Geographic Patterns of Interspecific Hybridization between Spotted Knapweed (*Centaurea stoebe*) and Diffuse Knapweed (*C. diffusa*)

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Hybridization between species has the potential to change invasion dynamics. Field observations suggest that spotted knapweed and diffuse knapweed, two ecologically and economically destructive invasive plants, hybridize in their introduced range. As a first step towards understanding whether hybridization has affected the dynamics of the invasion of these species, we conducted field surveys in the introduced (North American) and native (European) ranges to discern patterns of hybridization and measured fitness-related traits among field hybrids and parental species. In North America we detected plants with hybrid morphology in 97% of the diffuse knapweed sites (n = 40); such 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 (n = 22). In most regions surveyed in Europe, diffuse knapweed and spotted knapweed were isolated from each other and existed as distinct, nonhybridizing species. However, in Ukraine, the two species frequently coexisted within a site, resulting in hybrid swarms. On average, the plants from the North American diffuse knapweed sites (including plants with both diffuse and hybrid morphology), were larger than the apparently pure diffuse knapweed in the native range. The cross-continental patterns of hybridization likely are explained by differences in cytology. It recently has been confirmed that the spotted knapweed in North America is tetraploid whereas the diffuse knapweed is diploid. Genetic incompatibilities associated with these two cytotypes likely prevent ongoing hybridization. We hypothesize that hybrid individuals were introduced to North America along with diffuse knapweed. Because plants with hybrid morphology are found in nearly all North American diffuse knapweed sites, the introduction of hybrids likely occurred early in the invasion of diffuse knapweed. Thus, although the presence of hybrids might facilitate the ongoing invasion of diffuse knapweed into North America, elevated concern regarding their presence might not be warranted. Because such individuals are not likely to represent a new hybridization event, currently effective management strategies used in diffuse knapweed sites should not need alteration.

Nomenclature: Diffuse knapweed, *Centaurea diffusa* Lam.; spotted knapweed, *C. stoebe* L. (= *C. maculosa* Lam.)
Key words: Biological invasion, field surveys.

Intra- and interspecific hybridization are hypothesized to play an important role in plant invasion by creating evolutionary novelty, increasing genetic variation, decreasing genetic load, and/or causing heterosis (i.e., hybrid vigor) (Ellstrand and Schierenbeck 2000; Rieseberg et al. 2007). Ellstrand and Schierenbeck (2000) identified 28 plant species for which there is evidence of hybridization occurring prior to invasiveness. Although this suggests that hybridization might confer some advantage to an invasive species, the evidence is not definitive; hybridization is not unique to invasion, because many plant species hybridize naturally within their native ranges. However, even if hybridization occurs in each range, it might contribute more to the success of a species in the introduced range, which represents a new habitat and selection regime. One first step towards understanding whether hybridization has influenced invasion is to quantify the frequency and

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Interpretive Summary

This study serves as the first step towards understanding the role of hybridization in the invasions of spotted and diffuse knapweed. Based on extensive geographic surveys of these species, we conclude that diploid hybrids were most likely introduced along with diffuse knapweed. Hybrid-like plants are encountered in nearly all North American diffuse knapweed sites, and this suggests that hybrids might have been introduced into North America with the original introductions of diffuse knapweed around 1900. Differences in numbers of chromosomes between tetraploid spotted knapweed and diploid diffuse knapweed and largely nonoverlapping distributions likely prevent active hybridization. Therefore, because hybridization is not ongoing, and hybrid-like plants are likely to have been present since the initial stages of the invasion of diffuse knapweed, we suggest that management strategies that have been used effectively against diffuse knapweed do not require alteration if hybrid-like plants are detected at a site. This recommendation includes biological control, because we recently found that biological control agents readily attack hybrid-like plants (Blair et al. 2008). If diploid spotted knapweed is found or eventually introduced to North America, elevated concern might be warranted, because hybridization could result in the creation of unique phenotypes that do require altered management.

