim Kessinger (Fred Raish)
Date and Time: June 22, 2005 4pm

GPS Coordinates: N: 40° 06' 44.8" W: 103° 11' 27.6"

Elevation: 1412 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Edge of oat field along fence row. Grasses and thistles. Dense vegetation.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?): See above.

Size of knapweed population:
6m wide x 200 m long

Density: ranging from ____20________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4
Notes:
Some plants chewed.
Quad found on 2 plants – very windy day.
3 plants pressed.

Soil pH = 7.8

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 5H (diffuse-like)

Location and Contact: Yakima, WA. K-mart at exit 34 off I-82. Parking lot.
Date and Time:
6-28-05, 6:30pm

GPS Coordinates: N: 46° 34' 58.9" W: 102° 28' 15.8"

Elevation: 309m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffusa

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Highly disturbed – waste land around parking lot, near interstate.

Size of knapweed population:
36m wide x 93m long

Density: ranging from ____3________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken: 0

Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 6H (diffuse-like)

Location and Contact: Yakima Sportsman State Park – I-82 Nob Hill Exit East. Dick Jacobson, Co. weed agent

Date and Time: 6-29-05, 6:40am

GPS Coordinates: N:46° 35' 06.7" W: 120° 27' 33.0"

Elevation: 309m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Grass and diffusa

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Cotton wood trees along river. Sparse vegetation – dry grass and knapweed.

Size of knapweed population:
4m x 100m, sporadically 2 miles or so.

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0

Notes:
Along top of levee along Yakima River. Compacted soil – roadbed.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 7H (diffuse-like)

Location and Contact: Dick Jacobson, Off I-82, Zillag exit – South of Yakima, WA.

Date and Time: 6-29-05, 12:30pm

GPS Coordinates: N:46° 24' 0.34" W: 120° 16' 46.4"

Elevation: 238m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, Russian knapweed, curly doc

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
County owned gravel storage site.

Size of knapweed population:
120m x 126m

Density: ranging from ____7_______/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
1

Notes:
Added gravel, soil so sandy couldn't get a core to stick together.

pH 6.93

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 8H (diffuse-like)

Location and Contact: Roadway into Humane Society and Arboretum – Yakima, WA

Date and Time: 6-29-05

GPS Coordinates: N:46° 35' 06.4" W: 120° 28' 07.0"

Elevation: 314m Slope: gentle Aspect (slope faces): east

Dominant Plant Species:
Diffuse, grass, scrub trees

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed roadway

Size of knapweed population:
16m x 62m

Density: ranging from ____20________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0
Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 9H – (diffuse-like)

Location and Contact: Dick Jacobson, Sportsman Ground off I-82 exit 40 off frontage road through horse pasture – need key for 2 gates. South of Yakima, WA.

Date and Time: 6-30-05, 7am

GPS Coordinates: N: 46° 32' 32.5" W: 120° 27' 39.6"

Elevation: 277m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Cottonwood, gum weed, grass, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Near Yakima River next to a standing water marsh. Wild area for sportsmen next to horse pasture. Very weedy.

Size of knapweed population:
194m x 99m

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Diffuse throughout area. Large and widely spaced plants.

Very rocky – river rock size over much of area.

Plants in 2 very distinct forms – purple with polycarpy and white with no polycarpy (typical diffuse). Amy's guess is squarrose knapweed, although Cindy Roche has been sent a sample and declared it a hybrid.

# of purple flowers with polycarpy = 56
# of purple flowers w/out polycarpy = 3
# of white flowers (diffuse) with polycarpy = 0
# of white flowers (diffuse) w/out polycarpy = 16

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 10

Location and Contact: Dick Jacobson, Yakima, WA. Across from West Valley Station #4 (Fire). Tampico General Store parking lot – cross raods S. Fork and N. Fork. Mileage from Union Gap (Yakima) Main Street and Antanum cross roads = 19.5 miles.

Date and Time: 6-30-05, 8:30pm

GPS Coordinates: N: 46° 32' 07.4" W: 120° 52' 02.6"

Elevation: 653m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Weedy grass, diffuse, and spotted

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadway (grassy) into a field.

Size of knapweed population:
L 115m x W 19m

Density: ranging from ____20________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, <u>25-50</u>%, 50-100%

Number of soil samples taken:
3

Notes:
Pure diffuse at far end of site only. Hybrids more spotted-like – golden bracts, white flowers, no spines. Only a few plants blooming. Larinus present.

pH 6.97

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 11D (4 hybrids in entire patch)

Location and Contact: Dale Whaley, Along 97A, Wenatchee, WA. Roadway 7 miles North. Fresh fruit Homestead stand w. side of roadway.

Date and Time: 07-1-2005, 12:30pm

GPS Coordinates: N: 47° 33' 40.4" W: 120° 16' 11.3"

Elevation: 249m Slope: gentle Aspect (slope faces): east

Dominant Plant Species:
Sagebrush, grasses, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Along highway between frontage road to orchard and highway.

Size of knapweed population:
220m x 37m

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 very rocky soil

Notes:
Root mining insect in many samples – Sphenoptera?

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 12H (diffuse-like)

Location and Contact: Dale Whaley, South of Wenatchee, WA. Off Maluga-Alcoa Road. Near intersection on Stemilt Creek Road 1/4 mile west.

Date and Time: 7-2-05, 7:45am

GPS Coordinates: N: 47° 22' 32.6" W: 120° 14' 59.0"

Elevation: 212m Slope: gentle Aspect (slope faces): E/NE facing Columbia River

Dominant Plant Species:
Sage, grasses, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Pull-off near underground gas (maybe) pipeline – some past dumping – disturbed.

Size of knapweed population:
300m long x 65m wide

Density: ranging from ____5________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0, very rocky. Couldn't get 1"

Notes:
Diffuse all around this area – down Malaga-Alcoa Road. Hybrids few and dispersed through site. Purple flowers with obvious spots. Large plants.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 13H (diffuse-like)

Location and Contact: Dale Whaley, US Forest Service, Wenatchee, WA. AH.97 across from Honda Dealer – Ohm's Garden Road.

Date and Time: 07-2-05

GPS Coordinates: N: 47° 28' 14.4" W: 120° 20' 11.5"

Elevation: 232m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Buffer area between Forest Service parking lot and frontage road – very disturbed – some rock dumping.

Size of knapweed population:
100m long x 36m wide

Density: ranging from ____5-6________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 – rocky.

Notes:

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 14S (hybrids?)

Location and Contact: Dale Whaley. In mountains, S. of Leavenworth, WA along Icicle Road #7600 and Icicle Stream – about 1 mile before chatter creek guard station.

Date and Time: 7-3-05, 12pm

GPS Coordinates: N:47° 36' 31.3" W: 120° 52' 46.5"

Elevation: 792m Slope: gentle Aspect (slope faces): NE

Dominant Plant Species:
Clover, pine trees

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Along gravel road in national forest. Disturbed roadway – dense vegetation of weeds.

Size of knapweed population:
86m long both sides of road

Density: ranging from ___________/m^2 to ___________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Buds very small.
97 spotted plants.
111 golden plants.
5 certain hybrids.

Could be more hybrids, but couldn't tell because of bud size.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 15H (diffuse-like)

Location and Contact: Dale Whaley, Hwy 2 and Alpensee Strasse. E. edge of Leavenworth, WA.

Date and Time: 7-3-05 1:30pm

GPS Coordinates: N: 47° 35' 56.2" W: 120° 39' 04.5"

Elevation: 353m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Grass, diffuse, dalmation toadflax, pine trees

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Next to hotel with road on 3 sides – highly disturbed.

Size of knapweed population:
60m x 53m

Density: ranging from ____3________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 – sandy, very hard packed, rocky.

Notes:
Large plants. Great view!

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 16H (diffuse-like)

Location and Contact: Dale Whaley, East side of Hwy 97-2, north of Wenatchee, WA.

Date and Time: 7-3-05, 4pm

GPS Coordinates: N:47° 29' 06.0" W: 120° 18' 00.2"

Elevation: 231m Slope: moderate Aspect (slope faces): south

Dominant Plant Species:
Diffuse, sage, grass

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed roadway pull-off at base of steep rock hill.

Size of knapweed population:
54m x 14m x 82m x 45m

Density: ranging from ____3________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 rocky

Notes:
See actual sheet for shape of site.

109 hybrids: 479 diffuse

Hybrids more abundant near road. Scattered randomly.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 17S

Location and Contact: Rich Old. Corner of Yates St. and Ann St. (Larry St. west) off highway to Palouse, WA. Pullman, WA.

Date and Time: 7-5-05, 10am

GPS Coordinates: N: 46° 44' 47.9" W: 117° 10' 30.3"

Elevation: 760m Slope: steep Aspect (slope faces): N

Dominant Plant Species:
Grasses thick – patchy spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grass, residential area – empty steeply sloped lot.

Size of knapweed population:
28m x 38m

Density: ranging from ____2-3________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Better soil – not so rocky.

pH 6.47

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 18S

Location and Contact: Rich Old. Far east edge of WA on border of Idaho off Hwy 270 – S. side of road on old quarry loop road – across from electrical business along highway. Between Pullman and Moscow.

Date and Time: 7-5-05, 2:30pm

GPS Coordinates: N: 46° 44' 14.4" W: 117° 3' 4.0"

Elevation: 777m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Spotted knapweed, grasses, yellow mustard

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Fallow strip between roadway and bike path with access road in middle. Disturbed – dense grasses, weed, toadflax, horsetails.

Size of knapweed population:
372m x both sides of road

Density: ranging from ____________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Larinus and quad present.
Polycarpy evident.
Searched entire patch – no hybrids or diffuse.
Some variation in spot darkness – most dark. Could result from bud stage – smaller = lighter?
Purple flowers with white centers.
<1% flowers.

Idea – cross towards diffuse? Introgression easier in that direction?

pH 6.62

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 19S

Location and Contact: Rich Old. Pullman, WA. Above SYG nursery and landscaping. Edge of old gravel pit – north edge of Pullman.

Date and Time: 7-6-05

GPS Coordinates: N: 46° 44' 27.3" W: 117° 11' 42.4"

Elevation: 727m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Spotted knapweed, yarrow, bachelor's buttons.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Heavily disturbed – grass, weeds, trash. Spotted along fence line – 5-10m along fence. Old equipment and lumber.

Size of knapweed population:
56m long x 5m either side fence

Density: ranging from ____too dense to tell________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 rocky, dry soil.

Notes:
Rich said previously diffuse site – now spotted after spraying.

Rocky – rough terrain – hillside- much polycarpy.

Larinus present.

Some herbicide spraying.

Maybe one diffuse plant – sprayed?

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 20H

Location and Contact: Rich Old. Corner of Bishop and Hwy 270 to Moscow. Parking lot Quality Inn, along bike trail.

Date and Time: 7-6-05, 9:30am

GPS Coordinates: N: 46° 43' 16.9" W: 117° 09' 50.5"

Elevation: 717m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, grasses, bachelor's button

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Along road.

Size of knapweed population:
100m x 5-10m

Density: ranging from ____5________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0

Notes:
Rocky, highway department rock and levee between road and bike path.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 21H

Location and Contact: Don Sharratt – Patterson Ferry Road – off I-84. End of road at Columbia River and barge terminal.

Date and Time: 7-7-05,

GPS Coordinates: N:45° 54' 58.8" W: 119° 33' 31.8"

Elevation: 94m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, sage

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Bare ground, sandy, rocky, some bunch grass, very arid.

Size of knapweed population:
100m wide x 165m long. Diffuse throughout beyond this area.

Density: ranging from ____3________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0, sandy and many river rocks. Couldn't core.

Notes:
Along roadway closest to water, random sample.
500 diffuse, 29 hybrids.

Plants spread out.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 22D

Location and Contact: Dan Sharrar. Road to Freeway (I-84) west edge of Pendleton. Across from State Prison at Oak Creek Village parking lot.

Date and Time: 07-08-05

GPS Coordinates: N: 45° 40' 26.0" W: 118° 49' 15.5"

Elevation: 317m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, yellow star, white clover

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed parking lot area – very weedy.

Size of knapweed population:
150m long, surveyed vegetative area surrounding lot about 5m

Density: ranging from ____2________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
3 cores 4" deep

Notes:
69 all diffuse plants.

pH 8.79

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 23S

Location and Contact: Don in Condon, OR. Gilliam County, OR. 4 miles N of Condon, OR off Hwy 19 on Gwendolyn Rd – 2.5 miles through canyon.

Date and Time: 7-8-05, 3:40pm

GPS Coordinates: N: 45° 18' 06.8" W: 120° 11' 07.4"

Elevation: 760m Slope: steep Aspect (slope faces): south

Dominant Plant Species:
Grasses, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Cattle land around rock road – open range.

Size of knapweed population:
375m long along N side of road, about 20-30m

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
O, too rocky

Notes:
Larinus found often. Lots o f polycarpy seen. Tops of plants grazed by cattle in many places. One bull elk seems to live with small herd of cattle nearby. Multiple snakes. Some variation in bract coloration – related to age? Few flowers.

Diffuse found growing at edge of site (E. edge). Only hybrids found looked like diffuse with spotted bracts – purple version. Most spotted eaten, so hard to see hybrids (also lack of flowers).

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 24H

Location and Contact: in town of Condon, OR along Hwy 206 to Wasco behind downtown stores.

Date and Time: 7-9-05, 8am

GPS Coordinates: N: 45° 14' 14.6" W: 120° 11' 06.5"

Elevation: 850m Slope: moderate Aspect (slope faces): W

Dominant Plant Species:
Diffuse knapweed, grass

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Undeveloped weedy square section of land near town.

Size of knapweed population:
55m long x 21m wide

Density: ranging from ____10________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4 4" to 6" cores Some rocky soil

Notes:
591 diffuse
25 spotted bract diffuse – scattered

1/2 mile SW another town site-
159 diffuse, 15 spotted bract along roadway in front of houses and fenced county storage area.

NW corner of Main St. and Court – behind JB's Grill and Eatery and S. of bus parking lot of school in town of Condon, OR. 89 diffuse – 28 spotted bract hybrids

pH 8.07

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 25H (diffuse-like)

Location and Contact: Merril Co. Weed Office – The Dalles Port, OR – Road between I-84 and Columbia River. Wasco, County

Date and Time: 7-9-05, 1pm

GPS Coordinates: N:45° 36' 17.1" W: 121° 11' 02.3"

Elevation: 27m Slope: steep Aspect (slope faces): north

Dominant Plant Species:
Dry grasss, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadway bank, disturbed.

Size of knapweed population:
730m long roadway, 1-5m long

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 rocky

Notes:
Large plants, mostly widely spaced.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 26H

Location and Contact: The Dalles, OR – at junction of Hwy 30 and 197 near I-84

Date and Time: 7-10-05, 10am

GPS Coordinates: N:45° 35' 53.3" W: 121° 08' 19.5"

Elevation: 48m Slope: slight Aspect (slope faces): N

Dominant Plant Species:
Diffuse, some sage, grass

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Fenced area along roadway – backed by steep bank, ravine – small stream in ditch.

Size of knapweed population:
97m long x 25m wide

Density: ranging from ____5-8________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Knapweed dense
Counted 44m x 12m long area

506 diffuse
70 hybrid

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 27S (4 hybrids found)

Location and Contact: Bryan Dallolio, Boise, ID (Garden City Suburb). Walking path along Boise River near housing area and adjacent to junk yard.

Date and Time: 7-11-05, 11:30am

GPS Coordinates: N:43° 38' 35.5" W: 116° 15' 19.7"

Elevation:795 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Scrub trees, spotted knapweed and grasses.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Public trail behind junk yard fence.

Size of knapweed population:
37m long x 1-3m along trail

Density: ranging from ____5________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0 River Rock common

Notes:
Total of 4 hybrid plants found. Within spotted stand - 2 beside each other 2 times, 8m apart.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 28S (1 hybrid)

Location and Contact: Bryan Dallolio, corner of Yamhill and Federal – far E. side of Boise, ID.

Date and Time: 7-11-05

GPS Coordinates: N:43° 32' 55.0" W: 116° 09' 38.3"

Elevation: 894m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
White clover, sage, weeds, Rush skeleton weed, grasses, gum weed, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed, along railway bank, gravel parking area, near highway

Size of knapweed population:
140m x 39m wide

Density: ranging from ____1________ /m^2 to ______0______ /m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
30 spotted, 1 hybrid collected – specimen pressed. White flowers more diffuse-like. Large plants widely spaced.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 29H (diffuse-like)

Location and Contact: Terry Lee, Fairfield, ID – Soldiers Road Mountain Ranch and Resort off N. Highway 20 – W of Fairfield.

Date and Time: 7-13-05

GPS Coordinates: N:43° 24' 52.5" W: 114° 52' 17.3"

Elevation: 1589m Slope: moderate Aspect (slope faces): SE

Dominant Plant Species:
Dense grasses, sage, lupines, buck brush

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Along road at the resort. Plants scattered over property.

Size of knapweed population:
1/2 to 3/4 mile along roadway

Density: ranging from ____1________/m² to ______0______/m²

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0

Notes:
Larinus found.

Diffuse – 46
Hybrid – 6
Very rocky
Very sandy soil.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 30S/D

Location and Contact: Terry Lee, South of Fairfield, ID. Morman Rd. S. to 300 S. Right to 1st road – drive near barn.

Date and Time: 7-13-05, 7:30am

GPS Coordinates: N: 43° 18' 16.6" W: 114° 48' 06.4"

Elevation: 1528 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Knapweeds, grass, willow, weeds

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Dirt road between hay fields on high plains. Roadway, disturbed.

Size of knapweed population:
69m long x 4m wide on W. side of road

Density: ranging from ___________/m^2 to _____0_____/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Spotted and diffuse are spaced apart in clumps. Nice soil.

pH 6.71

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 31S

Location and Contact: Fairmont, MT – Fairmont Resort – land across from golf course along black top road.

Date and Time: 7-14-05, 11am

GPS Coordinates: N: 46° 02' 37.1" W: 112° 48' 52.2"

Elevation: 1538m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Thick grasses, cheat grass (?), other weeds, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Resort land, undeveloped along blacktop road behind main resort.

Size of knapweed population:
160m along roadway
18m wide

Density: ranging from ____4________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4 samples, 2-4" cores

Notes:
Rocky, pea gravel on top. Noted old black top and gravel at site. Perhaps area was used when original road was surfaced. In sample area, all spotted checked – no hybrids. Size of site is larger than visually surveyed, but nothing but spotted seen. Late bud stage on all plants.

pH 7.79

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 32H (spotted-like)

Location and Contact: Stacey Barta. 7 miles E of Big Timber, Montana. KOA parking lot – land between parking lot and highway near waterslide. Very near I-90.

Date and Time: 7-15-05, 10:20am

GPS Coordinates: N:45° 46' 23.7" W: 109° 47' 56.8"

Elevation: 1216m Slope: moderate Aspect (slope faces): N

Dominant Plant Species:
Clover, grass, weeds, spotted knapweed, bordered by willow sprouts

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Bank of roadway, greatly disturbed.

Size of knapweed population:
10m wide x 26m long – a few plants beyond, not many

Density: ranging from ____4________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Very rocky soil – sandy.
Across highway about 10 additional plants.
168 spotted
19 hybrids (white flowered, golden bracts)

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 33S

Location and Contact: Stacey Barta. MM18 – N on Hwy 191 – Big Timber, MT.

Date and Time: 7-15-05 7am

GPS Coordinates: N: 46° 04' 29.3" W: 109° 56' 08.7"

Elevation: 1507m Slope: slight Aspect (slope faces): NW

Dominant Plant Species:
Spotted knapweed, pasture grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Cow pasture, east side of highway

Size of knapweed population:
60m wide x 125m long

Density: ranging from ____4-6________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
3, 2 at 6", 1 at 3"

Notes:

Good soil, gravel on surface. Dense knapweed.

pH 7.56

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 34S

Location and Contact: Paul Dixon, N. of Laurel, MT – about 1 mile in country along roadside – E side of road – First Ave. and Lois Place (private drive).

Date and Time: 7-16-05, 8am

GPS Coordinates: N: 45° 41' 21.8" W: 108° 46' 17.2"

Elevation: 1076 Slope: slight Aspect (slope faces): S

Dominant Plant Species:
Grasses, sweet clover, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadside, disturbed. Grassy.

Size of knapweed population:
76m long x 8-10m in ditch

Density: ranging from ____1________/m^2 to ______0______/m^2

% Cover: <1%, <u>1-5</u>%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Rocky soil

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 35S (a few hybrids?)

Location and Contact: Paul Dixon, Billings, MT. Riverview Park – Exit 447 off I-90 – S. Billings exit – path around lake.

Date and Time: 7-17-05, 8:45am

GPS Coordinates: N:45° 44' 26.9" W: 108° 31' 56.9"

Elevation: 938m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Spurge, gasses, Canadian Thistle, large cottonwood trees, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Blacktop walking path in city/county park. Cottonwood trees dominate. Dense vegetation. Spotted knapweed along shaded path, often at corners with paths branching off from blacktop path.

Size of knapweed population:
230m long along path

Density: ranging from ____6________/m^2 to ______0______/m^2

% Cover: ≤1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Within 1 group of spotted found several hybrid looking plants, but not blooming. In general, different (hybrid?) plants not blooming. Could be young spotted, but unsure.

pH 7.74

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 36S

Location and Contact: Josh Shorb. West o f Cody, WY – very near edge of parking lot for Buffalo Bill Dam – between road (Hwy 20) and lake, across guard rail on lakeside.