patterns of hybridization in the introduced and native range. 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. Additionally, measures of plant performance in the field can provide initial information to evaluate the fitness consequences of hybridization; if interesting patterns are found (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 (*Centaurea stoebe* L. [= C. *maculosa* Lam.] and *C. diffusa* Lam.) 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 by a taxonomic expert (Ochsmann 2001a) suggested that hybrids between these two closely related species are present in the introduced range. Land managers and scientists expressed concern because hybridization could result in the management of a rapidly evolving target. As a first step in evaluating whether hybridization contributed to one or both of these species' success, we surveyed spotted and diffuse knapweed across the United States and Europe to answer the following questions: (1) Do floral and seed morphology reliably separate individuals into three biological groupings (i.e., the two parents plus plants with hybrid morphology)? If so, do those groups differ between the native (European) and introduced (North American) range? (2) How common are plants with hybrid morphology in the native and introduced ranges? Is the pattern of hybridization in North America similar to that found in the native range? (3) Do plants with hybrid morphology exhibit heterosis in the field? Are heterotic patterns similar in North America and Europe?

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. Our research focused on members of the *Centaurea* genus within the section *Acrolophus-Phalolepis* (Garcia-Jacas et al. 2006). More specifically, we focused on members of the *C. stoebe* (sensu lato) species group, which encompasses approximately 33 named taxa (Ochsmann 2000). We 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 as *C. diffusa* Lam. The tetraploid seems to be rare; it only has been reported twice in the literature, once from a specimen in Bulgaria (Löve 1979) and once from the former Yugoslavia (Löve 1978). The diffuse knapweed plants we worked with are likely to be diploid (A. C. Blair, unpublished data; Marrs et al. 2008a). The two cytotypes of spotted knapweed both are classified under the name *C. stoebe* L., which takes precedence over the commonly used *C. maculosa* Lam. (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 reliable way to distinguish the spotted knapweed subspecies (Ochsmann 2001b). In the literature, the few North American spotted knapweed plants assayed for chromosome number were tetraploids (Moore and Frankton 1954; Müller 1989; Ochsmann 2000). Thus, when we refer to spotted knapweed of North American origin, it is likely to be the tetraploid *C. stoebe* subsp. *micranthos*, whereas spotted knapweed from Europe might be either cytotype. When ploidy level is known, we clearly specify it.

Tetraploid spotted knapweed is a perennial (Ochsmann 2000), but diploid spotted knapweed is reported to be a biennial (Ochsmann 2000). Diffuse knapweed is an annual to short-lived perennial (Watson and Renney 1974). Spotted and diffuse knapweed are self-incompatible (A. C. Blair, personal observation; Harrod and Taylor 1995) 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). They

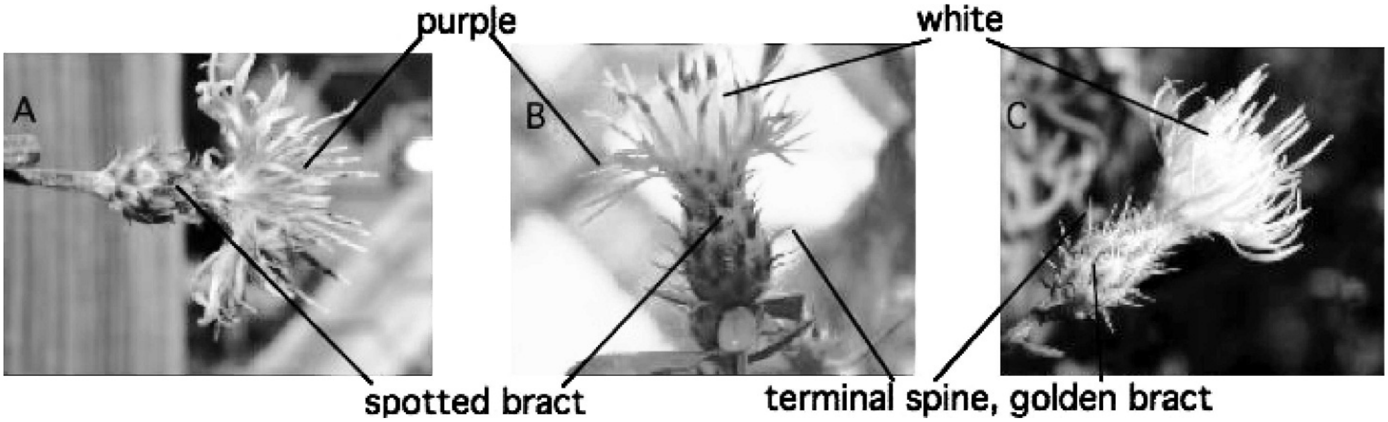

Figure 1. Floral morphology of (A) spotted knapweed, (B) spotted × diffuse hybrid, and (C) diffuse knapweed. Spotted bracts are diagnostic for spotted knapweed, and apical spines and golden bracts are diagnostic for diffuse knapweed. Note the spotted bracts with terminal spines in the hybrid. Flower color is a less reliable indicator of species status, but hybrids generally have both purple and white flowers.

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).

Hybrids between spotted and diffuse knapweed were first identified in the native range in 1909 (Gáyer 1909), and were given the name *C.* × *psammogena* Gáyer. It is thought that they occur only in two narrow zones of overlap in Romania and 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). Floral and seed (i.e., achene) morphology have been used to delineate the two parent species and the hybrids (Ochsmann 2000; Watson and Renney 1974; Figure 1; Table 1). In this study, after using floral characteristics to separate individuals into three groups (diffuse, spotted, and hybrid), we evaluate how various aspects of those groups differ. Because the designation of a plant as a hybrid or a parental species is based on floral morphological characters rather than molecular markers, we refer to the groups as hybrid-like, diffuse-like, or spotted-like to highlight that the classifications contain some uncertainty. Molecular work being gathered in a companion study has corroborated that the presence of intermediate individuals in a

Table 1. Floral characters used to delineate diffuse knapweed (*Centaurea diffusa*), spotted knapweed (*C. stoebe*), and diffuse × spotted knapweed hybrids (*C.* × *psammogena*) in North America (Watson and Renney 1974; Ochsmann 2000). Spotted knapweed plants in North America 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 (W) and length (L)	Achene size and pappus description
Diffuse knapweed	white, rarely pink	distinct terminal spine, ≈ 3 mm, **range 2.2–4.3 mm**	pale yellowish-green, **golden**	**W, 3.0–6.8 mm** **L, 5.5–12.0 mm**	small (2–3 mm) **(2–3.2 mm)** pappus absent to rudimentary
Spotted knapweed[a]	purple, rarely white	4–7 fringes per side, terminal spine absent	black triangular pointy tip	**W, 3.0–8.8 mm** **L, 6.2–12.6 mm**	large (3–3.5 mm) **(2.4–3.6 mm)** pappus persistent, 1–2.5 mm
Spotted × Diffuse hybrids	white, purple, **lavender**, or mixed	distinct terminal spine, ≈ **3 mm**, **range 1.7–4.3 mm**	light to dark tip, variable	**W, 2.6–6.8 mm** **L, 5.9–10.0 mm**	intermediate (2.5–3.5 mm) **(2.0–3.0 mm)** pappus absent to rudimentary

[a] Although these trait descriptions refer specifically to the North American tetraploid spotted knapweed, diploid European spotted knapweed traits overlap extensively (see Ochsmann 2001b for a comparison of floral morphology between the subspecies). Chromosome counts are the only way to definitively separate the two subspecies (Ochsmann 2001b).