Date and Time: 7-18-05, 1pm

GPS Coordinates: N:44° 30' 03.7" W: 109° 11' 27.9"

Elevation: 1649 Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Sage, spotted knapweed, grasses sparsely vegetated.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed roadside sparsely vegetated. Sandy, rocky soil.

Size of knapweed population:
55m long x 17m wide

Density: ranging from ____1________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Nice, large purple flowers. 20 plants total – no hybrids found.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 37S

Location and Contact: Josh Shorb. About 1-2 miles from Buffalo Bill Dam – N. side of Hwy 20 along private road to housing area.

Date and Time: 7-19-05, 6:15am

GPS Coordinates: N: 44° 29' 59.9" W: 109° 12' 44.3"

Elevation: 1664m Slope: steep Aspect (slope faces): S

Dominant Plant Species:
Sage, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Open range, undeveloped area of country housing development – shrub, grasses, pinyon pines.

Size of knapweed population:
78m long within 5m each side of gravel road

Density: ranging from ____________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
0

Notes:
Dense spotted along roadway – most dense near highway.

20-30 additional plants 138m above original site on same steep road.

Site identified last year for first time. Slated to be sprayed later in the week. Very rocky soil.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 38S

Location and Contact: Rod Litzel, About 2 miles N of Buffale, WY on Mainstreet across from Rock Creek Greenhouse.

Date and Time: 7-20-05, 7:20am

GPS Coordinates: N:44° 22' 38.7" W: 106° 42' 37.2"

Elevation: 1435m Slope: steep Aspect (slope faces): W

Dominant Plant Species:
Grasses, sage, spotted knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadway along blacktop road. Disturbed.

Size of knapweed population:
28m long x 10m wide. Along roadway ditch.

Density: ranging from ____2________$/m^2$ to ______0______$/m^2$

% Cover: $\leq$1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Plants are not evenly sampled at 1m intervals – small site so all plants surveyed.

Final 4 plants taken from E. side of greenhouse land – across from highway and S. of original site.

pH 6.12

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 39H

Location and Contact: Rod Litzel. I-25 exit W. of Kaycee, WY to 191 to Mayoworth. Then 2.0 miles up Slip Road (Road 67) Park at switch back S-curve.

Date and Time: 7-21-05, 7pm

GPS Coordinates: N:43° 50' 13.7" W: 106° 52' 28.6"

Elevation: 1849m Slope: moderate Aspect (slope faces): SE

Dominant Plant Species:
Sage, yucca, pine

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Rangeland side of large valley. Fairly undisturbed area, but plants along disturbed gravel road.

Size of knapweed population:
170m x 5m both sides

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Heavy bee activity on blooms. Very rocky – slate. Most plants in ditchway.

pH 8.53

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 40H

Location and Contact: Brian Connely, Casper, WY. Behind MirMac Inc. off 20 onto 119N – West side of town.

Date and Time: 7-21-07, 7:30pm

GPS Coordinates: N: 42° 53' 11.5" W: 106° 26' 24.8"

Elevation: 1609m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Diffuse, grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed empty lot behind industrial business.

Size of knapweed population:
200m long x 144m wide, patch likely beyond these dimensions

Density: ranging from ____5________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
In an area 27m x 28m, 191 diffuse, 32 hybrids.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 41H (diffuse-like)

Location and Contact: Brian Connely, Natrona Co. WY. West of Casper, WY off Hwy 20-26 to Co. 104N (MM 50) 19.4 miles N on gravel to 2 track to west about 1-2 miles into grassland.

Date and Time: 07-22-05, 8:45am

GPS Coordinates: N:43° 23' 07.9" W: 107° 03' 45.6"

Elevation: 1888 Slope: gentle Aspect (slope faces): E

Dominant Plant Species:
Grasses, sage (frigida and tridentata), western wheat grass, needle and thread grass, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Rangeland, rocky red soil.

Size of knapweed population:
51m x 17m

Density: ranging from ____7________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Very remote – in a small depression in middle of BLM grassland. Sprayed since 1987 – much smaller than normal plants. Likely these plants all came up from seed bank after wet spring.

pH 8.25

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 42S

Location and Contact: Brian Connely – Casper, WY. Gate 11 – International Airport – into secure area.

Date and Time: 7-23-05, 7:30am

GPS Coordinates: N:42° 54' 14.6" W: 106° 27' 09.7"

Elevation: 1628m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Spotted knapweed, grasses

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed patch of land along asphalt and fence.

Size of knapweed population:
148m long x 3-5m wide

Density: ranging from ____2________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Huge plants.

pH 8.36

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 43H (diffuse-like)

Location and Contact: Stephen Elzinga, Eagle Co. CO. Wolcott, CO behind Gallegos Corp. near RR tracks.

Date and Time: 7-25-05, 12pm

GPS Coordinates: N:39° 42' 10.2" W: 106° 40' 32.8"

Elevation: 2130 Slope: slight Aspect (slope faces): W

Dominant Plant Species:
Grasses, diffuse, rabbit brush, sage

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Very disturbed, piles of rocks

Size of knapweed population:
62m long x 80m wide

Density: ranging from ____5________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
Entire site mowed at sometime.

Soil pH = 7.8

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 44S

Location and Contact: Stephen Elzinga, Edwards, CO. Along Hwy 6 – W end of parking lot St. Clare of Assisi Catholic Church

Date and Time: 7-25-05, 3:50pm

GPS Coordinates: N: 39° 39' 27.0" W: 106° 38' 13.3"

Elevation: 2179m Slope: 0 Aspect (slope faces): 0

Dominant Plant Species:
Musk thistle, grasses, chamomile, Canadian thistle

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed, rocky, along Hwy 6

Size of knapweed population:
55m x 25m wide

Density: ranging from ____7________$/m^2$ to ______0______$/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
140 spotted knapweed plants – all typical
<5% bloom

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 45S

Location and Contact: Stephen Elzinga, Above Edwards, CO – I-70 exit – under interstate to Singleton Development – Beard Creek Road.

Date and Time: 7-26-05, 7:45am

GPS Coordinates: N:39° 39' 22.3" W: 106° 35' 59.9"

Elevation: 2262 Slope: slight Aspect (slope faces): S

Dominant Plant Species:
Sage, grasses, rabbit brush, spotted knapweed, Russian knapweed

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed, rocky in spots – other areas good soil.

Size of knapweed population:
18m wide x 83m long

Density: ranging from ____4________ $/m^2$ to ______0______ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4 total – 3 at 3", 1 at 5"

Notes:
Pure spotted.

Soil pH ≐ 7.95

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 46H (Diffuse-like)

Location and Contact: Steve Elzinga, Eagle-Vail, CO. Along Hwy 6 at Holy Cross Energy and Colorado State Patrol.

Date and Time: 7-26-05, 10am

GPS Coordinates: N: 39° 37' 18.0" W: 106° 28' 17.9"

Elevation: 2331m Slope: Moderate Aspect (slope faces): N

Dominant Plant Species:
Grasses, thistles, diffuse, rabbit brush, shrubs

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Rocky bank between Hwy 6 and fence for power company – disturbed.

Size of knapweed population:
8m wide x 130m long

Density: ranging from ____3________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4, 3 at 2" and 1 at 3"

Notes:
Soil above rocks in many places.

39 diffuse
98 hybrids
Some plants mowed.

Soil pH = 7.62

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 47D (a few hybrids)

Location and Contact: Andrew Norton, Rooney Gulch. Exit 259 off I-70 of Denver at beginning of Alameda Blvd. – N up Rooney Gulch Road to parking lot.

Date and Time: 7-27-05, 8am

GPS Coordinates: N: 39° 41' 48.5" W: 105° 11' 32.7"

Elevation: 1848 Slope: gentle Aspect (slope faces): SSW

Dominant Plant Species:
Yellow clover, parsnip, thistle

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed under heavy power lines. Lots of biking traffic in area. Prairie dog town.

Size of knapweed population:
70m x 50m

Density: ranging from ____12________/m^2 to ______0______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

Notes:
First 4 plants were sampled from along bike trail off S end of large parking lot. All others from area N of parking lot.

Soil pH = 7.45

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 48

Location and Contact: Rest area off 87 south just N or Westport. Mile marker 123. Ruth Hufbauer collector, east coast.

Date and Time: 08/13/05, 1:40pm

GPS Coordinates: N:44° 16' 42" W:073° 31' 52.6"

Elevation: 168m | Slope: 0 | Aspect (slope faces):

Dominant Plant Species:

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Exotic old field with grasses, some shrubs, golden rod

Size of knapweed population:
95 x 75 m, 49m == 55 paces

Density: ranging from 0 /m^2 to 15 /m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4

pH = 5.71

Notes:
Started raining at 20. Am going to collect more seed samples, but no more data. No visible sign of hybrids.

This site may have been mown early in the season, accounting for the (mostly) short plants. (or N limitation?)

Later noted PROBABLY NOT.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 49

Location and Contact:Bernd Blossey. "Alpine Junction" Just N of Diesel station. Corner of 13 and 224 between Ithaca and Elmira.

Date and Time:8/15/05, 10:50am

GPS Coordinates: N:42° 17' 33.9" W:076° 42' 49.0"

Elevation: 345m Slope: flat Aspect (slope faces):

Dominant Plant Species:

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
On edge of tractor trailer parking lot, v. rocky on upper edge of old field. Within the defined site knapweed dominates, but outside are golden rods and in marshy area below, cat tails.

Size of knapweed population:
137 x 4 paces

Density: ranging from ______0______/m^2 to ____________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
None. Ground is rocky and many plants are growing directly out of degraded asphalt.
Notes:
There is meadow knapweed growing along the road, approximately 200m N.

Most plants are scenesed, so to get flower characteristics, Ruth picked from among flowering ones only.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number:50

Location and Contact:Ontario, Canada. Town of Flamborough, N of Safari Road. W of Westover in Beverly Swamp Cons. Area.

Date and Time: 8/16/05

GPS Coordinates: N:43° 20' 58.5" W:080° 06' 43.6"

Elevation: 252 Slope: 0 Aspect (slope faces):

Dominant Plant Species:
In area – meadow knapweed, queen anne's lace, and vetch. Within defined site: spotted knapweed.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Dominated by vetch and meadow knapweed. Open areas between woods, some shrubs.

Size of knapweed population:
12 x 15 paces, 12 x 2 paces

Density: ranging from ____0________/m^2 to ____10________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
4 (3 of 2", 1 of 3", kept hitting rocks)

pH = 7.78

Notes:
Most plants have senesced entirely. Will only be able to record flower characters on a few. Try to get at least 20.

See hard copy for map of site shape.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Seeds collected 2006 from tagged plants .

Site Number: 1 2006 Ukraine

Location and Contact: River bank, Novoarchangelsk, Botanical Institute Uman

Date and Time: 9am 6-20-06

GPS Coordinates: N: 48° 38' 45.7" E: 30° 46' 30.3"

Elevation: 85m Slope: 30° Aspect (slope faces): south

Dominant Plant Species:
Festuca valesiaca, Achillea setaceae, Elyztrigia repens, Echium vulgare, Galium verum

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Riverbank, cattle grazed. Lightly disturbed.

Size of knapweed population:
120m x 70m. Probably larger scattered plants.

Density: ranging from ___0________/m^2 to ___5-6________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Seeds collected from tagged plants 2006.

Site Number: 2 2006 Ukraine

Location and Contact: Novoarchangelsk, Botanical Institute Uman

Date and Time: 6-20-06

GPS Coordinates: N: 48° 38' 45.7" E: 30° 46' 30.3"

Elevation:85m   Slope: 5°   Aspect (slope faces): south

Dominant Plant Species:
Medicago lufulina, Anisanta tectorum, Melilotus officianalis, Artemisia absinthium,

Lotus cosnuculatus

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Very disturbed, gravel, dug out.

Size of knapweed population:
105 m x 17m

Density: ranging from ___0________/m^2 to ___13-14________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
27 hybrid
47 diffuse in a subsample walking down the transect line. All plants at sites 1 and 2 were not flowering, but in the bud stage.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 3 Ukraine 2006

Location and Contact: Tal'ne

Date and Time: 06-21-06

GPS Coordinates: N: 48°52' 18.2" E: 30° 42' 40.3"

Elevation: 194m Slope: none Aspect (slope faces):

Dominant Plant Species:
Achillea, Festuca, Plantago lanceolata, Artemisia

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadside, potentially grazed and mowed.

Size of knapweed population:
122 m 5m

Density: ranging from ___0_________/m^2 to ___2-3_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 4 2006 Ukraine

Location and Contact: Talniv, Uman region, Memorial Tank

Date and Time: 6-21-06, noon

GPS Coordinates: N: 48° 53' 31.2" E: 30° 40' 33.2"

Elevation: 148m Slope: 1° Aspect (slope faces):

Dominant Plant Species:
Festuca, Trifolium, Hieracium,

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Public memorial area. Potentially mowed.

Size of knapweed population:
170m x 55m

Density: ranging from ___0_________/m^2 to ___3_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
x
Notes:

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 5 2006 Ukraine

Location and Contact: Talianka village. N of town near village sign.

Date and Time: 6-21-06

GPS Coordinates: N: 48° 48' 22.8" E: 30° 33' 54.9"

Elevation: 174m Slope: <5° Aspect (slope faces): south

Dominant Plant Species:
Festuca, Corovilla

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Roadside, grazed, disturbed. Grassy

Size of knapweed population:
152 m x 8 m

Density: ranging from ___0_________/m² to ___5-6_________/m²

% Cover: ≤1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Centaurea scabiosafolia also present.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 6 2006 Ukraine

Location and Contact: Donetsk, Krasnoe, Chasov Yar

Date and Time: 6-24-06

GPS Coordinates: N: 48° 34' 51.9" E: 037° 54' 36.9"

Elevation: 132m Slope: 1° Aspect (slope faces): south

Dominant Plant Species:
Salvia, Eurphorb, Festuca

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grazed, road passing through.

Size of knapweed population:
148 m x 10 m The site went further on.

Density: ranging from ___0_________/m^2 to ___1-2_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken: x

Notes:
At the sampled site, it is flatter and diffuse occurs with spotted. Further up the slope, only maculosa is found. One almost flowering: purple and white. Sampled to south of dirt of road. Chalky hills N of site. Limestone.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: 7 2006 Ukraine

Location and Contact: Donetsk, Konstantinovka

Date and Time: 24-06-06

GPS Coordinates: N: 48° 30' 42.7" E: 37° 44' 10.3"

Elevation: 104m Slope: 1-2° Aspect (slope faces): S/SW

Dominant Plant Species:
Centaurea diffusa, Ambrosia, Melilotus

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Abandoned rail track.

Size of knapweed population:
110m x 4m

Density: ranging from ___0_________ $/m^2$ to ___8_________ $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Length of one set of tracks: 178 typical diffuse, 13 hybrid.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Seeds collected 2006 from tagged plants.

Site Number: 8 Ukraine, 2006

Location and Contact: Donetsk, Amurosigevka

Date and Time: 25-06-2006

GPS Coordinates: N: 47°48' 24.7" E: 038°33'10.0"

Elevation: 67m Slope: 5° Aspect (slope faces): SE

Dominant Plant Species:
Elytrigia cretacea, Euphorbia, Festuca, Coronillararia

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Base of limestone hills. Many endemics. Reserve area – less disturbed.

Size of knapweed population:
146 m x 20-30 m

Density: ranging from ___0_________/m^2 to ___1-2_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Left side or road – 7 tall telephone poles down. 1st pole across from old ruin.

Diffuse also scattered around area. #1 and #2 hybrids.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Seeds collected 2006 from tagged plants.

Site Number: 9 Ukraine, 2006

Location and Contact: Donetsk, Blagodatnoevillage, Novoamurosievka

Date and Time: 6-24-06

GPS Coordinates: N: 47° 51' 43.2" E: 038° 27' 38.5"

Elevation: 84m Slope: none Aspect (slope faces):

Dominant Plant Species:
Coronillavaria, Melilotus, Festuca, Stipa capillata, Elytrigia

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Possibly grazed, moderate disturbance. Sandy soil.

Size of knapweed population:
90 x 10 m Probably plants beyond this surveyed area.

Density: ranging from ___0________ /m^2 to ___1-2________ /m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Alongside road below small hillside. Only 1-2 hybrids out of 50-60 plants.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Seeds collected 2006 from tagged plants.

Site Number: 10 Ukraine, 2006

Location and Contact: Donetsk, road Makecvka – Mineralnoe ravine forest

Date and Time: 6-25-2006

GPS Coordinates: N: 48° 06' 02.4" E: 037° 48' 58.0"

Elevation: 239m Slope: 1-2° Aspect (slope faces): S

Dominant Plant Species:
Euphorb, grassy, salvia

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Vegetated gully near interstate. Sparse diffuse.

Size of knapweed population:
72 m x 10 m

Density: ranging from ___0________/m^2 to ___1________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Centaurea orientalis and C. scabiosifolia present.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Seeds collected 2006 from tagged plants.

Site Number: 11 Ukraine, 2006

Location and Contact: Donetsk, Yasinovatoe, Peskovyjiles Ravine, Krutaja balka

Date and Time: 6-26-06

GPS Coordinates: N: 48° 09' 08.4" E: 037° 50' 26.1"

Elevation: 225 Slope: none Aspect (slope faces):

Dominant Plant Species:
Galatella dracenculoides, Agropyron, Potentilla argentea, Festuca

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Only found along disturbed road and paths. Out-competed

Size of knapweed population:

Density: ranging from ___0________/m² to ___1-2________/m²

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Along road and pasture. Very scattered. Site along two-track inside of forest near crop field.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: 12 Ukraine, 2006

Location and Contact: Donetsk city artificial lakes 15 min. from hotel.

Date and Time: 06-26-06

GPS Coordinates: N: 48° 00' 43.0" E: 037° 47' 16.5"

Elevation: 179m Slope: 1-2° Aspect (slope faces): south

Dominant Plant Species:
Elytrigia, Ambrosia artemisiifolia, Melilotus Achillea

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Dense vegetation with trail running through site.

Size of knapweed population:
24 m x 17 m

Density: ranging from ___0_________/m^2 to ___5-6_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
One strange plant pressed. #24 pressed also. Random sub-set of plants 40 typical diffuse, 17 putative hybrid.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Site Number: 13 Ukraine, 2006

Location and Contact: Petrivka railway station between electrician street and rail. KIEV

Date and Time: 06-28-06

GPS Coordinates: N: 50° 29' 01.2" E: 30° 30' 25.4"

Elevation: 98m Slope: 25° Aspect (slope faces): S

Dominant Plant Species:
Elytrigan, Salsea, Sisyumbrium, Tragopogon pratensis, Artemisia vulgaris, Plantago aranaria, Medicago, Bromus tectorum, C. diffusa, See hand-written sheet for more species listed by Mosyakin.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed roadside. Along rail tracks.

Size of knapweed population:
1/4 mile along road (5 m) and tracks.

Density: ranging from ___0________ /m^2 to ___10________ /m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:
x

Notes:
Random transect in mixed population: 10 diffuse, 7 spotted, 6 hybrid.

Centaurea maculosa, C. diffusa, or hybrid site (circle one)

Seed was collected fall 2006. Bags with 'A' notation were not sampled as part of the 30 surveyed in summer 2006 by Blair. They are additional supplements since not all of the initial plants were there at time of seed harvest (likely mowed). See Mosyakin's additional notes.

Site Number: 14 Ukraine, 2006

Location and Contact: Mixed food factory, Kurenlvka, AGROMARS. KIEV.

Date and Time: 06-28-06

GPS Coordinates: N: 50° 28' 50.7" E: 030° 29' 10.7"

Elevation: 90m Slope: none Aspect (slope faces):

Dominant Plant Species:
Spotted knapweed, Elytrigia repens, Echium vulgare, Erigeron annum, Reseda lutea, Achillea sp, Poa angustifolia See hand-written sheet for more species named by Mosyakin.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Abandoned railway tracks at the mixed food plant.

Size of knapweed population:
50 m x 20 m

Density: ranging from ___1_________/m^2 to ___?_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: 15 Switzerland, 2006

Location and Contact: Gentschenivil, Switzerland, Urs Schaffner

Date and Time: 07-03-06

GPS Coordinates: N: 47° 16' 58.5" E: 008° 08' 51.9"

Elevation: 513m Slope: 45° Aspect (slope faces):

Dominant Plant Species:
Hypericum perforatum, Mix of 2 grass species (Bromus erectus). Centaurea scabiosa

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grassy slope along rail track. Conserved space.

Size of knapweed population:
30 m x 10 m

Density: ranging from ___0_________/m^2 to ___3-5_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Previously noted spotted plants with white flowers. No plants in bloom, but look like typical spotted.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Adams 1, CO

Location and Contact: Adams County Parks and Rec Nature Preserve.

Date and Time: 08-10-2006

GPS Coordinates: N: 39° 56' 31.1" E:104° 51' 43.1"

Elevation: 1513m Slope: NA Aspect (slope faces): NA

Dominant Plant Species:

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Sandy roads and trails running through.

Size of knapweed population: area a couple miles

Density: ranging from ___0________ $/m^2$ to ___2________ $/m^2$

% Cover: ≤1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Low number of diffuse plants. Very few purple.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Adams county 2, CO

Location and Contact: Thornton Gravel Pit, Kelly Uhing

Date and Time: 08-10-2006

GPS Coordinates: N:39° 52' 02.5" E: 104° 55' 30.8"

Elevation: 1551m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Grasses.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Mined for amalgams. Now county land. Disturbed.

Size of knapweed population:
5-10 acres

Density: ranging from ___0_________/m^2 to ___2________/m^2

% Cover: ≤1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
8 miles from Adams. Spotted and diffuse mixed. (Few) Little hybrid plants.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Antelope Creek, OR

Location and Contact: 8-8-06, Eric Coombs

Date and Time: 08-08-2006

GPS Coordinates: N: 44° 50' 34.6" E: 120° 54' 01.7"

Elevation: 593m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed gravel pit.

Size of knapweed population:
1 acre

Density: ranging from ___0________/m^2 to ___5_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Plants collected for GH drying.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Condon, OR

Location and Contact: Eric Coombs

Date and Time: 08-08-2006

GPS Coordinates: N:45° 14' 35.7" E: 120° 10' 55.5"

Elevation:859m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Idahoe fescue, crested wheat, diffuse, sandberg blue, annual rye

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Vacant lot. Disturbance construction.

Size of knapweed population:
1 acrea

Density: ranging from ___________/m^2 to ___________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Plants collected to dry.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Douglas County, CO 1.

Location and Contact: Jonathan Rife, County Weed Agent. Behind Arby's off I-25, exit 184. Castle Rock, CO

Date and Time: 08-11-2006

GPS Coordinates: N: 39° 24' 36.7" E: 104° 52' 12.0"

Elevation: 1848m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Diffuse!

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Large disturbed area behind Arby's.

Size of knapweed population:
3-5 acres

Density: ranging from ___0_________/m^2 to ___8_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Large population. Loads of biocontrol. Little seed.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Douglas County, CO 2

Location and Contact: Jonathan Rife, Crystal Valley Parkway. Castle Rock, Exit 181

Date and Time: 08-11-2006

GPS Coordinates: N: 39° 20' 23.8" E: 104° 49' 53.3"

Elevation: 1960m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Planted grasses + diffuse along fence row.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Recently disturbed. Grated + re-seeded with grass. This entire area is experiencing a lot of development = disturbance, and diffuse has strong foothold. Just driving around, it is everywhere.

Size of knapweed population:
100 m along fence row. All over area.

Density: ranging from 0 /m^2 to 5 /m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Exit 147, I-84, Railyard

Location and Contact: Eric Coombs

Date and Time: 08-07-2006

GPS Coordinates: N: 45° 47' 28.2" E: 120° 01' 51.8"

Elevation: 96m Slope: NA Aspect (slope faces):

Dominant Plant Species:
DIFFUSE

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Railyard. Frequent burns/spray prevents knapweed bugs from establishing.

Size of knapweed population:
4 acres.

Density: ranging from ___1_________/m^2 to __5-10_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Heppner, OR

Location and Contact: Eric Coombs

Date and Time: 08-08-2006

GPS Coordinates: N:45° 20' 34.4" E: 119° 32' 58.1"

Elevation: 646m Slope: Aspect (slope faces):

Dominant Plant Species:
Diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Grazed pasture.

Size of knapweed population:

Density: ranging from ___________/m^2 to ___________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Plants collected to dry. Not quite mature.

Centaurea maculosa, C. *diffusa*, or hybrid site (circle one)

Site Number: Laramie, WY. South of Laramie about 10 miles on dirt road along the railroad tracks.

Location and Contact: Lindsey, Albany County Weed and Pest. 307-660-5399 cell

Date and Time: 09-05-2006

GPS Coordinates: N: 41° 04' 55.1" E: 105° 29' 06.2"

Elevation: 2275m Slope: NA Aspect (slope faces): NA

Dominant Plant Species:
Typical rangeland, grasses, diffuse, dalmation toadflax.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed coal dust on top of granite.

Size of knapweed population:
100m x 30m

Density: ranging from ___0________/m^2 to ___6-8________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:x

Notes:
Plants clumped in distribution. Only 10-11 purple plants/ 100-200 normal diffuse. Purple plants clumped together – in 1 patch really. Strange phenotypes. Some very spotted-like.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Mosier, OR

Location and Contact: I-84, Eric Coombs

Date and Time: 08-07-2006

GPS Coordinates: N: 45° 41' 01.9" E: 121° 24' 08.3"

Elevation: 43m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Mowed, diffuse, dry grasses.

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Highly disturbed.

Size of knapweed population:
50m x 20m

Density: ranging from 0 $/m^2$ to 3-5 $/m^2$

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Entrance of trailer park. Sphenoptera, Larinus minutus, Pterolonche, Flies (both species), Banagasternus.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Rivergate District Port of Portland

Location and Contact: Eric Coombs

Date and Time: 08-09-2006

GPS Coordinates: N:45° 38' 08.8" E: 122° 45' 24.4"

Elevation: 20m Slope: NA Aspect (slope faces):

Dominant Plant Species:
Trifolium, diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):
Disturbed shipping yard.

Size of knapweed population:
1 mile scattered

Density: ranging from ___0_________/m^2 to _____5-10_______/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Plants are spread out in vacant lots.

Centaurea maculosa, *C. diffusa*, or hybrid site (circle one)

Site Number: Tygh Valley, OR

Location and Contact: Eric Coombs

Date and Time: 08-07-2006

GPS Coordinates: N:45° 15' 14.9" E: 121° 09' 05.8"

Elevation: 361m Slope: Aspect (slope faces):

Dominant Plant Species:
Diffuse

Description of surrounding vegetation and land use (e.g. grass, shrubby, mostly native or exotic, disturbed?):

Size of knapweed population:
20 acres

Density: ranging from ___0,3_________/m^2 to ___3_________/m^2

% Cover: <1%, 1-5%, 6-25%, 25-50%, 50-100%

Number of soil samples taken:

Notes:
Pasture area. Thick diffuse. Eric has a transect at this site.

APPENDIX 2 – CHAPTER 1

SOIL ANALYSES AND RESULTS

METHODS

Soil pH

Soil was collected at field sites when possible. Four 6" soil cores were taken randomly throughout the site and combined in a brown paper bag. The soil was air-dried. Soil pH was measured by combining 2 g of soil with 4 ml of distilled water. The solution equilibrated 20 minutes prior to taking the pH.

C:N

Carbon and nitrogen were analyzed on a Leco TruSpec Elemental Determinator (Leco Corp., St. Joseph, MI). Soils were prepared by grinding 10 g with mortar and pestle, and 0.2 g of the homogenized soil was analyzed. Standards and blanks were run initially, in the middle, and at the end of each run. Prior to analysis, soils were assayed for carbonates by adding several drops of 1 M HCl to the soil; if it bubbled, carbonates were present. If present, the carbon in the inorganic calcium carbonates was calculated and subtracted from the total carbon to obtain the organic carbon value. Microbes are only able to utilize organic carbon for their source of food. Therefore, organic carbon cycles on a much faster time scale than inorganic carbon (i.e. found in calcium carbonate). Briefly, to calculate the amount of carbonates, I used the pressure transducer method, which involved weighing out 1 g of soil, adding acid (6 N HCl + 3% ferrous chloride), letting it sit for 2 h, and measuring the pressure that came off of the reaction in that time with a voltage meter (Sherrod et al. 2002). I constructed a standard curve by running soils with known carbonate values ranging from 0.25 to 10%. I also ran blanks and accounted for the voltage produced by subtracting this amount off of my values. I could then calculate the % of carbonates in each sample based on that curve. Samples that had % carbonates beyond the standard curve were rerun with smaller amounts of soil and recalculated.

RESULTS

Soil pH

Soil pH was measured at 21 sites (8 spotted, 11 diffuse). The average pH of all sites measured was 7.47 ± 0.166, with a maximum pH of 8.79 and a minimum pH of 6.12. Soil pH did not differ between spotted and diffuse/hybrid sites ($F_{1,17} = 1.00$, $p = 0.33$).

Soil C:N

I analyzed C:N from 23 sites (11 diffuse, 11 spotted, and 1 diffuse/spotted). Two of the sites had aberrantly high C:N values (110 and 50) and were excluded from the analyses. The overall C:N average was 13.52 ± 0.97, with an upper value of 28.87 and a lower value of 8.76. The C:N did not differ between diffuse and spotted sites ($F_{1,18} = 0.008$, $p = 0.93$). I hypothesized that higher C:N values may be associated with smaller plants; indeed, when looking at population-level mean plant size within spotted knapweed sites (n = 8 sites), all plant size measures were negatively associated with increased C:N, although plant diameter was not a significant association (stem number:

$R^2 = 0.50$, $P = 0.05$; plant height: $R^2 = 0.54$, $P = 0.037$; and plant diameter: $R^2 = 0.23$, $P = 0.23$). While I only had five field sites with both C:N data and plant measurements for diffuse knapweed, the trend was quite different. There was no association with C:N and plant height ($R^2 = 0.008$, $P = 0.88$) and plant diameter ($R^2 = 0.018$, $P = 0.83$), and there was a strong positive association with C:N and stem number($R^2 = 0.96$, $P = 0.003$).

CHAPTER II

HYBRIDIZATION AND INVASION: ONE OF NORTH AMERICA'S MOST DEVASTATING INVASIVE WEEDS HAS UNDERGONE INTERSPECIFIC HYBRIDIZATION

Abstract

Hybridization is hypothesized to play an important role in invasion success. To rigorously examine this hypothesis for a given invasive species, it is critical to verify the presence of hybridization at the molecular level. *Centaurea diffusa* Lam. (diffuse knapweed) and *C. stoebe* L. (spotted knapweed) are two problematic invasive plants in western North America. Some individuals within most diffuse knapweed sites in North America exhibit intermediate diffuse x spotted morphology. I set out to determine if such individuals are indicative of hybridization at the molecular level. I generated 417 polymorphic amplified fragment length polymorphism markers. The Bayesian clustering program, STRUCTURE, correctly placed the parent species and a more distantly related outgroup (*C. pratensis* Thuill) into non-overlapping genetic groups. Approximately one-third of the assayed North American diffuse knapweed individuals exhibited detectable introgression from spotted knapweed. Counter to expectation, the putative hybrids did not show evidence of mixed ancestry more often than the plants that looked like typical diffuse knapweed. Evidence from recent studies suggests that diffuse knapweed was likely introduced with admixed individuals, and the hybrids are not newly created post-introduction. A century of back-crossing with diffuse knapweed has likely decoupled the relationship between morphology and admixture at the molecular level. In regions in the native range where diploid diffuse and spotted knapweed overlap, hybrid swarms are common. In such sites with on-going hybridization, the phenotype aligns more closely with the genotype. In conclusion, I demonstrate for the first time at the molecular level that the invasive plant diffuse knapweed contains admixture from a closely related congener.

Introduction

Hybridization can have profound evolutionary consequences (Stebbins 1959; Arnold 1992; Rieseberg *et al.* 2003; Gompert *et al.* 2006). Recently, attention has focused on the role that hybridization may play in successful biological invasions (Ellstrand & Schierebeck 2000; Rieseberg *et al.* 2007). Hybridization may result in evolutionary novelty and/or increased genetic variation, either of which may provide the genetic material for rapid adaptation to new biotic and abiotic conditions (Ellstrand & Schierenbeck 2000; Rieseberg *et al.* 2007). Additionally, hybridization can cause increased heterozygosity, which may increase fitness (Reed & Frankham 2003). If such heterozygosity is fixed, for example via clonal reproduction or allopolyploidy, a more vigorous line may be permanently established in the new range (Ellstrand & Schierenbeck 2000). The outcomes of hybridization, however, are not always positive and can often result in negative effects like outbreeding depression, as two disparate genomes are brought together (Price & Waser 1979). Yet, even if low fitness is the rule for most early generation hybrid individuals, gene flow and the creation of new evolutionary lineages is still possible (Arnold *et al.* 1999).

In a review of plant hybridization and invasion, 28 examples were found where invasiveness occurred after interspecific hybridization, and approximately 24 additional examples were found but not supported with molecular evidence (Ellstrand & Schierenbeck 2000). For example, Gaskin and Schaal (2002) discovered through DNA sequence data that the invasive *Tamarix* in North America is a hybrid undetected in the native range. The authors posit that multiple introductions brought together historically isolated genotypes from the native range. Another example is *Spartina anglica*, an allopolyploid hybrid capable of invading salt marshes and becoming a dominant species across a variety of such habitats (Thompson 1991). This hybrid differs significantly from its parent species, which do not demonstrate this aggressive, dominating capability (Thompson 1991). Thus, it appears hybridization may play an important role in some invasions. Presently, hybridizing non-native species warrant intense scrutiny and should be 'guilty until proven innocent,' as enough is not yet known about the importance of this mechanism in invasion.

This chapter focuses on spotted and diffuse knapweed, two of the most ecologically and economically devastating invasive plants in western North America (Watson & Renney 1974; Rochè & Rochè 1991; Sheley *et al.* 1999). The diploid variants of these species are capable of hybridization (Ochsmann 1998, 1999), and almost 100 years ago in the native range, Gáyer (1909) described and named the spotted x diffuse hybrid *Centaurea* x*psammogena*. I refer to such individuals here and elsewhere (Chapters 1 and 3) as hybrid-like, as the designation of 'hybrid' is based on morphological, and not molecular data. In a recent study conducted across western North America, such hybrid-like plants were found in 38 out of 39 diffuse knapweed sites, but none of the spotted knapweed sites (Chapter 1). In that same chapter, it was suggested that the hybrids were introduced with diffuse knapweed and created post-introduction (discussed below). While the plants with intermediate morphology in North America are suggestive of hybridization between the two knapweeds, their presence has been interpreted in a variety of ways. For example, Watson and Renney (1974) suggested that 'the degree of variation within the diffuse knapweed populations is possibly due to more than one introduction of the species into the area, and that the variable genotypes expressed by flower color in diffuse knapweed populations may be due to loose multiple gene control' rather than hybridization. Moore and Frankton (1954) reached a similar conclusion that putative hybrids are simply morphological variants of diffuse knapweed in North America. Contrary to these conclusions, Ochsmann (2001a) argued that *C.* x*psammogena* is present in North America based on herbaria records from seven different states in the USA. By conducting cross-continent comparisons of the species in their native range,(Chapter 1) I provided several lines of evidence suggesting that such plants are likely of hybrid origin; for example, regions exist in the native range where typical diffuse knapweed is present in the absence of putative hybrids. This is different from the nearly ubiquitous presence of hybrid-like plants in North American diffuse knapweed sites and suggests something more than morphological variation.

The goal of this chapter was to definitively resolve this debate by examining whether hybrid-like individuals are indicative of hybridization at the molecular level. I also wanted to gain a deeper understanding of morphological patterns encountered in the native and introduced ranges. Thus, I used Amplified Fragment Length Polymorphisms (AFLPs) (Vos *et al.* 1995) to examine hybridization between spotted and diffuse knapweed at the genome level.

Materials and methods

Study Species

The genus *Centaurea* L. (Asteraceae) contains approximately 300 species (Garcia-Jacas *et al.* 2006), a number of which have been introduced globally and become invasive. In North America, at least 34 *Centaurea* species have been introduced, at least 14 of which are defined as noxious weeds in one or more states (http://plants.usda.gov/). The taxonomy of the genus is complicated: sections within the genus are still being revised, and relationships within sections are not well resolved (Garcia-Jacas *et al.* 2006). Thus, there is some uncertainty regarding which taxa are actually present in North America, and the nomenclature is complex.

My research focused on members of the *Centaurea* genus within the section Acrolophus-Phaelolepsis (Garcia-Jacas *et al.* 2006). More specifically, I focused on two members, *C. stoebe* (sensu stricto) and *C. diffusa* and their hybrids, of the *C. stoebe* (sensu latto) species group. This group encompasses approximately 33 named taxa (Ochsmann 2000). It is reported that both species have diploid (2n = 18) and tetraploid (4n = 36) cytotypes (Ochsmann 2000). Both cytotypes of diffuse knapweed are referred to simply as *C. diffusa* Lam. The tetraploid has only been reported twice in the literature from one specimen in Bulgaria (Löve 1979) and one in Yugoslavia (Löve 1978). The diploid is more common and likely the only cytotype in North America (A.C. Blair, unpublished data; Marrs et al. 2008). The two cytotypes of spotted knapweed are both under *C. stoebe* L., a name that takes precedence over the commonly used *C. maculosa* (Ochsmann 2000). The monocarpic diploid is designated *C. stoebe* subsp. *stoebe* L., and the polycarpic tetraploid is designated *C. stoebe* subsp. *micranthos* (Gugler) Hayek (for which *C. biebersteinii* DC. is a synonym). Ploidy number is the only way to unambiguously distinguish these sub-species (Ochsmann 2001b). In the literature the few North American spotted knapweed plants that have been assayed for chromosome number are tetraploids (Moore & Frankton 1954; Müller 1989). Both spotted and diffuse knapweed are self-incompatible (A.C. Blair, personal observation; Harrod & Taylor 1995).

Floral traits are often used to distinguish species in the *Centaurea* genus. Spotted knapweed has larger flowering heads than diffuse knapweed, and is characterized by a pronounced dark spot on each bract, while diffuse knapweed has a terminal spine on each bract and no pigmentation (Watson & Renney 1974; Ochsmann 2000). Spotted knapweed flowers are purple (rarely white), while diffuse knapweed flowers are white (rarely pink). The hybrid *C.* x*psammogena* is characterized by distinct spotted bracts in addition to a terminal spine (Ochsmann 2000). Individual inflorescences often have both purple ray flowers and white disc flowers.

Spotted and diffuse knapweed were accidentally introduced into North America from Eurasia in the late 1800s or early 1900s (Watson and Renney 1974; Roché and Roché 1991); both species were likely introduced several times (Hufbauer & Sforza 2008). They have become major threats to rangeland productivity and quality across western North America (Watson & Renney 1974; Roché & Roché 1991; Sheley *et al.* 1999). These plants increase soil erosion (Lacey *et al.* 1989; Sheley *et al.* 1997), can alter plant community composition (Tyser & Key 1988), negatively impact biodiversity (Ortega *et al.* 2006), and are thought to have allelopathic effects on other plants (Fletcher & Renney 1963; Callaway & Aschehoug 2000; but see Locken & Kelsey 1987; but see Blair *et al.* 2005, 2006).

Collection Sites and Specimens

Tissue for molecular analysis was collected across Europe and North America (Table 2.1), following the sampling approach successfully employed to study interspecific hybridization at the molecular level by O'Hanlon *et al.* (1999), Kronforst *et al.* (2006), and Gompert *et al.* (2006). To evaluate hybridization at the interspecific level, it is critical to obtain samples of the relevant parent species. Spotted x diffuse hybrids are diploid (A.C. Blair, unpublished data; Ochsmann 1998, 1999), so morphologically typical diploid diffuse knapweed and diploid spotted knapweed from the native range were included in the molecular analysis as the parent species (hereafter, 'spotted knapweed' and 'diffuse knapweed' refer to the diploid variants unless otherwise stated). Additionally, to determine if the AFLP technique could reliably detect recent hybridization, individuals from apparently active hybrid zones in the Ukraine were included as positive controls; such sites were never encountered in North America (Chapter 1). To obtain the parental species and the positive controls, I therefore sampled individuals in Europe (Fig. 2.1; Table 2.1) from three site types: 1) diffuse knapweed, 2) spotted knapweed and 3) active hybrid zones of spotted x diffuse knapweed. To determine if interspecific hybrids are present in North American diffuse knapweed sites, I analyzed individuals from sites that contained both morphologically typical diffuse knapweed and hybrid-like plants. I included approximately equal numbers of typical diffuse knapweed and hybrid-like individuals from each site (see Cluster Analysis below). Additionally, I included a more distantly related outgroup species, meadow knapweed (*C. pratensis* Thuill), to test the ability of my markers to distinguish between the closely related spotted and diffuse knapweed. If I could not clearly distinguish the two species with AFLP markers, inclusion of this outgroup would help discern between inherent technique and analysis problems versus actual difficulty in distinguishing between the species because of close relatedeness. Thus, in North America (Fig. 2.2; Table 2.1) I sampled individuals from two site types: 1) diffuse knapweed + hybrid-like plants and 2) meadow knapweed. Tissue was either collected in the field and dried by temporary storage in Drierite (W.A. Hammond Drierite Co., Xenia, OH) prior to transfer to a -80 freezer, or collected fresh from plants grown from seed in the greenhouse. As floral traits are critical in distinguishing hybrids and parental species, in sites in the Ukraine and North America that contained hybrid-like plants, I recorded the following floral traits per plant included in the molecular analyses: flower color ranked 1 (white) to 5 (solid purple), bract pigmentation ranked 0 (no spot, golden) to 3 (deeply pigmented), capitula width, capitula length, and spine length averaged from three spines per plant.

DNA Extraction

Total DNA was extracted from 95 individuals from fresh (100 mg) or dry (25 mg) leaf tissue with QIAGEN Mini Plant Extraction kits (QIAGEN Inc., Valencia, CA USA). Leaf tissue from individual plants was ground under liquid nitrogen with a mortar and pestle, and then the Qiagen protocol was followed.