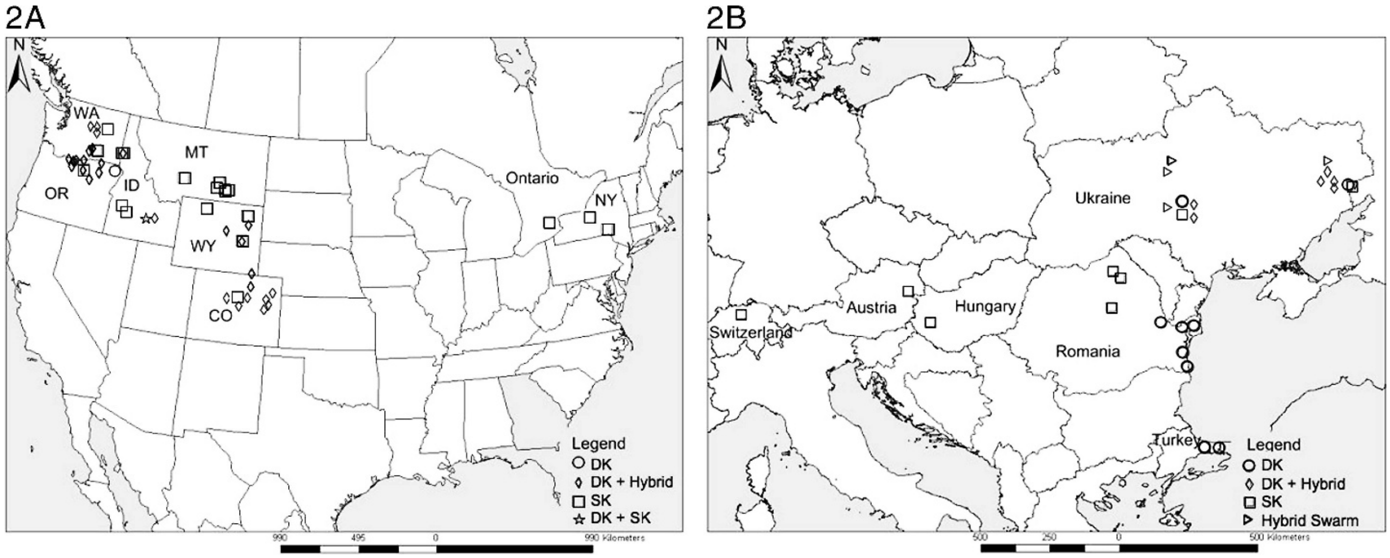

Figure 2. Maps of sites for the field survey of spotted (SK) and diffuse knapweed (DK) conducted in 2005 and 2006 in (A) North America and (B) Europe.

region indicates interspecific hybridization between spotted and diffuse knapweed (A. C. Blair, unpublished data).

Field Surveys of Spotted and Diffuse Knapweed. *Introduced Range—North America 2005 and 2006.* During summer 2005, we surveyed a total of 50 knapweed sites (22 spotted knapweed, 27 diffuse knapweed, and one site with both species) in seven states (Colorado, Idaho, Montana, New York, Oregon, Washington, Wyoming) and Ontario, Canada (Figure 2A; Appendix 1). The focus of this research was on the western United States 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, we contacted county weed supervisors, and sites were selected either by driving until encountering a site or from directions from a weed supervisor. Prior information on whether hybrid-like individuals were present at a site was generally not available.

We visually inspected each site for the presence of hybrid-like individuals. When they were present, we estimated their proportional abundance by either counting hybrid-like and parental plants exhaustively, or for large sites, running transects through the site and counting 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), we conducted more intensive measurements on 30 individual plants. We haphazardly ran a 50-m transect through the population and surveyed plants every meter (or more if plants were spaced further apart). For each plant, we measured plant height and diameter; polycarpy (i.e., evidence of last season's flowering stalks); inflorescence color (ranked 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 to 3 for light to dark bract pigmentation); apical spine length (three spines were measured from three different seedheads midcapitula); capitula width and length from a freshly opened flower; seed length averaged from three seeds; 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 we visited an additional 13 diffuse knapweed sites in Colorado, Oregon, and Wyoming (Appendix 1) to measure the seed characters noted above (*n* approximately 30 plants per site; approximately two-thirds were hybrid-like and one-third were diffuse-like).

Native Range—Europe 2005 and 2006. To compare patterns of hybridization in North America to those in the native range, we conducted field surveys in Europe. We surveyed spotted knapweed sites in Austria and Hungary in 2005 and Switzerland in 2006, and diffuse knapweed sites in Turkey in 2006 (Figure 2B; Appendix 1); it was suggested a priori that these regions did not likely contain hybrids (U. Schaffner, personal communication). We also surveyed both species in Romania in 2005 and Ukraine in 2006, where hybrids were thought to occur (U. Schaffner, personal communication). Thus, although we had general predictions whether or not we would detect hybridization within a region, individual sites were surveyed without prior knowledge of the presence of hybrids. Either local botanists identified sites for us, or we found sites while driving through the countryside. The basic procedures for sampling and obtaining morphological measures described above for North America in 2005 were followed, except in Turkey, where a local botanist inspected the sites exhaustively for hybrid-like individuals.

Because of an unexpectedly cool spring in 2006, plants were just starting to flower during the Ukraine survey, so we could not record the flower color for most plants. Additionally, capitulum measurements and spine length were measured mostly on immature seedheads, so the data for floral traits are not directly comparable to those from North America and other sites in Europe. At seven of the Ukraine sites, plants were marked, and Ukrainian colleagues returned later in the summer to collect seed.