AFLP Protocol

The AFLP method followed Vos *et al.* (1995) but included the following changes: restriction and ligation were performed during a single step in an 11-µl reaction containing genomic DNA, 1 U *Mse*I, 5 U *Eco*RI, 1X T4 DNA ligase buffer, 60 U T4

DNA ligase, 0.05 *M* NaCl, 0.5X BSA, 4.5 μ*M* MseI adaptor, 0.45 μ*M* EcoRI adapter, and water. This mixture was incubated at room temperature overnight. The next day, 5.5 μl of the reaction was diluted to 100 μl in TE (15 m*M* Tris and 0.1 m*M* EDTA). A preselective polymerase chain reaction (PCR) was performed in a 20-μl reaction containing the following: 4 μl of the diluted restriction-ligation product, 1X PCR buffer, 1.5 m*M* $MgCl_2$, 0.2 m*M* each dNTP, 0.2 μ*M* of each preselective amplification primer (*Mse*I + C and *Eco*RI + A), 0.5 U Taq polymerase, and water. The preselective PCR cycles were as follows: 20 cycles of 30 s at 94 C, 60 s at 56 C, and 60 s at 72 C. Ten microliters of the preselective amplification product was diluted to 200 μl in TE (15 *m*M Tris and 0.1 *m*M EDTA). The selective amplification was performed in a 20 μl reaction with 3 μl of the diluted preselective amplification product. The following reagents were included in the reaction: 1X PCR buffer, 1.5 *m*M $MgCl_2$, 0.2 *m*M each dNTP, 0.1 μ*M* *Mse*I selective primer, 0.05 μ*M* *Eco*RI selective primer dye-tagged with D4 (blue), 0.5 U of Taq polymerase, and water. The selective PCR cycles were as follows: 120 s at 94 C, 10 cycles of 20 s at 94 C and 30 s at 66 C (decreasing by 1 C each cycle), 120 s of 72 C; 25 cycles of 20 s at 94 C, 30 s at 56 C, and 120 s at 72 C, and a final 30 minutes at 60 C. One microliter of each selective PCR product was combined with 0.3 μl of 600 bp size standard and 28.7 μl of deionized formamide. All selective primer combinations of *Mse*I + CAA, CAC, CAT, CTA, or CTC and *Eco*RI + AAG, ACC, or ACT were prescreened with five individuals, and the three most polymorphic primer pairs were chosen (*Mse*I + CAC/*Eco*RI + AAG; *Mse*I + CAT/*Eco*RI + AAG; and *Mse*I + CTA/*Eco*RI + AAG). Samples were analyzed on a Beckman Coulter CEQ 8000 fragment analyzer.

AFLP Data Analyses

AFLP fragments between 100 and 600 bp were scored using the fragment analysis software Genemarker® (Softgenetics®, State College, PA). Initially, I set the program to call only peaks above 200 reflectance units; thus, bins for markers were created that had at least one peak > 200 reflectance units. I then ran these data with the new bin set and lowered the threshold to 100 reflectance units. This approach was used to minimize ambiguity in subjectively defining 'real' peaks. After these two passes of the data, I went through each electropherogram trace by hand to ensure that peaks were correctly called and placed in the appropriate bins. If a peak was a borderline call (i.e. around 100 reflectance units), I compared it to other traces to see if the shape and position matched other individuals for that marker.

To test the repeatability of the method, 10 individuals (≈ 10%) were selected at random, and AFLP fragments were generated starting from the restriction/ligation step for each of the three primer pairs. Repeat runs were scored blindly and compared to original runs to calculate the error rate.

Statistical Analysis

I used a Bayesian clustering method (STRUCTURE v. 2.2; Pritchard *et al.* 2000; Falush *et al.* 2007) to determine if the AFLP markers could (1) distinguish amongst the three species (spotted knapweed, diffuse knapweed, and meadow knapweed) and (2) detect interspecific hybridization between spotted and diffuse knapweed (e.g. Kronforst et al. 2006). Briefly, STRUCTURE works as follows: a model is used which assumes there are *K* populations (either known or unknown), and each of these *K* populations is defined by a unique set of allele frequencies at each locus. STRUCTURE then assigns individuals to these populations based on their allele frequencies, while at the same time, estimating population allele frequencies. The most recent version of STRUCTURE (v. 2.2) can analyze dominant markers, like AFLPs, by defining a null allele at each locus (Falush *et al.* 2007). STRUCTURE assumes Hardy-Weinberg equilibrium within populations and linkage equilibrium between loci within populations (Pritchard *et al.* 2000). The populations in this study are three species and likely violate those assumptions. The program should, however, still perform adequately as species level differences should overwhelm violations of the assumptions. For each analysis, I had a burn-in length of 10,000 iterations; an adequate burn-in length is critical to minimize the effect of the starting configuration. This was followed by 100,000 iterations of data collection; an appropriate number of iterations is necessary to obtain accurate parameter estimates. These two values produced highly consistent results across runs, and the summary statistics were stable before the end of the burn-in. For all runs, I provided only genetic data to the model with no prior information about the location of collection or morphological species status.

I assumed that 3 genetic clusters would likely best explain the data, as among species differences would presumably be greater than within species differences. I validated that $K = 3$ clusters yields the highest log-likelihood probability by running replicated runs of $K = 2, 3, 4, 5$, and 6 with the admixture model in STRUCTURE (Fig. 2.3). I then used the admixture model ($K = 3$) to estimate the proportion of each individual's genotype (q) from the K populations. Using the ANCESTDIST option in STRUCTURE, I computed the 95% posterior probability interval around each individual's admixture proportion. If an individual's probability proportion did not include one, introgression was likely to have occurred.

All other statistical analyses were conducted using JMP v. 6.0 (SAS Institute Inc., Cary, North Caroline, USA). Based on both morphology and the molecular data (see below), hybridization was detected within the North American diffuse knapweed sites. I therefore wanted to know if the phenotype matched the genotype (i.e. did morphological hybrids demonstrate significant admixture at the molecular level?). In North American diffuse knapweed sites (n = 9 sites), individuals included in the molecular analysis were visually classified as 'diffuse' or 'hybrid' based on observations of floral characters. To objectively classify plants and to determine if my field observations resulted in two distinct clusters (i.e. diffuse and hybrid), I used hierarchical clustering with the five floral traits recorded in the field. Prior to analysis, data were standardized by the variable mean and standard deviation. I used 'Ward's minimum variance' clustering method. Thirty-one out of the 36 North American individuals had data for all five traits and were included in the analysis.

To determine if North American individuals in the morphological hybrid cluster (see above) exhibited greater admixture from spotted knapweed than plants that morphologically clustered as typical diffuse knapweed, ANOVA was used to compare the posterior mean proportion of ancestry associated with the diffuse knapweed cluster between the two plant types. I then used this same approach to compare plants from three active hybrid swarms in the Ukraine. Due to the relatively small number of individuals (n=12), I used data from a cluster analysis that included several hundred individuals to place the plants in to one of three morphological clusters: spotted, diffuse, or hybrid (Chapter 1). I then used a Tukey-Kramer post-hoc test to determine which plant types had different posterior mean proportions of ancestry associated with the diffuse knapweed cluster.

Results

AFLP Analyses

I used a total of 417 AFLP loci after removing 67 uninformative markers that were either present in all individuals surveyed or found in only one individual. In the duplicated runs to examine the consistency of this technique, ≈ 94% of the bands were scored similarly across the three primer pairs.

With the admixture model in STRUCTURE (v. 2.2), all European spotted and diffuse knapweed and North American meadow knapweed individuals had a population of origin genome probability interval that included one, indicating that admixture was unlikely in those groups (Fig. 2.4). As predicted based on morphology, those species groups seem genetically isolated, and the admixture model performed well at distinguishing at the species level.

Within the actively hybridizing sites in the Ukraine, 11 out of 12 individuals had population of origin genome probability intervals that did not include one, and all individuals had pure ancestry proportions < 0.9 (Fig. 2.4). The admixture model found many individuals with mixed ancestry in the North American diffuse knapweed sites (Fig. 2.4). Thirteen out of 36 individuals had population of origin genome probability intervals that did not include one (Table 2.1), and eighteen out of the same 36 individuals had pure ancestry proportions < 0.9.

Based on the hierarchical cluster analysis of the floral characters from the plants within the North American diffuse knapweed sites, two clusters were identified with 17 and 14 members. The first cluster was dominated by plants identified visually as diffuse knapweed in the field (15 out of 17), while the second cluster contained only plants identified as hybrid-like in the field (14 out of 14) (Fig. 2.5). While the presence of individuals with intermediate floral morphology encountered in North American diffuse knapweed sites correctly suggested interspecific hybridization, the floral morphology did not correctly predict the genetic classification within the North American sites (Fig. 2.5). Out of the 31 individuals included in the hierarchical cluster analysis, STRUCTURE only identified half (16/31) as predicted by the phenotype (i.e. a plant that looked like typical diffuse had a population of origin genome probability interval that included one). In fact, counter to expectation, six out of 17 individuals in the morphological diffuse cluster demonstrated admixture from spotted knapweed, while nine of the 14 individuals in the morphological hybrid cluster did not exhibit evidence of mixed ancestry. The two morphological clusters had the same posterior mean proportion of ancestry associated with the diffuse knapweed cluster ($F_{1,29} = 0.003$, $P = 0.95$; morphological diffuse cluster = 0.86, morphological hybrid cluster = 0.86).

Of the 12 individuals included from the actively hybridizing Ukraine sites, based on floral morphology, three plants were classified as diffuse knapweed, six as hybrid-like, and three as spotted knapweed (Chapter 1). Contrary to the North American data, plants that appeared more like typical diffuse knapweed had a significantly greater posterior mean proportion of ancestry associated with the diffuse knapweed cluster than those that appeared visually as typical spotted knapweed (Fig. 2.6). As predicted by morphology, hybrids were intermediate between the two. Interestingly, the plants that appeared as typical diffuse knapweed in the hybrid swarms exhibited greater levels of admixture than either the pure European diffuse knapweed or the North American diffuse + hybrid-like plants (Fig. 2.6).

Discussion

I have shown at the molecular level that some individuals of the North American noxious weed, diffuse knapweed, contain detectable admixture from a closely related species, spotted knapweed. STRUCTURE identified evidence of mixed ancestry in 36% of the assayed plants in the North American diffuse knapweed sites. Thus, I have definitively identified a new example of an invasive organism that has undergone interspecific hybridization. Neither parental species from the native range or the outgroup meadow knapweed demonstrated such admixture, while 92% of the plants in the hybrid swarms in the Ukraine were of hybrid origin.

Recent evidence most parsimoniously suggests that the hybrid-like plants encountered in North American diffuse knapweed sites are not from recent spotted x diffuse knapweed hybridization events in the introduced range (Chapter 1). It now seems that only tetraploid spotted knapweed is likely present in North America (H. Müller-Schärer, personal communication; R.A. Marrs, unpublished data), while the diffuse knapweed is diploid (A.C. Blair, unpublished data; R.A. Marrs *et al.* 2008). Triploids have never been found in North America (A.C. Blair, unpublished data; Moore & Frankton 1954), and multiple attempts to create F1 hybrids between North American tetraploid spotted and diploid diffuse knapweed from various populations failed, in spite of the successful production of other crosses (i.e. European diploid spotted knapweed x North American diploid diffuse knapweed). Apparently, genetic incompatibilities between tetraploid North American spotted and diploid diffuse knapweed largely prevent successful mating. Therefore, hybrids were most likely introduced with diffuse knapweed at the turn of the century. While the specific location(s) of where diffuse knapweed was introduced from are unknown, it seems conceivable that this plant was introduced one or multiple times from the regions where diploid spotted and diffuse knapweed overlap and hybridize in certain parts of Romania or the Ukraine (U. Schaffner, personal communication).

Floral traits correctly suggested the presence of hybridization in both the Ukraine and North America; however, individuals in North America with intermediate floral traits were no more likely to show evidence of mixed ancestry than those that appeared as typical diffuse knapweed (Fig. 2.5). It is likely that the diagnostic floral traits are controlled by a small number of genes, and the randomly distributed AFLP markers were probably not located within those genes. This is similar to the situation that Kronforst *et al.* (2006) encountered; a butterfly they morphologically identified as a hybrid had a population of origin genome proportion interval that included one when admixture clustering was implemented with AFLP data in STRUCTURE, indicating the individual was not of hybrid origin. They used wing pattern to diagnose hybridization between butterflies, and concluded that 'within a few generations of initial hybridization, many individuals with hybrid ancestry are unlikely to be distinguishable based on phenotype alone.' Wing patterning provides few loci for determining ancestry of a butterfly (Kronforst *et al.* 2006), perhaps similar to floral morphology in the knapweeds.

Additionally, in the introduced range diffuse knapweed has been isolated from diploid spotted knapweed for approximately 100 years. Reproductive barriers do not exist between hybrid-like individuals and those with typical diffuse knapweed morphology within a site; seeds from hybrid-like plants often result in plants with typical diffuse knapweed morphology and vice versa (Chapter 1). There has been ample time for genetic shuffling, and the floral hybrid traits may no longer be strongly associated with hybridization, *per se*. It is interesting that after a century, portions of the spotted knapweed genome have been retained in some diffuse knapweed individuals. This long period of time might also explain why I did not detect hybridization in some of the diffuse knapweed sites that contained morphological hybrids. Extensive back-crossing and drift have possibly erased the signature of hybridization in some locations.

In the Ukraine, I encountered several sites where there were morphologically typical parental species and a gradient of intermediate plants. Individuals within those sites that grouped with the diffuse or spotted knapweed clusters based on floral characters still demonstrated admixture. Different than within North America, however, plants that appeared more similar to diffuse knapweed had a greater posterior mean proportion of ancestry associated with the diffuse knapweed cluster, while plants that appeared more similar to spotted knapweed had a greater posterior mean proportion of ancestry associated with the spotted knapweed cluster. In locations of recent and/or on-going hybridization, it seems that the floral morphology is associated with the predicted species at the genetic level. These data further support that the hybrid-like plants in North America are not newly created.

Two individuals from a diffuse knapweed site near Yakima, WA, USA had a significant portion of their genome that grouped with the meadow knapweed cluster (Fig. 2.5). Interestingly, when I ran the same analysis but with $K = 4$ clusters (data not shown), the fourth cluster was only present in those same two individuals, which no longer were assigned to the meadow knapweed cluster. This suggests that some of the diffuse knapweed individuals from this site have hybridized with a presently unidentified species. As *Centaurea* species are known to hybridize frequently in their native range (Ochsmann 2000), it is possible that I have detected a separate instance of diffuse knapweed either introduced as an inter-specific hybrid or currently undergoing hybridization with a different introduced *Centaurea* species.

In conclusion, some diffuse knapweed plants in North America contain detectable admixture from diploid spotted knapweed, and I found one instance that suggests diffuse knapweed may contain introgression from a presently unidentified species. While morphological floral traits in the field correctly suggested the presence of hybridization in North America, the individual phenotype did not align with the genotype; individuals with diffuse knapweed morphology often showed evidence of mixed ancestry, while hybrid-like plants often did not. These discrepancies likely result from the long time period since the hybridization event prior to introduction. In sites where hybridization is ongoing in the Ukraine, the genotype and phenotype were more closely aligned. Further research is exploring whether the inclusion of hybrids in the introduction of diffuse knapweed influenced the invasion process, as predicted by Ellstrand and Schierenbeck (2000).

References

Arnold ML (1992) Natural hybridization as an evolutionary process. Annual Review in Ecology and Systematics, 23, 237-261.

Arnold ML, Bulger MR, Burke JM, Hempel AL, Williams JH (1999) Natural hybridization: how low can you go and still be important? Ecology, 80, 371-381.

Blair AC, Hanson BD, Brunk GR, Marrs RA, Westra P, Nissen SJ, Hufbauer RA (2005) New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecology Letters, 8, 1039-1047.

Blair AC, Nissen SJ, Brunk GR, Hufbauer RA (2006) A lack of evidence for a role of the putative allelochemical (±)-catechin in spotted knapweed invasion success. Journal of Chemical Ecology, 32, 2327-2331.

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science, 290, 521-523.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the USA, 97, 7043-7050.

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes, 7, 574-578.

Fletcher RA, Renney AJ (1963) A growth inhibitor found in *Centaurea* spp. Canadian Journal of Plant Science, 43, 475-481.

Gaskin JF, Schaal BA (2002) Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proceedings of the National Academy of Sciences of the USA, 99, 11256-11259.

Garcia-Jacas J, Uysal T, Romashchenko K, Suarez-Santiago VN, Ertugrul K, Susanna A (2006) *Centaurea* revisited: a molecular survey of the *Jacea* group. Annals of Botany, 98, 741-753.

Gáyer G (1909) Vier neue Centaureen der Flora Botanikai von Ungarn. Magyar Botanikai Lapok, 8, 58-61.

Gompert Z, Fordyce JA, Forister ML, Shapiro AM, Nice CC (2006) Homoploid hybrid speciation in an extreme habitat. Science, 314, 1923-1925.

Harrod RJ, Taylor RJ (1995) Reproduction and pollination biology of *Centaurea* and *Acroptilon* species, with emphasis on *C. diffusa*. Northwest Science, 69, 97-105.

Hufbauer RA, Sforza R (2008) Multiple introductions of two invasive *Centaurea* taxa inferred from cpDNA haplotypes. Diversity and Distributions, 14, 252-261.

Lacey JR, Marlow CB, Lane JR (1989) Influence of spotted knapweed (*Centaurea maculosa*) on surface water runoff and sediment yield. Weed Technology, 3, 627-631.

Lee CE (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution, 17, 386-391.

Locken LJ, Kelsey RG (1987) Cnicin concentrations in *Centaurea maculosa*, spotted knapweed. Biochemical Systematics and Ecology, 15, 313-320.

Löve A (1978) IOPB Chromosome Number Reports LIX. Taxon, 27, 53-61.

Löve A (1979) IOPB Chromosome Number Reports LXIV. Taxon, 28, 391-408.

Kronforst MR, Young LG, Blume LM, Gilbert LE (2006) Multilocus analyses of admixture and introgression among hybridizing *Heliconius* butterflies. Evolution, 60, 1254-1268.

Marrs RA, Sforza R, Hufbauer RA (2008) When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biological Invasions. DOI 10.1007/s10530-007-9153-6.

Moore RJ, Frankton C (1954) Cytotaxonomy of three species of *Centaurea* adventive in Canada. Canadian Journal of Botany, 32, 182-186.

Müller H (1989) Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.:Cochylidae). Weed Research, 29, 103-111.

Ochsmann J (1998) Ein bestand von *Cenaurea* x*psammogena* Gáyer (*Centaurea diffusa* Lam. x *Centaurea stoebe* L.) am NSG Sonnenstein (Thüringen). Florist. Rundbr., 31, 118-125.

Ochsmann J (1999) Chromosomenzahlen einiger europäischer *Centaurea*-Sippen. Haussknechtia, 7, 59-65.

Ochsmann J (2000) Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. - Diss. Bot. 324 (Ph.D. Dissertation).

Ochsmann J (2001a) An overlooked hybrid in North America: *Centaurea* x *psammogena* Gáyer (diffuse knapweed x spotted knapweed). Oral presentation abstract In: The First International Symposium of the Twenty-First Century (ed. Smith L), p. 76. Coeur d'Alene, Idaho.

Ochsmann J (2001b) On the taxonomy of spotted knapweed (*Centaurea stoebe* L.). In: The First International Knapweed Symposium of the Twenty-First Century (ed. Smith L). pp. 33-41. Couer d'Alene, ID.

O'Hanlon PC, Peakall R, Briese DT (1999) Amplified fragment length polymorphism (AFLP) reveals introgression in weedy *Onopordum* thistles: hybridization and invasion. Molecular Ecology, 8, 1239-1246.

Ortega YK, McKelvey KS, Six DL (2006) Invasion of an exotic forb impacts reproductive success and site fidelity of a migratory songbird. Oecologia, 149, 340-351.

Price MV, Waser NM (1979) Pollen dispersal and optimum outcrossing in *Delphinium nelsoni*. Nature, 277, 294-297.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conservation Biology, 17, 230-237.

Rieseberg LH, Raymond O, Rosenthal DM *et al.* (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. Science, 301, 1211-1216.

Rieseberg LH, Kim SC, Randell RA *et al.* (2007) Hybridization and the colonization of novel habitats by annual sunflowers. Genetica, 129, 149-165.

Roché BF, Roché CT (1991) Identification, introduction, distribution, and economics of *Centaurea* species. In: Noxious Range Weeds (eds. James LF, Evans JO, Ralphs MH, Child RD), pp. 274-291. Westview Press, Boulder, CO.

Sheley RL, Olson BE, Larson LL (1997) Effect of weed seed rate and grass defoliation level on diffuse knapweed seedlings. Journal of Range Management, 50, 39-43.

Sheley RL, Jacobs JS, Carpinelli ML (1999) Spotted knapweed. In: Biology and Management of Noxious Rangeland Weeds (eds. Sheley RL, Petroff JK), pp.350-361. Oregon State University Press, Corvallis, OR.

Stebbins GL (1959) The role of hybridization in evolution. Proceeding of the American Philosophical Society, 103, 231-251.

Thompson JD (1991) The biology of an invasive plant. BioScience, 41, 393-401.

Tyser RW, Key CH (1988) Spotted knapweed in natural areas fescue grasslands – an ecological assessment. Northwest Science, 62, 151-160.

Vos P, Hogers R, Bleeker M *et al.* (1995) A new technique for DNA-fingerprinting. Nucleic Acids Research, 23, 4407-4414.

Watson AK, Renney AJ (1974) The biology of Canadian weeds: *Centaurea diffusa* and *C. maculosa.* Canadian Journal of Plant Science, 54, 687-701.

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Table 2.1. Sites of European diffuse knapweed, spotted knapweed (verified diploid), and spotted x diffuse knapweed hybrid swarms, and North American diffuse knapweed + hybrid-like plants and meadow knapweed used in the AFLP analyses.

Site ID	State/Country	Species	GPS	number of introgressed individuals†/total
Europe				
Ro 6	Romania	Diffuse knapweed	N 45°11'8.8" E 28°47'8.3"	0/4
Ro 5	Romania	Diffuse knapweed	N 44°94'34.3" E 28°91'4.5"	0/4
Ro 4	Romania	Diffuse knapweed	N 44°23'22.8" E 28°31'35.9"	0/4
Crimea 21	Ukraine	Diffuse knapweed	N 44°33'0.0" E 34°"16'0.0"	0/4
Crimea 20	Ukraine	Diffuse knapweed	N 44°36'0.0" E 34°10'0.0"	0/3
Rus 1119	Russia	Diffuse knapweed	N 44°3'0.0" E 43°3'36"	0/4
Rus 1142	Russia	Diffuse knapweed	N 51°22'48" E 56°48'0.0"	0/4
UA 2-2n-SK	Ukraine	Spotted knapweed	N 49°55'48.5" E 24°50.1'8.9"	0/5
UA 5-2n-SK	Ukraine	Spotted knapweed	N 49°46"19.2' E 27°17.5'27.6"	0/5
SUAC-2n-SK	Ukraine	Spotted knapweed	N 49°13'13.4" E 24°42.3'17.6"	0/6
UA 6	Ukraine	Active hybrid zone	N 48°34'51.9" E 37°54'36.9"	4/4
UA 4	Ukraine	Active hybrid zone	N 48°53'31.2" E 30°40'33.2"	3/4
UA 14	Ukraine	Active hybrid zone	N 50°28'50.7" E 30°29'10.7"	4/4
North America				
1 W.USA	CO, USA	Diffuse knapweed + hybrid-like	N 39°40'17.0" W102°33'01.3"	1/4
6 W.USA	WA, USA	Diffuse knapweed + hybrid-like	N 46°35'06.7" W120°27'33.0"	2/4
11 W. USA	WA, USA	Diffuse knapweed + hybrid-like	N 47°33'40.4" W120°16'11.3"	0/4
13 W.USA	WA, USA	Diffuse knapweed +	N 47°28'14.4" W120°20'11.5"	1/4

		hybrid-like		
20 W.USA	WA, USA	Diffuse knapweed + hybrid-like	N 46°43'16.9" W117°9'50.5"	0/4
41 W.USA	WY, USA	Diffuse knapweed + hybrid-like	N 43°23'07.9" W107°03'45.6"	4/4
43 W. USA	CO, USA	Diffuse knapweed + hybrid-like	N 39°42'10.2" W106°40'32.8"	4/4
25 W.USA	OR, USA	Diffuse knapweed + hybrid-like	N 45°36'17.1" W121°11'02.3"	1/4
21 W.USA	OR, USA	Diffuse knapweed + hybrid-like	N 45°54'58.8" W119°33'31.8"	0/4
Cow Creek	OR, USA	Meadow knapweed	N 42.9131 W123°31.6'36.8"	0/2
Wyeth	OR, USA	Meadow knapweed	N 45°41'20.0" W121°47'56.6"	0/2

†Significant introgression is assumed if the 95% posterior probability interval around the individual's admixture proportions did not include 1.

Figure 2.1. European site locations for diffuse knapweed, spotted knapweed (2n), and active hybrid swarms (i.e. both parent species and a morphological gradient of hybrids) used in AFLP analyses.

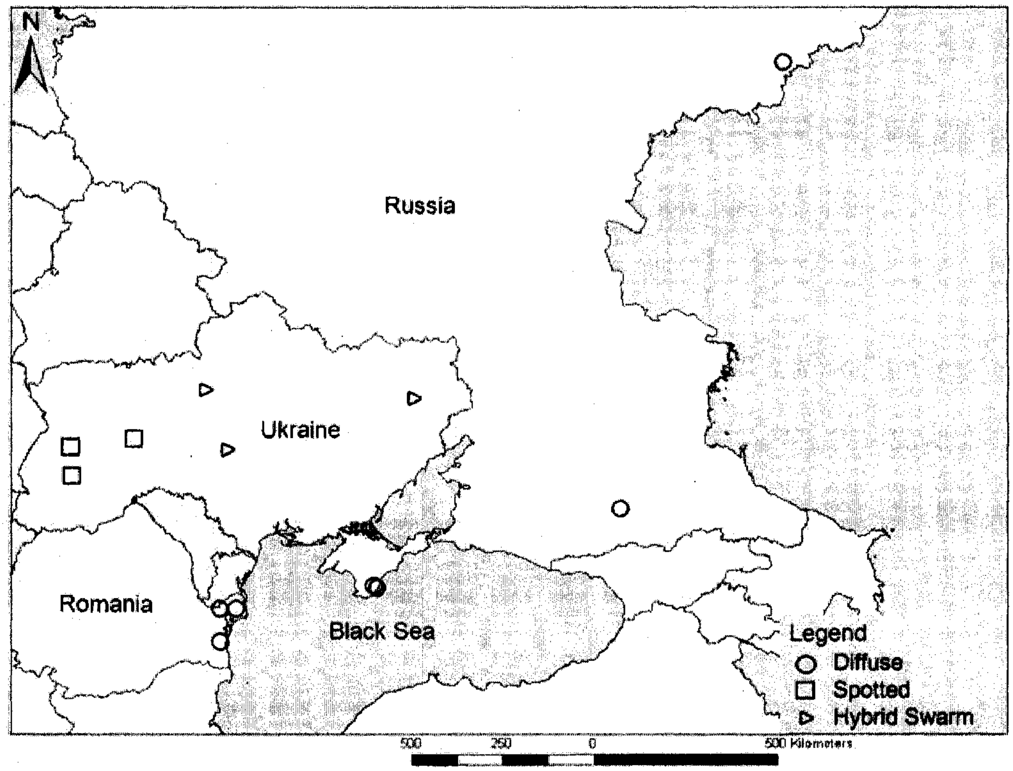


Figure 2.2. North American site locations for diffuse knapweed + hybrid-like plants and meadow knapweed used in AFLP analyses. All diffuse knapweed sites contained hybrid-like plants at varying frequencies.

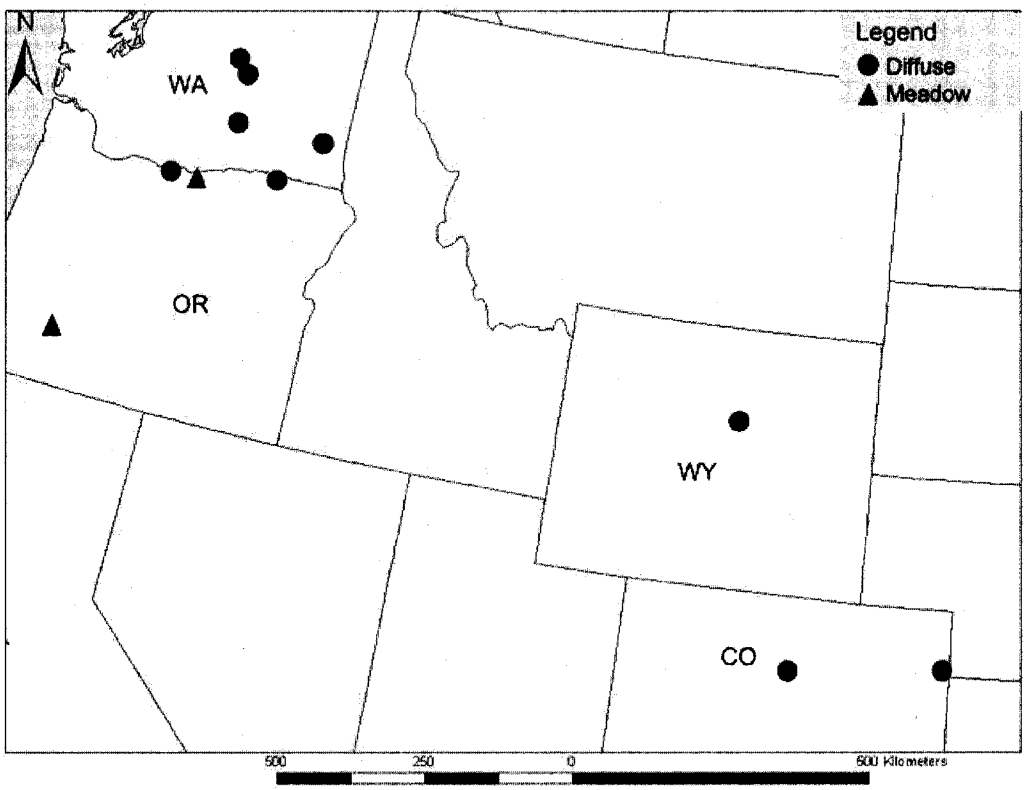


Figure 2.3. Log-likelihood probability of the number of clusters (*K*) for two independent series of *K* = 2 through 6 estimated using STRUCTURE v. 2.2 with admixture (Pritchard et al. 2000; Falush et al. 2007).

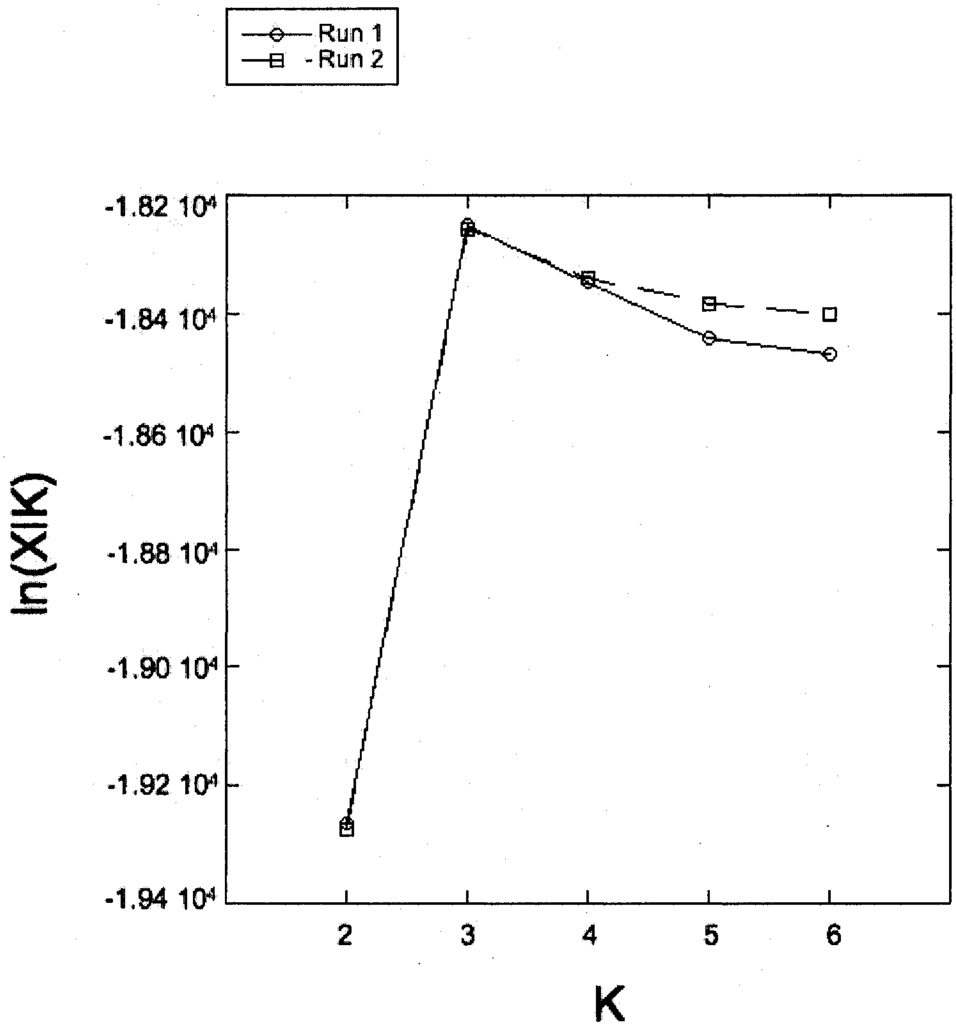
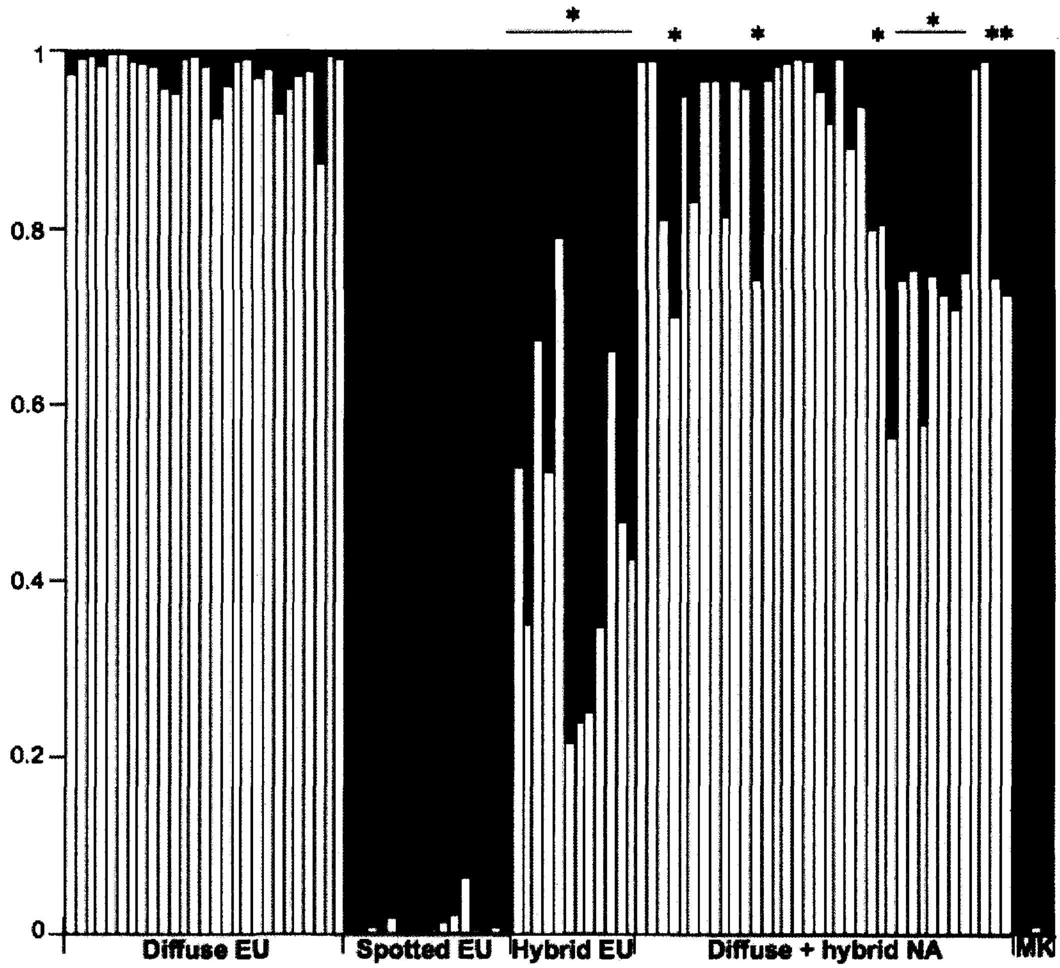


Figure 2.4. Bayesian assignment probabilities with admixture ($K = 3$) [STRUCTURE v. 2.2; Pritchard et al (2000); Falush et al. (2007)]. Each vertical bar represents one individual. The white, black, and grey coloring represents the posterior mean proportion of ancestry from diffuse knapweed, spotted knapweed, and meadow knapweed, respectively. * = population of origin genome probability interval does not include one, indicating hybridization. EU = Europe; NA = North America; Hybrid EU = individuals from spotted x diffuse hybrid swarms in the Ukraine; MK = meadow knapweed (out-group). Based on morphology, diffuse and hybrid-like plants were included in approximately equal numbers in North America (see text).



*
*
*
*
*
**
1
0.8
0.6
0.4
0.2
0
Diffuse EU
Spotted EU
Hybrid EU
Diffuse + hybrid NA
MK

Figure 2.5. A phenogram from hierarchical cluster analysis (Ward's method) of diffuse knapweed and hybrid-like plants surveyed across nine diffuse knapweed sites in western North America in 2005. Five morphological floral characters were analyzed. The top cluster includes plants with typical diffuse knapweed morphology, while the bottom cluster includes plants with hybrid morphology. The numbers to the left of the branches are the posterior mean proportion of ancestry associated with the diffuse knapweed group [($K = 3$ with admixture, STRUCTURE v. 2.2; Pritchard et al (2000); Falush et al. (2007)]. * = population of origin genome probability interval does *not* include one, indicating interspecific hybridization. See text for details.

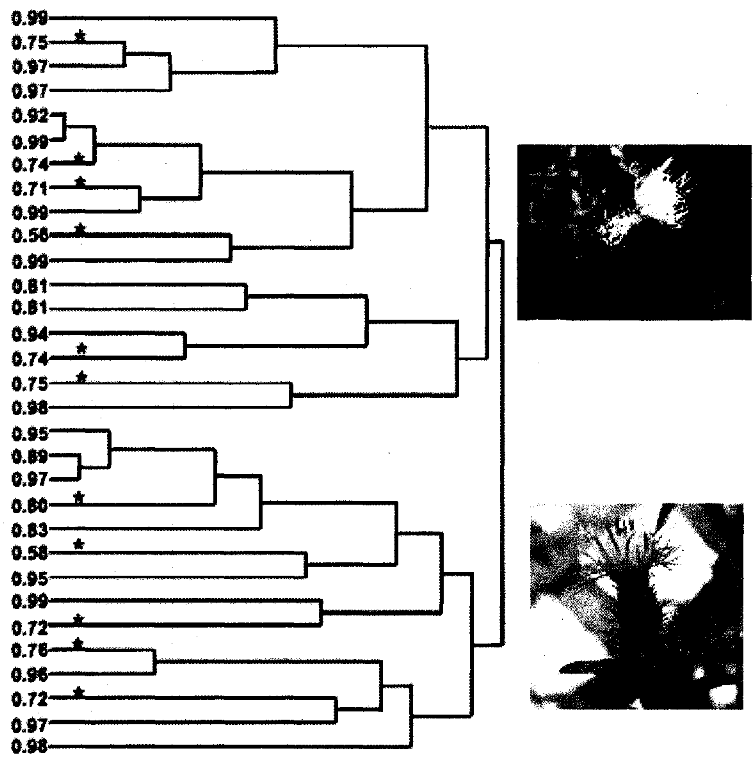
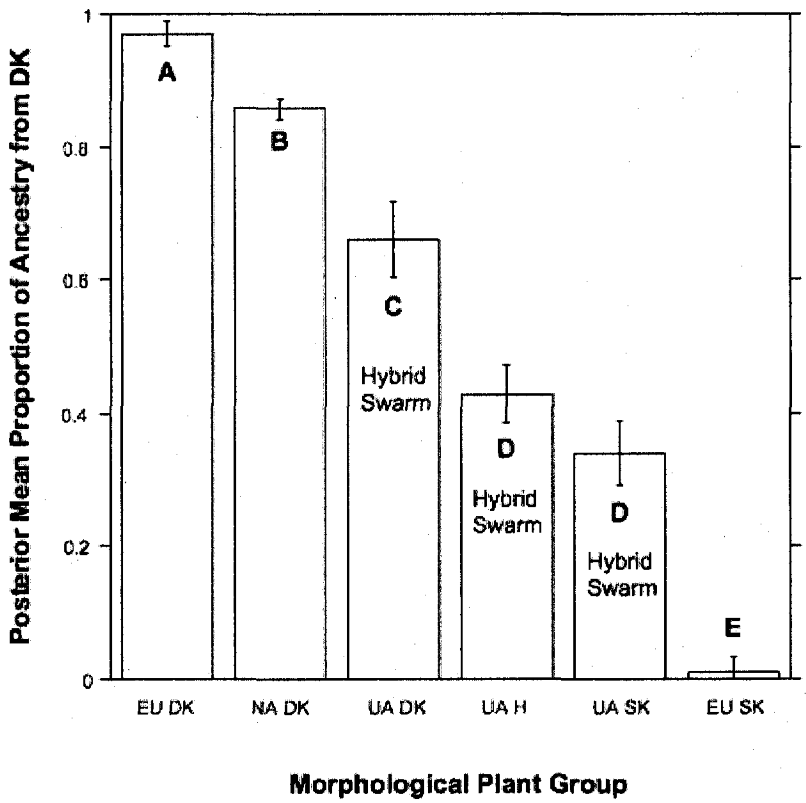

0.99
0.75
0.97
0.97
0.92
0.99
0.74
0.71
0.99
0.56
0.99
0.81
0.81
0.94
0.74
0.75
0.98
0.95
0.89
0.97
0.80
0.83
0.58
0.95
0.99
0.72
0.76
0.96
0.72
0.97
0.98

Figure 2.6. The posterior mean proportion of ancestry associated with the diffuse knapweed cluster, as calculated by STRUCTURE v. 2.2 [($K = 3$ with admixture; Pritchard et al (2000); Falush et al. (2007)]. EU = Europe; NA = North America; UA = Ukraine; DK = diffuse knapweed; H = hybrid; SK = spotted knapweed. Based on morphology, the European diffuse and spotted knapweed sites did not contain any hybrid-like plants. Diffuse and hybrid-like plants from North America were combined into one group for this analysis because they had identical posterior mean proportion of ancestry values from the diffuse knapweed cluster (see text). Individuals from the Ukraine came from apparently active hybrid swarms, and the morphological grouping of an individual plant from these sites as spotted, diffuse, or hybrid was done by hierarchical cluster analysis (Blair and Hufbauer, In Review). Values represent mean ± 1 SE. Different letters denote significantly different means (Tukey's test $P < 0.05$).