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 objectively classify plants prior to further analyses, we used hierarchical clustering to identify natural groupings based on the five floral characters (flower color, bract pigmentation, capitula width, capitula length, and spine length). Thus, the null hypothesis underlying this statistical analysis was that all plants form a single group; the alternative was that the five floral characters indicate three natural groupings. We had complete floral data for 538 of the 822 plants surveyed in North America 2005. We used a similar approach to classify the Ukrainian plants (the only location in Europe we surveyed with hybrids, see below), except that many plants did not have open flowers, so we could not include flower color. We had data on the remaining floral traits for 208 out of 419 plants surveyed in Ukraine in 2006. Prior to analysis, data were standardized by the variable mean and standard deviation. We selected "Ward's minimum variance" clustering method, because it has been employed frequently in the literature and has been found to outperform other hierarchical methods (Khattree and Naik 2000 p. 442). We also compared this method to "average linkage" and the "centroid method"; all methods produced similar patterns. For these analyses, we used JMP version 6.0 (SAS Institute 2003).

Global Patterns of Hybridization. We conducted all other statistical analyses in SAS version 9.1 (SAS Institute 2002). We 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 any hybrid-like plants, we used contingency table analysis (G-tests). Sites with any 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, we used ANOVA with region as a fixed effect and the proportion of hybrid-like plants within a site as the response variable.

Performance and Seed Trait Comparisons. In North America, hybrid-like plants were found only within diffuse knapweed sites (see below). Thus, the relevant performance and seed trait comparisons were between North American diffuse-like and hybrid-like plants, but not spotted-like plants. We compared the following traits from plants within North American diffuse knapweed field sites: plant diameter, plant height, and number of stems (n = 15 sites sampled intensively in 2005), and seed length (n = 13 sites, 2006 data). 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. We analyzed these traits with a mixed model (PROC MIXED) with population as a random effect and classification (diffuse-like or hybrid-like) as a fixed effect. We used a mixed model (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.

We observed hybrid swarms with individuals ranging from spotted knapweed to diffuse knapweed in several of the Ukraine sites. Thus, we compared performance and seed traits between spotted-like, hybrid-like, and diffuse-like plants based on the groups found by the cluster analysis described above. We examined plant size (plant diameter, plant height, stem number) and seed length among the three groups with a mixed model (PROC MIXED) with population as a random effect and plant type (spotted-like, diffuse-like, or hybrid-like) as a fixed effect. If the model was significant, we used Tukey-Kramer posthoc tests to determine which groups differed. Again, plant height, diameter, and stem number + 1 were log-transformed prior to analysis. We 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, we used Tukey-Kramer posthoc tests to determine which classes differed.

Because intermediate 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 further evaluate whether diffuse knapweed performance and seed traits might be influenced by hybridization, we compared measures of size (plant diameter, plant height, and stem number) and seed length between diffuse knapweed sites in Europe lacking hybrid-like individuals (n = 7 sites, 5 in Romania, 2 in Ukraine) to the assayed North American diffuse knapweed sites with hybrid-like individuals sampled in 2005 (n = 15 sites). For these analyses we first combined diffuse-like and hybrid-like individuals from North America, because we were interested in the overall differences at the continent scale instead of among morphological variants within a site. Then we tested whether the same patterns were observed when hybrid-like individuals were excluded from analysis. All of the size measures were log-transformed, and as above, the reported means have been back-transformed. Traits were analyzed with a mixed model (PROC MIXED) with population nested within continent as a random effect and continent (North America or Europe) as a fixed effect. We 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.

Table 2. The proportion of field-collected seed bearing a pappus from North American and Ukrainian diffuse-like, hybrid-like, and spotted-like maternal plants, and the proportion of plants that exhibited polycarpy (i.e., evidence of last year's flowering stalks).

Trait	Diffuse-like[a]	Hybrid-like	Spotted-like
Proportion with pappus			
North America	0.29 A	0.30 A	—
Ukraine	0.15 A	0.69 B	1.0 B†
Proportion with polycarpy			
North America	0.04 A	0.17 B	—
Ukraine	0.011 A	0.017 A	0.17 B

[a] Different letters denote significantly different means (Ukrainian data, Tukey's test, $P < 0.05$, † $P < 0.1$). See the text for details regarding the statistical analyses.