Posterior Mean Proportion of Ancestry from DK
1
0.8
0.6
0.4
0.2
0
A
B
C
Hybrid Swarm
D
Hybrid Swarm
D
Hybrid Swarm
E
EU DK
NA DK
UA DK
UA H
UA SK
EU SK
Morphological Plant Group

CHAPTER III

IMPACTS OF BIOLOGICAL CONTROL AND HYBRIDIZATION ON ENEMY ESCAPE

Abstract

Two mechanisms often linked with plant invasions are escape from enemies and hybridization. Classical biological control aims to reverse enemy escape and impose top-down population control. However, hybridization has the potential to alter interactions with enemies and thus affect biological control. I examined how introductions of biological control agents affect enemy escape by comparing specialist enemy loads between the native and introduced ranges of two noxious weeds (spotted and diffuse knapweed; *Centaurea stoebe* L. and *C. diffusa* Lam.) that have been the targets of an extensive biological control program. Hybrids between spotted and diffuse knapweed are often found within diffuse knapweed sites, so I also compared enemy loads on plants that appeared morphologically like diffuse knapweed and hybrids. Finally, I tested the preference for diffuse knapweed, hybrids, and spotted knapweed of one of the agents thought to be instrumental in control of diffuse knapweed (*Larinus minutus*; Curculionidae). Spotted knapweed has largely escaped its root herbivores, while seedhead herbivore loads are comparable in the introduced and native ranges. Diffuse knapweed exhibited seedhead herbivore loads five times higher in the introduced compared to native range. While this pattern of seedhead herbivory is expected with successful biological control, increased loads of specialist insect herbivores in the introduced range have rarely been reported in the literature. This finding may partially explain the better population control of diffuse versus spotted knapweed. Within North America diffuse-like and hybrid-like plants carried similar herbivore loads. However, in paired feedings trials, the specialist *Larinus minutus* demonstrated a preference for newly created artificial hybrids over North American diffuse knapweed and for European diploid spotted knapweed over North American tetraploid spotted knapweed. Overall though, hybridization does not appear to disrupt biological control in this system.

Keywords: Biogeographical approach; Biological control; *Centaurea diffusa*; *Centaurea maculosa*; *Centaurea stoebe*; Diffuse knapweed; Enemy escape; Hybridization; Invasion; *Larinus minutus*; Spotted knapweed.

1. Introduction

When an organism leaves its native range, it escapes its natural predators and parasites (Keane and Crawley, 2002), although new, mainly generalist natural enemies may be acquired (Cripps et al. 2006). Overall, introduction to a new range often results in significantly reduced enemy damage (Maron and Vilá, 2001; Keane and Crawley, 2002; Mitchell and Power, 2003; Torchin et al., 2003). Enemy escape appears to be common for invasive plants: comparisons of the native and introduced ranges show that enemy load is often significantly lower in the introduced range (Wolfe, 2002; Vilá et al., 2005; Liu and Stiling, 2006). The enemy release hypothesis, one of the most often cited hypotheses to explain biological invasions, is based on the assumption that, due to the reduced enemy load (often measured as the number of individual enemies per host or biomass of enemies per host) in the introduced range, invasive species experience a decrease in top-down regulation by natural enemies, resulting in rapid increase in population growth rate and distribution (Keane and Crawley, 2002; White et al., 2008).

Classic biological control of weeds is also based on the observation of enemy escape (DeBach, 1964; Wapshere et al., 1989; Coombs et al., 2004; van Klinken and Raghu, 2006). Natural enemies of an invasive weed are imported from the native range of that pest in an attempt to increase enemy load and establish top-down population control in the introduced range. As Elton (1958) noted, many introduced agents fail to establish or are ineffective, while others 'have done splendid work in ameliorating disastrous situations.' When classical biological control is implemented, typically only one or a few specialist natural enemies from the plant's component community (sensu

Root, 1973) are introduced. Thus, even with biological control, invasive weeds might still experience reduced enemy load relative to the native range due to the reduction in total specialist enemy diversity. Alternatively, introduced biological control agents (i.e. specialist enemies) might attain considerably higher densities in the introduced range than in their native range for numerous abiotic and biotic reasons (Keane and Crawley, 2002), leading to an increase in specialist enemy load despite the lower diversity of natural enemies. One mechanism in particular that might result in greater enemy densities in the introduced range is that the introduced agent escapes its own natural enemies and competitors. Thus, following biological control, it is not obvious whether invasive species will have lower enemy load than in the native range due to a drop in specialist diversity or higher enemy load than in the native range due to changes in the population dynamics of the specialist biological control agents themselves.

Evolutionary changes within populations of invasive weeds might also affect the degree of enemy escape following the introduction of biological control agents. In particular, hybridization can alter interactions with natural enemies (Whitham, 1989; Fritz et al., 1999). Hybrids may be more, less, or equally resistant to enemy attack than their parental species (Hjälten, 1998; Fritz et al., 1994, 1999, 2001), although greater resistance seems to be rare. For example, Roley and Newman (2006) found that a native weevil had highest survival on an introduced watermilfoil, lowest survival on the native watermilfoil, and intermediate survival on the hybrid between the two species. In contrast, Whitham (1989) found that hybrid cottonwoods were more susceptible to aphid attack than either pure parent species, and these hybrid trees acted as sinks for the aphids, perhaps preventing adaptation to the more numerous parental trees; although hybrids comprised only 3% of the population, they contained 85-100% of the aphid population. Thus, hybridization may have important consequences for biological control, as hybrids may be attacked differently than one or both parent species.

This study focuses on spotted and diffuse knapweed, which were inadvertently introduced to North America from Eurasia in the late 1800s or early 1900s, and have become a major threat to rangeland productivity and quality across western North America (Watson and Renney, 1974; Roché and Roché, 1991; Sheley et al., 1999). Additionally, diploid spotted knapweed and diploid diffuse knapweed can hybridize (Gáyer, 1909; Ochsmann, 1999), and hybrids are present within diffuse knapweed sites in North America (Chapter 1). As these weeds infest more than two million hectares (Sheley et al., 1998; Duncan et al., 2004), it is difficult or impossible to control them with cultivation, and herbicide application is often not economically feasible because of the low productivity of the land. Therefore, 13 specialist herbivorous insects from the native range have been introduced in an effort to impose biological control (Rosenthal et al., 1991; Sheley et al., 1999). These 13 species feed on both diffuse and spotted knapweed, and each is considered to be a biological control agent for both weeds. Overall, diffuse knapweed seems to be better controlled by these herbivores than spotted knapweed (Smith, 2004; Seastedt et al., 2007). Using a biogeographical approach (e.g. Hinz and Schwarzlaender, 2004; Hierro et al., 2005), I conducted cross-continental field surveys to quantify seedhead and root herbivore loads in the introduced and native range of each species to determine if the introduction of specialist insects has resulted in different outcomes for the two invasive plants, which may serve as a first step towards understanding the greater control of diffuse knapweed. In North America, seedhead and root herbivory is almost certainly attributed to specialist insects, as there are no known native generalist herbivores that damage these knapweeds in those specific plant parts. Similarly in Europe, most seedhead and root herbivory is likely to be due to specialists (U. Schaffner and P. Häfliger, personal observation), especially given the complex defense chemistry of the genus *Centaurea* (Djeddi et al. 2007 and references within). Thus, overall my study most likely compares herbivory between regions by specialists and not generalists.

To examine the consequences of hybridization for biological control efforts, I surveyed herbivore loads on diffuse knapweed and hybrids across western North America and experimentally tested the preference of an important biological control agent, *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) for the parent species and the hybrids.

The research presented here addressed the following questions:

1) How has implementation of biological control affected specialist herbivore loads in the introduced range relative to the native range of diffuse and spotted knapweed?
2) Within North American diffuse knapweed sites, do hybrid and diffuse knapweed plants experience different or equivalent specialist herbivore loads?
3) Do adults of the seedhead weevil *Larinus minutus* show a feeding preference for diffuse knapweed, hybrids, or spotted knapweed?

2. Materials and methods

2.1 Study Species

Centaurea stoebe L. subspecies *micranthos* (Gugler) Hayek (= *C. maculosa* Lamarck; = *C. biebersteinii* de Candolle) (spotted knapweed) and *C. diffusa* Lamarck (diffuse knapweed) are problematic weeds in North America that can increase soil erosion (Lacey et al., 1989; Sheley et al., 1997), alter plant community composition (Tyser and Key, 1988), negatively impact biodiversity (Ortega et al., 2006), and are thought to have allelopathic effects on other plants (Fletcher and Renney, 1963; Callaway and Aschehoug, 2000; but see Locken and Kelsey, 1987; but see Blair et al., 2005, 2006). Both species appear to have been introduced several times, as introduced populations harbor high levels of genetic diversity (Hufbauer and Sforza, 2008; Marrs et al., 2008).

It is reported that both species have diploid (2n = 18) and tetraploid (4n = 36) cytotypes (Ochsmann, 2000). Both cytotypes of diffuse knapweed are referred to simply as *C. diffusa* Lam. The tetraploid seems to be rare, as it has only been reported twice in the literature from one specimen in Bulgaria (Löve 1979) and one in the former Yugoslavia (Löve 1978). All diffuse knapweed in this study are likely to be diploid. Diffuse knapweed is an annual to short-lived perennial. The two cytotypes of spotted knapweed both fall under the name *C. stoebe* L., which takes precedence over the commonly used *C. maculosa* (Ochsmann, 2000). The biennial diploid is designated *C. stoebe* subsp. *stoebe* L., and the polycarpic tetraploid is designated *C. stoebe* subsp. *micranthos* (Gugler) Hayek (for which *C. biebersteinii* DC. is a synonym). The spotted knapweed plants that have been surveyed in North America are tetraploids (i.e. *C. stoebe* subsp. *micranthos*) (Moore and Frankton, 1954; Ochsmann, 2000; H. Müller-Schärer, personal communication). Thus, when I refer to spotted knapweed of North American origin, it is likely to be the tetraploid *C. stoebe* subsp. *micranthos*, while spotted knapweed from Europe may be either cytotype. When ploidy level of European spotted knapweed is known, I clearly specify it. Diploid hybrids between diploid spotted and diffuse knapweed were first identified in the native range in 1909 (Gáyer, 1909; Ochsmann, 1999), and were given the name *C. xpsammogena* Gáyer. They tend to occur only in a narrow zone of overlap between the two diploid parent species ranging from Romania to the Ukraine (U. Schaffner, personal communication). Through field observations and molecular techniques, I have recently found that diffuse knapweed in North America was introduced with hybrid individuals containing significant introgression from diploid spotted knapweed (Chapters 1 and 2). Individuals of hybrid origin are found in most North American diffuse knapweed sites, but not in spotted knapweed sites, and hybridization does not appear to be ongoing (Chapters 1 and 2).

Floral traits are used to diagnose species in this complicated genus (Ochsmann, 2000). Diffuse knapweed has smaller white flowers (rarely pink), no bract pigmentation, and a pronounced terminal spine, while spotted knapweed has larger purple flowers, obvious bract pigmentation, and lacks a terminal spine (Watson and Renney, 1974; Ochsmann, 2000). Hybrids typically have intermediate morphology with purple ray flowers and white disc flowers, pigmented bracts, and terminal spines. In this paper, designation of a plant as a hybrid or typical diffuse knapweed is based on floral morphological characters. As this classification has not been confirmed at the molecular level for the specific plants studied here, I denote the plants as hybrid-like or diffuse-like to highlight that the classifications contain some uncertainty at the genome level. Molecular work has corroborated that the presence of intermediate individuals within a region correctly predicts interspecific admixture (Chapter 2).

2.2 Biological control program

The thirteen specialist insects that have been introduced to North America from the native range can all attack both spotted and diffuse knapweed. The larvae of these insects damage the plant either in the root (n = 5; three moth and two beetle species) or the seedhead (n = 8; four fly, one moth, and three beetle species). The seedhead weevil *L. minutus* was first introduced to North America in 1991 from Greece (Lang et al., 1996). This weevil seems to be reducing some infestations of diffuse knapweed (Seastedt et al., 2003; Smith, 2004) but appears to play a relatively smaller role in spotted knapweed control (e.g. Smith, 2004; Story et al., 2006). Seastedt et al. (2007) reported that upon introduction of *L. minutus*, seed production of diffuse knapweed declined from 4400 seeds/m^2 in 1997 to zero seeds/m^2 in 2006. Other reports of successful control of diffuse knapweed have come out of Montana, Oregon, Washington, and British Columbia (Smith, 2004; Story and Coombs, 2004; Myers, 2004), and *L. minutus* may have played a large role in each area (Seastedt et al., 2007). The weevil is univoltine; adults overwinter in leaf litter and emerge in late spring/early summer. While all of the biological control agents introduced against the knapweeds cause damage as larvae, adult *L. minutus* are also able to significantly defoliate knapweed plants prior to flowering (Wilson and

Randall, 2003; Piper, 2004; Norton et al., 2008). At flowering, the weevils switch to feeding on knapweed flowers. Eggs are laid in open flowers, and developing larvae can destroy all of the seeds in a diffuse knapweed capitulum and 25-100% of the seeds in spotted knapweed capitulum (Lang et al., 1996). *Larinus minutus* is capable of developing in several *Centaurea* species including *C. stoebe* (spotted knapweed), *C. diffusa* (diffuse knapweed), *C. arenaria,* and *C. calcitrapa* (Jordan, 1995). Several studies have explored the preference and performance of *L. minutus* on spotted versus diffuse knapweed, but found conflicting results (Table 3.1).

2.3 Cross-continental field surveys of spotted and diffuse knapweed specialist herbivore loads

Three sets of surveys were conducted: summer 2005 North America, summer 2005 Central Europe, and summer 2006 North America. The main focus of the 2005 surveys was to compare seedhead and root herbivore loads of spotted and diffuse knapweed between the native and introduced range (Table 3.2). The history of releases of biological control agents was largely unknown for nearly all surveyed sites in North America 2005. The main focus of the 2006 surveys was to compare seedhead herbivore loads between diffuse-like and hybrid-like individuals within North American diffuse knapweed sites. About half of the 2006 sites were surveyed without prior knowledge of where biological control agents had been released, while the other half were < 6 km from seedhead herbivore release sites (Table 3.2). To obtain a relative measure of herbivore loads for comparisons across sites and between plant types (i.e., diffuse-like and hybrid-like), I recorded herbivores or evidence of herbivores as present/absent data within individual seedheads and roots (described further below).

Between June and September 2005, I surveyed seven spotted knapweed sites and five diffuse knapweed sites across North America to assay specialist seedhead herbivory (Table 3.2). Surveys focused on regions where these plants are considered quite invasive. I found sites with spotted and/or diffuse knapweed by contacting county weed agents prior to the collection trip, and sites were then selected either by driving until encountering a site or from directions from a weed agent. At each site I haphazardly ran a 50 m transect through the population and surveyed approximately 30 plants on one side of the tape measure every 1-meter (or more if plants were spaced further apart). This sampling scheme ensured the inclusion of a representative sub-sample of the site. To measure herbivore load of seedhead feeders, I surveyed from 13 to 30 plants per site (Table 3.2), depending on the availability of mature seedheads, and I opened five seedheads per plant. Spring 2005 was unseasonably cool, so flowering was delayed across much of the west. Therefore, although all sites had >30 plants, not all sites had 30 plants with mature seedheads at the time of the survey. I recorded whether seedhead feeders were present or absent per seedhead. In addition to actual larvae or insects, I scored seedheads as having seedhead feeders present if I found freshly laid egg(s) or damage with frass (even in the absence of an insect). Within the diffuse knapweed sites, approximately half of the plants surveyed were diffuse-like (n=61), and half were hybrid-like (n=63).

During the same set of surveys across North America, I also measured herbivore load of specialist root miners in eight diffuse knapweed sites, nine spotted knapweed sites, and one diffuse + spotted knapweed site (Table 3.2). I evaluated whether root miners were present by excavating the root from the soil and opening it. I surveyed from 11 to 30 plants per site (Table 3.2). Within the diffuse knapweed sites, again approximately half of the plants sampled were diffuse-like (n=114), and the other half hybrid-like (n=99).

To quantify seedhead and root miner loads in the native range, in August 2005 I visited five spotted knapweed sites and five diffuse knapweed sites in Europe and in 2006 one diffuse knapweed site in the Ukraine (Table 3.2). Either local botanists identified sites for me, or I found sites while driving through the countryside. Only one of the six diffuse knapweed sites contained hybrid-like plants, and at that site those plants made up < 5% of the population; therefore, I consider these sites to be relatively pure diffuse knapweed. Ploidy was assayed for three of the five spotted knapweed sites; one of these sites was diploid, while the other two were tetraploid (H. Müller-Schärer, unpublished data; Table 3.2). Approximately thirty plants were assayed per site, as described above in North America 2005 (Table 3.2).

To further examine if seedhead herbivores respond to the different floral morphology between diffuse-like and hybrid-like plants, in 2006 I compared seedhead herbivore loads between diffuse-like and hybrid-like plants in North America. Between July and September 2006, I visited an additional 11 diffuse knapweed sites throughout western North America (Table 3.2). I assayed seedhead feeders as described above for 14 to 31 plants per site (≈ 2/3 hybrid-like and 1/3 diffuse-like, see Table 3.2). On average, I assayed ≈ 30 seedheads per plant, for a total of 8,649 seedheads. Surveys for root herbivores were not conducted in 2006.

2.4 Larinus minutus collection and colony conditions for leaf preference tests

Larinus minutus weevils were collected during the first week of June 2007 at Hughes Stadium, Fort Collins, CO (N 40° 33' 27.88" W 105° 7' 59.60") from a large diffuse knapweed infestation. Insects were kept in mesh cages with one or two live plants. The plants used for feeding the weevils were from a diffuse knapweed site in North America not used in the choice study (Roosevelt, WA, USA). As plants were defoliated, new ones were added. The cage was kept under artificial lights that were set on a 14 h light, 10 h dark cycle. Collecting weevils from and maintaining them on diffuse knapweed may have introduced a bias for preference of diffuse over spotted knapweed; however, the results suggest this was not a problem (see Results and Discussion). Limited spotted knapweed infestations in the area prevented collection of weevils from that species.

2.5 Plant material for Larinus minutus feeding preference trials

Plants from the following sites were grown for this experiment:

- two pure diffuse knapweed sites in Crimea, Ukraine
- two diffuse knapweed sites that contained both hybrid-like and diffuse-like plants in North America
- three verified diploid spotted knapweed sites in the Ukraine
- two verified tetraploid spotted knapweed sites in North America

Additionally, twenty Back Cross 1 (BC1) seeds created in the lab were planted. As the hybrid-like individuals found within diffuse knapweed sites are likely to have experienced many generations of introgression since their introduction at the turn of the century, BC1 seeds were included to better understand how individuals with a greater portion of their genome derived from spotted knapweed may influence preference. BC1 seeds were created by crossing North American diffuse knapweed with European diploid spotted knapweed. An F1 from this cross was then back-crossed with diffuse knapweed to create BC1 seed. I used microsatellite markers to confirm the identity of the F1 and BC1 individuals (A.C. Blair, unpublished data).

Plants were grown in pots (diameter 1.5", depth 8.25") in Sunshine Mix #3 potting soil. The plants were started in a greenhouse in May 2007 and subsequently they were moved outside to a lath house. Pots were misted daily until most seeds had germinated (approximately 2 weeks) and then watered daily. Plants were fertilized (Osmocote Classic 14-14-14 Scotts) as needed. At three weeks all plants were sprayed to control a thrips outbreak (Borer, Bagworm, Leafminer, and Tent Caterpillar Spray, Ferti•lome).

2.6 Larinus minutus leaf preference tests

Two sets of feeding trials were conducted (July and Sept. 2007). Weevils were starved for 24 h prior to the feeding trials, and then were presented with pairs of fresh leaves that were similar in age, size, and shape. Harvested leaves were placed in a 150 x 15 mm polystyrene Petri dish; the cut end of the leaf was placed in a moistened paper towel to keep the leaf fresh. Twenty weevils were then added to each Petri dish and allowed to feed for 24 h.

Leaves were scanned with a flatbed scanner (Microtek, ScanMaker 6800) pre- and post-feeding. I calculated the area (mm^2) of the leaf pre- and post-feeding with the software program VegMeasure (v.1.6, D.E. Johnson, Oregon State University). This program uses an algorithm to select pixels that correspond to green vegetation from color photographs. I then calculated the relative amount of damage per leaf as follows: [initial green area – final green area]/initial green area.

Twenty replicates of the following four paired feeding trials were conducted in July 2007 with the plants grown in the lath house (described above):

1) European diffuse knapweed vs. North American diffuse knapweed
2) European diffuse knapweed vs. BC1
3) European diploid spotted knapweed vs. North American diffuse knapweed
4) European diploid spotted knapweed vs. BC1

The second set of feeding trials was performed in September 2007 with newly emerged weevils collected from the same site. I performed 20 choice feeding trials for the following three pairs:

1) North American diffuse knapweed vs. BC1
2) North American tetraploid spotted knapweed vs. European diploid spotted knapweed
3) North American diffuse knapweed vs. North American tetraploid spotted knapweed

2.7 Statistical analyses

Field Surveys

To compare specialist herbivory of diffuse knapweed between North America (2005) and Europe (2005), I combined all North American hybrid-like and diffuse-like plants into one category – North American diffuse knapweed, as I was interested in the overall differences at the continent scale instead of among morphological variants within a site. Each seedhead or root was assigned a 0 if there was no evidence of herbivory or a 1 if there was evidence of herbivory. For each plant, I then analyzed the seedhead data as x seedheads with presence of herbivory out of a total of y seedheads (i.e. events/trials = response variable). Thus, the unit of measure is the seedhead, while the plant is the unit of analysis. Data in this events/trials format are properly analyzed with a binomial distribution and a logit link function (Littell et al., 2006, pp. 542-543). For both seedhead and root infestation, I used mixed models in SAS (PROC GLIMMIX) with continent as a fixed effect and site nested within continent as a random effect. I used the same mixed model to compare seedhead and root infestation of spotted knapweed across continents. To compare seedhead (2005 and 2006) and root herbivory (2005) between North American hybrid-like and diffuse-like plants, I used a model with plant classification as a fixed effect and site as a random effect. In 2005 I collected size data on all of the plants surveyed for biological control (Chapter 1). Initially, I included plant size (using the equation for the volume of a cylinder = [π*plant diameter^2*plant height]/4) as a covariate for the 2005 data analyses, but I removed this term from the models, as it did not alter patterns of significance.

Leaf Preference Test

I used PROC TTEST to compare the percent change of leaf tissue between the seven paired plant types. The percentage change data were normally distributed. I used the same approach to compare the total amount eaten (initial green area – final green area) between pairs; the same results were found, so I only report the findings from the analyses of percent change.

3. Results

3.1 Cross-continental field surveys of spotted and diffuse knapweed specialist herbivore loads

Question 1. How has implementation of biological control affected specialist herbivore loads in the introduced range relative to the native range of diffuse and spotted knapweed?

The 2005 surveys revealed less seedhead damage on European than on North American diffuse knapweed ($F_{1,8} = 5.62$, $P = 0.04$; Fig. 3.1A). Attack by root miners showed the opposite pattern; a greater proportion of diffuse knapweed plants were attacked in Europe than North America ($F_{1,13} = 5.32$, $P = 0.04$; Fig. 3.1B).

For spotted knapweed, no difference was found in the 2005 surveys for attack by seedhead feeders ($F_{1,10} = 0.01$, $P = 0.94$; Fig. 3.1C). However, European spotted knapweed was much more often attacked by root miners than North American plants

($F_{1,17} = 21.62$, $P < 0.001$; Fig. 3.1D). In fact, only a total of four spotted knapweed plants in two out of ten sites in North America had root herbivory.

Question 2. Within North American diffuse knapweed sites, do hybrid and diffuse knapweed plants experience different or equivalent specialist herbivore loads?

Across the sites in 2005, hybrid-like and diffuse-like plants did not differ in the amount of seedhead ($F_{1,118} = 0.01$, $P = 0.94$; hybrid-like = 44%, diffuse-like = 58%) or root herbivory ($F_{1,201} = 0.41$, $P = 0.52$; hybrid-like = 18%, diffuse-like = 34%). Within diffuse knapweed sites from the North American 2006 field surveys, the percentage of seedheads with herbivory ranged from 1 to 95% (Table 3.2). Across all 2006 sites hybrid-like and diffuse-like plants did not differ in the level of seedhead herbivory ($F_{1,274} = 1.97$, $P = 0.16$; hybrid-like = 66%, diffuse-like = 62%).

3.2 Larinus minutus leaf preference tests

Question 3. Do adults of the seedhead weevil *Larinus minutus* show a feeding preference for diffuse knapweed, hybrids, or spotted knapweed?

In the first set of paired feeding trials (July 2007), weevils consumed comparable amounts of leaf tissue between the paired plants (Fig. 3.2A-D). In the second set of paired feeding trials (Sept. 2007), the amount of tissue consumed significantly differed for two of the three pairs (Fig. 3.2E-G). European diploid spotted knapweed was consumed more than North American tetraploid spotted knapweed, and North American diffuse knapweed was consumed more than hybrid BC1 individuals.

4. Discussion

In this study my goal was to examine how biological control affects enemy escape and what role hybridization might play. I found that whether or not enemy escape was observed depended on both the plant species (i.e. spotted or diffuse knapweed) and the type of herbivory by specialist insects (i.e. seedhead or root). Hybrid-like plants in the field in North America had similar herbivore loads to diffuse-like plants. The specialist insect *L. minutus* showed a preference for North American admixed diffuse knapweed over hybrids created in the lab that contained more of the diploid spotted knapweed's genome. These findings, discussed below, could have implications for successful management of these noxious weeds by biological control agents and lend to a better understanding of how biological control interacts with mechanisms put forward to explain invasion success.

4.1 Biological control of spotted knapweed: impacts on enemy loads and management implications

Spotted knapweed now infests more than 1.2 million hectares in North America (Sheley et al., 1998), in spite of a rigorous biological control program begun in North America more than 30 years ago (reviewed in Müller-Schärer and Schroeder, 1993).

Although biological control introductions have largely reversed the escape from specialist seedhead herbivores, this type of herbivory may not be a strong top-down regulator of spotted knapweed. Spotted knapweed capitula are relatively large, and even when attacked, seeds are often still able to develop (Maddox, 1982; Smith and Mayer, 2005). Indeed, Story et al. (1989) concluded that the 36-41% reduction of seed production by the seedhead fly *Urophora affinis* documented in a field study would not likely exert a strong enough impact to control spotted knapweed alone. A similar conclusion was reached when Story (1989) reported seed reduction by the seedhead flies around 50-75%. The seedhead weevil, *Larinus minutus*, has had a significant impact on introduced diffuse knapweed (discussed below), and it is currently not having such a dramatic impact on spotted knapweed (Seastedt et al., 2007).

Unlike the high seedhead herbivore loads found on spotted knapweed, it appears that this species still largely escapes its specialist root herbivores in North America. Other studies have found that the root weevil *Cyphocleonus achates* has the potential to decrease infestations of spotted knapweed at some locations (Corn et al., 2006; Jacobs et al. 2006; but see Clark et al., 2001). Story et al. (2006) found that at two sites, spotted knapweed decreased by 99% and 77% as *C. achates* numbers increased. Reductions of spotted knapweed density did not take place at six additional sites where *C. achates* was absent, although six other biological control agents were present (Story et al., 2006). Recently, it was shown that *C. achates* can reduce spotted knapweed size regardless of drought conditions, and it seems unlikely that recent declines observed in western Montana have resulted just from persistent drought conditions (Corn et al., 2007). It is possible that as populations of root feeding *C. achates* grow and spread, the patterns of root miner loads I observed here may shift and reduction of spotted knapweed may occur.

Agapeta zoegana, a root mining moth introduced for biological control, seems less likely to successfully control spotted knapweed, as infested plants were found to compensate for herbivory (Steinger and Müller-Schärer, 1992; Calaway et al., 1999; Newingham et al., 2007). Müller (1989) found that spotted knapweed from North America infested with *A. zoegana* increased root growth, but he also found that this insect reduced survival of immature plants. In conclusion, spotted knapweed appears to have reduced root herbivore loads in North America, as predicted by the enemy escape hypothesis, and recent evidence suggests that at least one, but perhaps not all, of the introduced root-feeding biological control agents may shift that pattern and has the potential to successfully control this plant species.

4.2 Biological control of diffuse knapweed: impacts on enemy loads and management implications

Diffuse knapweed infests at least 700,000 hectares in North America (Duncan et al., 2004). Compared to spotted knapweed, this species seems to be better controlled by biological control agents (Coombs et al., 2004; Seastedt et al., 2003, 2007), and this may partly be attributed to adult feeding damage by *L. minutus* that causes water stress and consequently death of plants. Additionally, the smaller capitulum size of diffuse knapweed may result in this better control, as seedhead herbivores often consume all of the seeds in an infested seedhead. Additionally, the significantly greater seedhead herbivore loads in North America relative to Europe may partly explain this phenomenon. I detected seedhead herbivory at a level of 9% across European sites. This low level of seedhead infestation in the native range is interesting because the introduced biological control agents represent only a subset of the specialists enemies found to attack seedheads in Europe (Schroeder, 1985), and generalists may cause some damage. Within North America across two field seasons, I found that approximately 60% of the seedheads showed evidence of herbivory, with a high of 95%; this damage can be attributed to the introduced specialist insects because there are no known native seedhead herbivores of the knapweeds in North America. Similarly, Smith (2004) reported seedhead infestation of 99% and 59% within two sites in Montana, USA. As intended, biological control of diffuse knapweed has increased enemy loads, resulting in seedhead herbivory in North America exceeding that found in Europe. Plants in the native range may not be controlled by top-down seedhead feeders; one plant in North America can produce 925 seeds in one season (Watson and Renney, 1974), even if one assumes seed production is roughly half of that in the native range due to disease and herbivory, a 9% reduction would still leave approximately 420 seeds for dispersal. This scenario supports the idea that top-down regulation of a plant by a certain enemy or guild of enemies in the native range is not requisite for successful population control by such specialists in the introduced range (Müller-Schärer and Schaffner, 2008).

The elevated rates of specialist seedhead herbivory on diffuse knapweed in North America compared to Europe could be due to one or more of the following: (1) the introduced biological control agents have escaped their enemies and competitors upon introduction to North America, allowing them to attain higher population densities, (2) larger plant populations in North America have allowed the insects to attain higher densities (Root, 1973; Kareiva, 1985) (3) the abiotic conditions are more favorable for seedhead herbivores in the introduced range, (4) plants in North America may have experienced selection for a trade-off of reduced defenses for increased growth [i.e. the 'Evolution of Increased Competitive Ability' (Blossey and Nötzold, 1995)], (5) hybridization has altered enemy dynamics (Fritz et al., 1999), and/or (6) regulation in the native range by specialist seedhead feeders fluctuates through time.

Increased enemy loads by specialist herbivores in the introduced versus native range is a phenomenon that may be commonly found when biological control is successful; however, such a pattern has rarely been reported in the literature. While Young (2003) similarly found higher levels of floral herbivory by specialists in the introduced (Nebraska, USA) versus native range (United Kingdom) on the introduced thistle *Cirsium vulgare*, the elevated herbivory in the introduced range resulted from a shift of specialists native to North American thistles (Takahashi, 2006), and not from introduced biological control agents. Counter to my findings, Wolfe (2002) found that two specialist herbivores of the weed *Silene latifolia* were either absent or found at very low levels in the introduced range. Unlike the knapweeds though, the *S. latifolia* specialists have not been introduced as part of a biological control program. Sheppard et al. (1994) found comparable levels of an important biological control weevil of *Carduus nutans* in its native and introduced regions.

Diffuse knapweed has, to some extent, escaped its specialist root herbivores in North America compared to Europe, but the difference is less pronounced than with spotted knapweed. Compared to other reported values of root herbivore loads (i.e. 74%

and 69% in MT) (Smith, 2004), I found overall lower levels of diffuse knapweed root herbivory (24%). However, two of my sites had levels nearing the previously reported values (57% and 72%), and it seems likely that these insects are regionally rare, but locally abundant when present. While *C. achates* preferentially attacks spotted knapweed because of its larger root diameter (Stinson et al., 1994), it may play a role in diffuse knapweed control, as it might be more damaging to smaller roots (Smith, 2004). The root feeding beetle *Sphenoptera jugoslavica* also has the potential to reduce some infestations of diffuse knapweed (Powell and Myers, 1988). No research has been done to examine whether diffuse knapweed compensates for root herbivory like spotted knapweed has the potential to do (Steinger and Müller-Schärer, 1992). A recent study found that instead of compensation, sub-lethal above-ground feeding of diffuse knapweed by two biological control agents reduced knapweed performance. This reduction resulted in a small, but significant, increase in the performance of two native species (Norton et al., 2008).

4.3 Hybridization, enemy load, and biological control

Hybridization has the potential to alter interactions with enemies (Fritz et al., 1999). As a number of invasive plant species are both of hybrid origin (Ellstrand and Schierenbeck 2000) and the target of control efforts through biocontrol introductions (e.g., over 20 agents have been targeted at and established on the hybrid weed *Lantana camara* (Zalucki et al. 2007)), it is important to understand how biological control and hybridization interact. In this study, in the field, hybrid-like plants did not exhibit different levels of specialist seedhead or root herbivore loads compared to diffuse-like plants. Similarly, in laboratory preference tests *L. minutus* did not discriminate between individuals from North American admixed diffuse knapweed sites and European genetically 'pure' diffuse knapweed (P = 0.54). Recent hybridization in the BC1 individuals did not alter the preference when compared to 'pure' European diffuse knapweed (P = 0.59). However, BC1 individuals were consumed significantly less when paired with North American diffuse knapweed (P = 0.004). This finding raises the possibility that hybridization between the two species may initially result in a reduction of herbivore damage on hybrid individuals. If hybrids are rare within a population though, introgression with diffuse knapweed would likely erase that advantage quickly through time. As the introduced diffuse knapweed is not currently undergoing hybridization with spotted knapweed, these findings do not presently affect the population dynamics of diffuse knapweed in North America. If diploid spotted knapweed were to invade North America, it may warrant further investigation.

4.4 Larinus minutus preference of spotted versus diffuse knapweed

Previous studies found various patterns of preference and/or performance of *L. minutus* for spotted versus diffuse knapweed (Table 3.1). Jordan (1995) found that *L. minutus* preferred European spotted knapweed over North American and European diffuse knapweed. Jordan (1995) also found that *L. minutus* did not feed on European diffuse knapweed leaves; in contrast, I found that *L. minutus* did consume European diffuse knapweed. In Greece, Groppe et al. (1990) found that the rate of attack by *L. minutus* was 12.6% on diffuse knapweed and 27.7% on spotted knapweed. Counter to these two European studies, in a field cage study in North America, Smith and Mayer (2005) found greater *L. minutus* establishment, infestation of capitula, and progeny production on diffuse instead of spotted knapweed.

I did not find a strong feeding preference when I paired either diploid European or tetraploid North American spotted knapweed with North American diffuse knapweed (Fig. 3.2D and 3.2F). I predicted that *L. minutus* would favor diffuse over spotted knapweed because *L. minutus* was collected for release from diffuse knapweed, and to a lesser extent, from a closely related species *C. grisenbachii* (U. Schaffner, personal communication). Additionally, I collected and maintained weevils on diffuse knapweed, which may have resulted in a bias for diffuse knapweed. While not significant, in both feeding trials with spotted and diffuse knapweed, *L. minutus* tended to prefer spotted over diffuse knapweed. If rearing conditions influenced host preference, inclusion of weevils maintained on spotted knapweed may have revealed a significant preference for that species.

Finally, *L. minutus* showed a significant preference for diploid European spotted knapweed over tetraploid North American spotted knapweed (Fig. 3.2G). While ploidy alone may be driving this pattern, it would be interesting in further studies to include European tetraploid spotted knapweed to tease apart whether ploidy or other differences cause this preference.

4.5 Summary

Many hypotheses have offered mechanistic explanations for why an organism flourishes in its introduced region. Less is known about how biological control programs interact with these mechanisms. Regarding spotted knapweed, biological control has resulted in equivalent levels of seedhead herbivory in the introduced and native ranges, but this knapweed species has still largely escaped its root herbivores. As root herbivores have potential to regulate spotted knapweed, I predict that control may occur as root miner loads attain or exceed levels found in the native range. Introduced seedhead feeders of diffuse knapweed have not only reversed enemy escape, but these enemies now occur at levels higher than those encountered in the native range. This enhanced enemy load may explain the control of diffuse knapweed in some locations in the introduced range. Diffuse knapweed has also escaped its root enemies, and even greater biological control of this species may result as root agents spread throughout the region.

Hybridization has the potential to stimulate invasibility (Ellstrand and Schierenbeck, 2000) and alter interations with herbivores (Fritz et al., 1999; Roley and Newman, 2006), but I did not find a strong role for hybridization influencing biological control in the field. In the laboratory, recent hybridization did alter herbivory, but this phenomenon is likely to be short-lived in the field as introgression occurs.

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References

Blair, A.C., Hanson, B.D., Brunk, G.R., Marrs, R.A., Westra, P., Nissen, S.J., Hufbauer, R.A., 2005. New techniques and findings in the study of a candidate allelochemical implicated in invasion success. Ecology Letters 8, 1039-1047.

Blair, A.C., Nissen, S.J., Brunk, G.R., Hufbauer, R.A., 2006. A lack of evidence for a role of the putative allelochemical (±)-catechin in spotted knapweed invasion success. Journal of Chemical Ecology 32, 2327-2331.

Blossey, B., Nötzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. Journal of Ecology 83, 887-889.

Callaway, R.M., DeLuca, T.H., Belliveau, W.M., 1999. Biological control herbivores may increase competitive ability of the noxious weed *Centaurea maculosa*. Ecology 80, 1196-1201.

Callaway, R.M., Aschehoug, E.T., 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290, 521-523.

Clark, S.E., Van Driesche, R.G., Sturdevant, N., Kegley, S., 2001. Effect of root feeding insects on spotted knapweed (*Centaurea maculosa*) stand density. Southwestern Entomologist 26, 129-135.

Coombs, E.M., Clark, J.K., Piper, G.L., Cofrancesco, A.F., 2004. Biological Control of Invasive Plants in the United States. Oregon State University Press, Corvallis.

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

Corn, J.G., Story, J.M, White, L.J., 2007. Effect of summer drought on the impact of the root weevil *Cyphocleonus achates* on spotted knapweed. Environmental Entomology 36, 858-863.

Cripps, M.G., Schwarzlander, M., McKenney J.L., Hinz, H.L., Price, W.J., 2006. Biogeographical comparison of the arthropod herbivore communities associated with *Lepidium draba* in its native, expanded, and introduced ranges. Journal of Biogeography 33, 2107-2119.

DeBach, P. (Ed.), 1964. Biological Control of Insect Pests and Weeds. Reinhold Publishing Corporation, New York.

Djeddi, S., Karioti, A., Sokovic, M., Stojkovic, D., Seridi, R., Skaltsa, H., 2007. Minor sesquiterpene lactones from *Centaurea pullata* and their antimicrobial activity. Journal of Natural Products 70, 1796-1799.

Duncan, C.A., Jachetta, J.J., Brown, M.L., Carrithers, V.F., Clark, J.K., DiTomaso, J.M., Lym, R.G., McDaniel, K.C., Renz, M.J., Rice, P.M., 2004. Assessing the economic, environmental, and societal losses from invasive plants on rangeland and wildlands. Weed Technology 18, 1411-1416.

Ellstrand, N.C., Schierenbeck, K.A., 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97, 7043-7050.

Elton, C.S., 1958. The Ecology of Invasions by Animals and Plants. University of Chicago Press, Chicago, p. 131.

Fletcher, R.A., Renney, A.J., 1963. A growth inhibitor found in *Centaurea* spp. Canadian Journal of Plant Science 43, 475-481.

Fritz, R.S., Nichols-Orians, C.M., Brunsfeld, S.J., 1994. Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. Oecologia 97, 106-117.

Fritz, R.S., Moulia, C., Newcombe, G., 1999. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. Annual Review of Ecology and Systematics 30, 565-591.

Fritz, R.S., Hochwender, C.G., Lewkiewicz, D.A., Bothwell, S., Orians, C.M., 2001. Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. Oecologia 129, 87-97.

Gáyer, G., 1909. Vier neue Centaureen der Flora Botanikai von Ungarn. Magyar Botanikai Lapok 8, 58-61.

Groppe, K., Sobhian, R., Kashefi, J., 1990. A field experiment to determine host specificity of *Larinus curtus* Hochhut (Col., Curculionidae) and *Urophora sirunaseva* Hg. (Dipt. Tephritidae), candidates for the biological control of *Centaurea solstitialis* L. (Asteracea), and *Larinus minutus* Gyllenhal, a candidate for biological control of *C. maculosa* Lam. and *C. diffusa* Lam. Journal of Applied Entomology 110, 300-306.

Hierro, J.L., Maron, J.L., Callaway, R.M., 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. Journal of Ecology 93, 5-15.

Hinz, H.L., Schwarzlaender, M., 2004. Comparing invasive plants from their native and exotic range: what can we learn for biological control? Weed Technology 18, 1533-1541.

Hjälten, J., 1998. An experiment test of hybrid resistance to insects and pathogens using *Salix caprea*, *S. repens*, and their F1 hybrids. Oecologia 117, 127-132.

Hufbauer, R.A., Sforza, R., 2008. Multiple introductions of two invasive *Centaurea* taxa inferred from cpDNA haplotypes. Diversity and Distributions 14, 252-261.

Jacobs, J.S., Sing, S.E., Martin, J.M., 2006. Influence of herbivory and competition on invasive weed fitness: observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environmental Entomology 35, 1590-1596.

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

Kareiva, P., 1985. Finding and losing host plants by *Phyllotreta* – patch size and surrounding habitat. Ecology 66, 1809-1816.

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

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

Lang, R.F., Story, J.M., Piper, G.L., 1996. Establishment of *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) for biological control of diffuse and spotted knapweed in the western United States. Pan Pacific Entomology 72, 209-212.

Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R.D., Schabenberger, O., 2006. SAS® for Mixed Models, Second Edition. SAS Institute Inc., Cary, NC.

Liu, H., Stiling, P., 2006. Testing the enemy release hypothesis: a review and meta-analysis. Biological Invasions 8, 1535-1545.

Locken, L.J., Kelsey, R.G., 1987. Cnicin concentrations in *Centaurea maculosa*, spotted knapweed. Biochemical Systematics and Ecology 15, 313-320.

Löve, A., 1978. IOPB Chromosome Number Reports LIX. Taxon 27, 53-61.

Löve, A., 1979. IOPB Chromosome Number Reports LXIV. Taxon 28, 391-408.

Maddox, D.M., 1982. Biological control of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*C. maculosa*). Weed Science 30, 76-82.

Maron, J.L., Vilá, M., 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypothesis. OIKOS 95, 361-373.

Marrs, R.A., Sforza, R., Hufbauer, R.A., In Press. When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biological Invasions. DOI 10.1007/s10530-007-9153-6

Mitchell, C.E., Power, A.G., 2003. Release of invasive plants from fungal and viral pathogens. Nature 421, 625-627.

Moore, R.J., Frankton, C., 1954. Cytotaxonomy of three species of *Centaurea* adventive in Canada. Canadian Journal of Botany 32, 182-186.

Müller, H., 1989. Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.:Cochylidae). Weed Research 29, 103-111.

Müller-Schärer, H., Schaffner, U. 2008. Classical biological control: exploiting enemy escape to manage plant invasions. Biological Invasions. DOI 10.1007/s10530-008-9238-x.

Müller-Schärer, H., Schroeder, D., 1993. The biological control of *Centaurea* species in North America: Do insects solve the problem? Pesticide Science 37, 343-353.

Myers, J.H., 2004. A silver bullet in the biological control of diffuse knapweed. ESA 2004 Annual meeting abstract. http://abstracts.co.allenpress.com/pweb/esa2004.

Newingham, B.A., Callaway, R.M., Bassirirad, H., 2007. Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. Oecologia 153, 913-920.

Norton, A.P., Blair, A.C., Hardin, J.G., Nissen, S.J., Brunk, G.R., 2008. Herbivory and novel weapons: no evidence for enhanced competitive ability or allelopathy induction of *Centaurea diffusa* by biological controls. Biological Invasions 10, 79-88.

Ochsmann, J., 1999. Chromosomenzahlen einiger europäischer *Centaurea*-Sippen. Haussknechtia 7, 59-65.

Ochsmann J., 2000. Morphologische und molekularsystematische Untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae-Cardueae) in Europa. - Diss. Bot. 324 (Ph.D. Dissertation).

Ortega, Y.K., McKelvey, K.S., Six, D.L., 2006. Invasion of an exotic forb impacts reproductive success and site fidelity of a migratory songbird. Oecologia 149, 340-351.

Piper, G.L., 2004. Biotic suppression of invasive weeds in Washington state: a half-century of progress. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L., Scott, J.K. (Eds.), Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO, Canberra, pp. 584-588.

Powell, R.D., Myers, J.H., 1988. The effects of *Sphenoptera jugoslavica* Obenb. (Col., Buprestidae) on its host plant *Centaurea diffusa* Lam. (Compositae). Journal of Applied Entomology 106, 25-45.

Roché, B.F., Roché, C.T., 1991. Identification, introduction, distribution, and economics of *Centaurea* species. In: James, L.F., Evans, J. O., Ralphs, M. H., Child, R. D. (Eds.), Noxious Range Weeds. Westview Press, Boulder, CO, pp. 274-291.

Roley, S.S., Newman, R.M., 2006. Developmental performance of the milfoil weevil, *Euhrychiopsis lecontei* (Coleoptera: Curculionidae), on Northern watermilfoil, Eurasian watermilfoil, and hybrid (Northern x Eurasian) watermilfoil. Environmental Entomology 35, 121-126.

Root, R.B., 1973. Organization of a plant-arthropod association in simple and diverse habitats – fauna of collards (*Brassica oleracea*). Ecological Monographs 43, 95-120.

Rosenthal, S. S., Campobasso, G., Fornasari, L., Sobhian, R., Turner, C. E., 1991. Biological control of *Centaurea* spp. In: James, L.F., Evans, J. O., Ralphs, M. H., Child, R. D. (Eds.), Noxious Range Weeds. Westview Press, Boulder, CO, pp. 292-302.

Schroeder, D., 1985. The search for effective biological control agents in Europe 1. Diffuse and spotted knapweed. In: Delfosse, E.S. (Ed.), Proceedings of the VI International Symposium on Biological Control of Weeds. Agriculture Canada, Ottawa.

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

Seastedt, T.R., Knochel, D.G., Garmoe, M., Shosky, S.A., 2007. Interactions and effects of multiple biological control insects on diffuse and spotted knapweed in the Front Range of Colorado. Biological Control 42, 345-354.

Sheley, R.L., Jacobs, J.S., Carpinelli, M.L., 1999. Spotted knapweed. In: Sheley R.L., Petroff, J.K. (Eds.), Biology and Management of Noxious Rangeland Weeds. Oregon State University Press, Corvallis.

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

Sheley, R.L., Olson, B.E., Larson, L.L., 1997. Effect of weed seed rate and grass defoliation level on diffuse knapweed seedlings. Journal of Range Management 50, 39-43.

Sheppard, A.W., Cullen, J.M., Aeschlimann, J.P., 1994. Predispersal seed predation on *Carduus nutans* (Asteraceae) in southern Europe. Acta Oecologia International Journal of Ecology 15, 529-541.

Smith, L., Mayer, M., 2005. Field cage assessment of interference among insects attacking seedheads of spotted and diffuse knapweed. Biocontrol Science and Technology 15, 427-442.

Smith, L., 2004. Impact of biological control agents on *Centaurea diffusa* (diffuse knapweed) in central Montana. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L., Scott, J.K. (Eds.), Proceedings of the XI International Symposium on Biological Control of Weeds. CSIRO, Canberra, pp. 589-593.

Steinger, T., Müller-Schärer, H., 1992. Physiological and growth-responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil-nitrogen availability. Oecologia 91, 141-149.

Stinson, C.S.A., Schroeder, D., Marquardt, K., 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., 1989. The status of biological control of spotted and diffuse knapweed. In: Proceedings of the 1989 Knapweed Symposium, Bozeman, MT, pp. 37-42.

Story, J.M., Boggs, K.W., Nowierski, R.M., 1989. Effect of two introduced seed head flies on spotted knapweed. Montana Agresearch 6, 14-17.

Story, J.M., Coombs, E.M., 2004. *Larinus minutus*: In: Coombs, E.M., Clark, J.K., Piper, G.L., Cofrancesco, A.F. Jr. (Eds.), Biological control of invasive plants in the United States. Oregon State University Press, Corvallis Oregon, USA, pp. 214-217.

Story, J.M., Callan, N.W., Corn, J.G., White, L.J., 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.

Takahashi, M., 2006. Insect community composition in ecological resistance to invasiveness of bull thistle in eastern Nebraska. Masters thesis, University of Nebraska, Lincoln, NE.

Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628-630.

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

van Klinken, R.D., Raghu, S., 2006. A scientific approach to agent selection. Australian Journal of Entomology 45, 253-258.

Vilá, M., Maron, J.L., Marco, L., 2005. Evidence for the enemy release hypothesis in *Hypericum perforatum*. Oecologia 142, 474-479.

Wapshere, A.J., Delfosse, E.S., Cullen, J.M., 1989. Recent developments in biological control of weeds. Crop Protection 8, 227-250.

Watson, A.K., Renney, A.J., 1974. The biology of Canadian weeds: *Centaurea diffusa* and *C. maculosa*. Canadian Journal of Plant Science 54, 687-701.

Whitham, T.G., 1989. Plant hybrid zones as sinks for pests. Science 244, 1490-1493.

White, E.M., Sims N.M., Clarke, A.R. 2008. Test of the enemy release hypothesis: The native magpie moth prefers a native fireweed (*Senecio pinnatifolius*) to its introduced congener (*S. madagascariensis*). Austral Ecology 33, 110-116.

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

Wolfe, L.M., 2002. Why alien invaders succeed: Support for the escape-from-enemy hypothesis. The American Naturalist 16, 705-711.

Young, L.R., 2003. Native Insect Herbivory Provides Resistance to Invasive Spread by an Exotic Thistle. Thesis. University of Nebraska, Lincoln.

Zalucki, M.P., Day, M.D., Playford, J., 2007. Will biological control of *Lantana camara* ever succeed? Patterns, processes, & prospects. Biological Control 42, 251-261.

Table 3.1. A summary of the studies that compare the preference or performance of *Larinus minutus* for spotted (SK) vs. diffuse knapweed (DK). EU = plants of European origin, NA = plants of North American origin. The preferred plant type or the plant type with greater *L. minutus* performance is shown in bold.

Author(s)	Measure of Preference or Performance	Preferred Species
Smith and Mayer 2005	Establishment of *L. minutus* in release cages	**DK NA– 100%** SK NA – 69%
	L. minutus infested capitula in release cages	**DK NA – 30 ± 4%** SK NA – 11 ± 3%
	L. minutus progeny production in release cages	**DK NA – 30.9/100 capitulum** SK NA – 11.1/100 capitulum
Groppe et al. 1990	Dispersal preference in native range	DK EU – Insignificant preference over SK EU
	Rate of attack in the field in the native range	**SK EU – 27.7%** DK EU – 12.6%
Jordan 1995	Adult feeding on leaf tissue	**SK NA, EU – Yes** **DK NA – Yes** DK EU - No
	Percentage of flowers attacked, choice test	**SK EU – 66.7%** DK EU – 27.8%
	Number of eggs laid, choice test	**SK EU – 19** DK EU - 8
	Percentage of flowers attacked, choice test	**SK EU – 66.7%** DK NA – 16.7%
	Number of eggs laid, choice test	**SK EU – 12** DK NA – 3
	Percentage of flowers attacked, choice test	SK EU – 66.7% SK NA – 55.6%
	Number of eggs laid, choice test	SK EU – 19 SK NA – 20
	Survival pupa to adult, performance test	**SK NA – 70%** SK EU – 55% DK NA – 55% DK EU – 50%

Table 3.2. The proportion of seedheads and roots with evidence of herbivory per surveyed diffuse and/or spotted knapweed site in the introduced (North America) and native (European) ranges in 2005 and 2006. DK = diffuse knapweed, SK = spotted knapweed, n = number of plants surveyed within the site. Plants within North American diffuse knapweed sites in 2006 were classified as either DL = diffuse-like or HL = hybrid-like based on floral morphology.

Site	GPS location	Altitude (m)	Seedhead	Root
2005 North America diffuse knapweed				
Wolcott, CO	N39°42'10.2" W106°40'32.8"	2130	--	0.04 n=24
Denver, CO	N39°41'48.5" W105°11'32.7"	1848	0.69 n=29	0.28 n=29
Idalia, CO	N39°40'30.5" W102°33'22.3"	1260	--	0.0 n=16
Pendleton, OR	N45°54'58.8" W119°33'31.8"	94	0.68 n=30	0.57 n=30
Dalles, OR	N45°36'17.1" W121°11'02.3"	27	0.58 n=27	--
Wenatchee, WA	N47°33'40.4" W120°16'11.3"	249	--	0.73 n=26
Wenatchee, WA	N47°28'14.4" W120°20'11.5"	232	--	0.32 n=28
Kaycee, WY	N43°50'13.7" W106°52'28.6"	1849	0.13 n=16	0.0 n=20
Natrona, WY	N43°23'07.9" W107°03'45.6"	1888	0.05 n=22	0.0 n=25
2005 North America spotted knapweed		(all sites likely 4n)		
Eagle, CO	N39°39'22.3" W106°35'59.9"	2262	--	0.0 n=18
Boise, ID	N43°38'35.5" W116°15'19.7"	795	--	0.0 n=12
Big Timber, MT	N45°46'23.7" W109°47'56.8"	1216	--	0.04 n=21
Big Timber, MT	N46°04'29.3" W109°56'08.7"	1507	--	0.0 n=27
Laurel, MT	N45°41'21.8" W108°46'17.2"	1076	0.83 n=21	0.0 n=23
Westport, NY	N44°16'42" W073°31'52.6"	168	0.56 n=20	0.15 n=20
Ithaca, NY	N42°17'33.9" W076°42'49"	345	0.68 n=30	--
Buffalo, WY	N44°29'59.9" W109°12'44.3"	1664	0.08 n=13	0.0 n=11
Buffalo, WY	N44°22'38.7" W106°42'37.2"	1435	0.66 n=14	0.0 n=18
Casper, WY	N42°54'14.6" W106°27'09.7"	1628	0.64 n=27	--
Flamborough, Ontario	N43°20'58.5" W080°06'43.6"	252	0.23 n=20	0.0 n=20
2005 North America diffuse + spotted knapweed				
Fairfield, ID	N43°18'16.6" W114°48'06.4"	1528	--	0.13 DK n=15 0.0 SK n=13

2005 Europe diffuse knapweed				
Romania 3	N43°54'8.76" E28°34'26.1"	17	0.03 n=29	0.43 n=30
Romania 4	N44°23'22.8" E28°31'35.9"	17	0.15 n=29	0.63 n=30
Romania 5	N44°94'34.3" E28°91'4.9"	26	0.08 n=30	0.37 n=30
Romania 6	N45°11'8.8" E28°47'8.3"	9	0.07 n=30	0.37 n=30
Romania 7	N45°29'52.3" E27°54'42.5"	17	0.12 n=30	0.57 n=30
Ukraine 5	N48°48'22.8" E30°33'54.9"	174	--	0.83 n=30
2005 Europe spotted knapweed				
Austria 1 (ploidy unknown)	N47°53'3.0" E16°16'40.9"	573	0.32 n=30	0.40 n=30
Hungary 1 (2n)	N46°48'1.7" E17°12'20.0"	151	0.27 n=30	0.20 n=30
Romania 2 (4n)	N47°13'59.9" E26°30'57.4"	327	0.80 n=30	0.87 n=29
Romania 8 (4n)	N47°28'30.3" E26°16'6.0"	401	0.67 n=30	0.37 n=30
Romania 9 (ploidy unknown)	N46°21'33.1" E25°47'38.7"	727	0.43 n=30	0.23 n=30
2006 North America diffuse knapweed				
Adams 2, CO	N39°52'02.5" W104°55'30.8"	1551	0.40 DL=16, HL=4	--
Doug1, CO	N39°24'36.7" W104°52'12.0"	1848	0.79 DL=9, HL=19	--
Doug2, CO	N39°20'23.8" W104°49'53.3"	1960	0.68 DL=9, HL=19	--
Estes, CO	N40°22'09.5" W105°31'54.2"	2183	0.53 DL=10, HL=19	--
Mosier, OR†	N45°41'01.9" W121°24'08.3"	43	0.95 DL=6, HL=8	--
Tygh, OR†	N45°15'14.9" W121°09'05.8"	361	0.80 DL=9, HL=22	--
I84, OR†	N45°47'28.2" W120°01'51.8"	96	0.31 DL=9, HL=20	--
Hepner, OR†	N45°20'34.4" W119°32'58.1"	646	0.69 DL=8, HL=19	--
Condon, OR†	N45°14'35.7" W120°10'55.5"	859	0.83 DL=11, HL=18	--
Antelope, OR†	N44°50'34.6" W120°54'01.7"	593	0.40 DL=8, HL=19	--
Laramie, WY	N41°04'55.1" W105°29'06.2"	2275	0.01 DL=15, HL=10	--

†Sites < 6 km from seedhead herbivore (*Larinus minutus* and *Bangasternus fausti*) release points.

Figure 3.1. The proportion of diffuse knapweed (white bars) A) seedheads and B) roots and spotted knapweed (gray bars) C) seedheads and D) roots with evidence of herbivory in Europe vs. North America. $^{*}P < 0.05$, $^{***}P < 0.001$

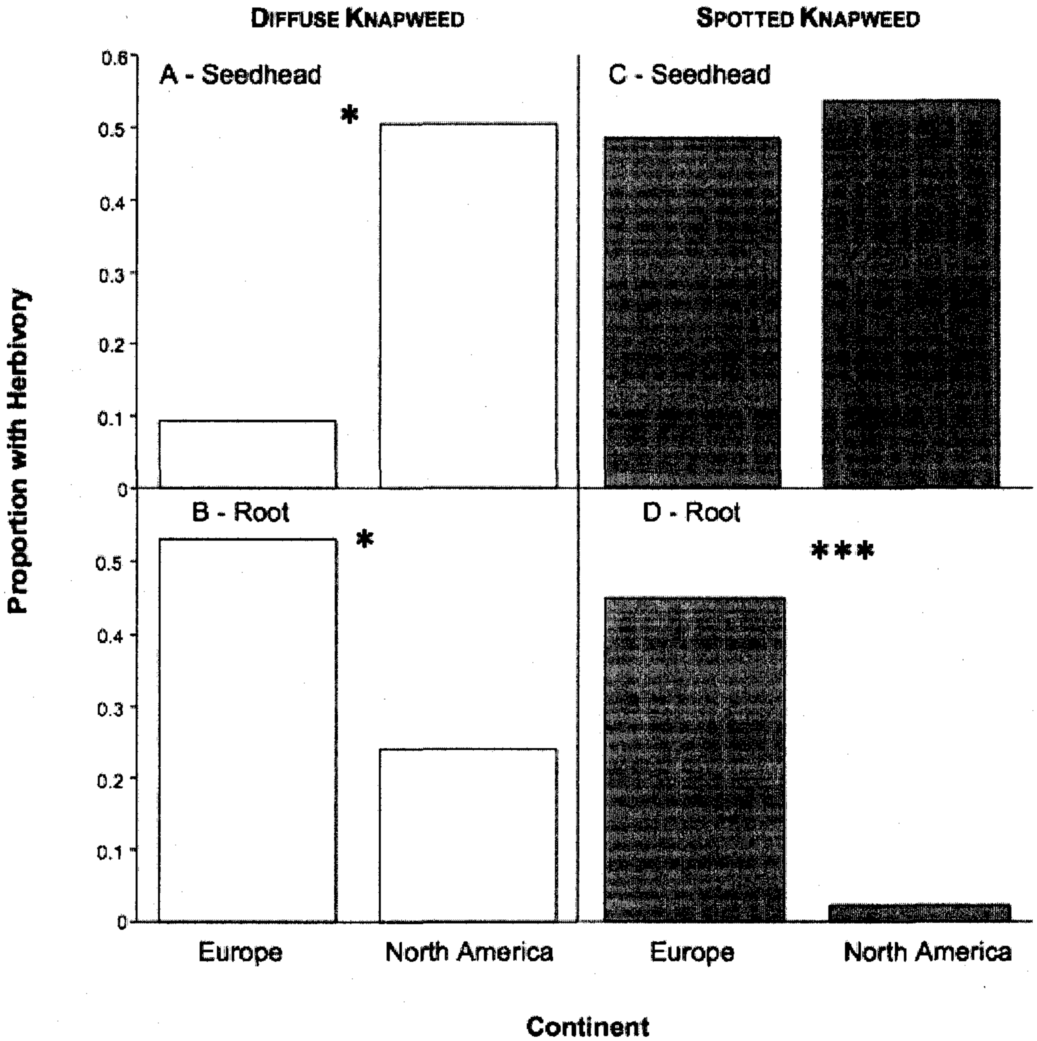
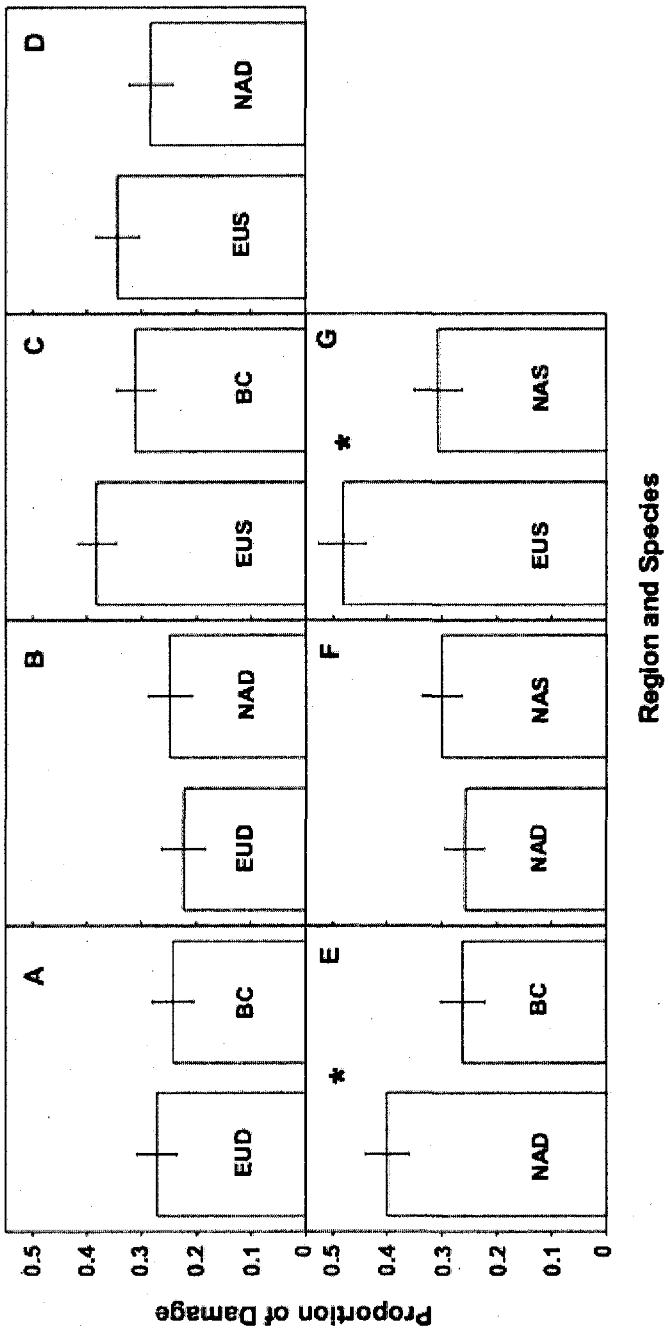

DIFFUSE KNAPWEED
SPOTTED KNAPWEED
A - Seedhead
C - Seedhead
B - Root
D - Root
*
*
***
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion with Herbivory
Europe
North America
Europe
North America
Continent

Figure 3.2. The proportion of damage (i.e. leaf tissue consumption) by the biological control agent *Larinus minutus* in two sets of paired preference tests (A-D = set 1; E-G = set 2). Pairs of trials were analyzed with paired t-tests. BC = Back Cross 1 hybrid individuals; EUD = European diffuse knapweed; EUS = European spotted knapweed (verified diploid); NAD = North American diffuse knapweed; NAS = North American spotted knapweed (verified tetraploid). Values represent the mean ± 1 SE, *$P < 0.01$.


A
B
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0.5
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0.1
0
Proportion of Damage
Region and Species