Results

Field Surveys of Spotted and Diffuse Knapweed. *1) Do Floral and Seed Morphology Reliably Separate Individuals into Three Biological Groupings (i.e., the Two Parents Plus Plants with Hybrid Morphology)? If So, Do Those Groups Differ Between the Native (European) and Introduced (North American) Range?* Based on the North American floral data, the Ward's dendrogram revealed three major clusters: cluster 1 contained only individuals that we had classified as diffuse-like in the field (100%); cluster 2 was comprised largely of what we had classified as hybrid-like in the field (73.8% hybrid); and cluster 3 was comprised mainly of individuals we had classified as spotted-like (98.8%) (dendrogram not shown). The cluster 2 (hybrid-like) individuals were all found in the diffuse knapweed sites. We used these three statistical clusters to categorize individuals for which we had data on all five floral characteristics prior to further analyses. We lacked a complete set of floral data on 284 individuals. To assess whether our field classification would be an appropriate substitute for the statistical classification, we compared how well the two corresponded. Classification based on the cluster analysis matched our field classifications 92% of the time; thus we grouped the 284 plants not included in the cluster analysis based upon our field observations. This is conservative, in that it might add to the variance in our dataset.

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

Seeds from diffuse-like and hybrid-like plants measured at 13 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 seed types had pappi ($F_{1,243}$ = 0.10, P = 0.75) (Table 2). Similarly, seed length did not differ significantly among the three Ukrainian plant types ($F_{2,219}$ = 0.99, P = 0.37). The presence of pappi did differ among the classes ($F_{2,222}$ = 3.98; P = 0.02). Plants in the spotted-like and hybrid-like clusters produced seeds with a pappus more often than plants in the diffuse-like cluster (Table 2).

A comparison of the floral and seed traits between the North American diffuse knapweed sites with hybrid-like individuals and the European diffuse knapweed sites without hybrids (Appendix 1) supported that hybrids are present in North American diffuse knapweed sites. 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 (Table 3), as predicted if plants contain introgression from spotted knapweed (Table 1). When hybrid-like plants were removed from these analyses, the results did not change qualitatively (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 larger proportion of North American seeds had pappi ($F_{1,20}$ = 9.62, P =

Table 3. Floral and vegetative trait values for North American and European field-surveyed diffuse knapweed. Floral values are lsmeans ± 1 SE. The three vegetative traits were ln transformed prior to analysis and the reported values have been back-transformed (back-transformed lsmean [lower and upper limits for back-transformed SE]). See the text for details on the statistical analyses.

Trait	North America	Europe
Floral		
Capitula width (mm)	4.68 ± 0.15**	3.10 ± 0.22
Capitula length (mm)	7.85 ± 0.16*	7.03 ± 0.23
Terminal spine length (mm)	3.04 ± 0.07*	2.62 ± 0.10
Vegetative		
Number of stems	3.90 (3.45, 4.41)†	2.63 (2.20, 3.14)
Plant diameter (cm)	44.18 (40.53, 48.16)*	25.79 (22.73, 29.26)
Plant height (cm)	48.40 (44.56, 52.57)*	30.10 (26.67, 33.97)

* $P < 0.01$;
** $P < 0.0001$;
† $P < 0.1$.

0.0056; North America = 0.30; Europe = 0.077), also expected if some North American diffuse knapweed plants contain introgression from spotted knapweed.

2) How Common Are Plants with Hybrid Morphology 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 39 of the 40 diffuse knapweed sites contained hybrid-like individuals (Table 4). The percentage of hybrid-like plants in a diffuse knapweed site ranged from 1 to 95%, with most sites containing less than 20% hybrid-like individuals (median value = 13.5%, mean ± SE = 24 ± 5%). None of the 22 spotted knapweed sites contained hybrid-like plants.

Hybridization was not detected in regions where diploid spotted and diffuse knapweed were not thought to overlap (Austria, Hungary, Switzerland, and Turkey). Although we 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 ongoing in Romania, our surveys did not capture it.

Spotted and diffuse knapweed overlapped more extensively in Ukraine, with four of fourteen sites containing a gradient from pure spotted-like to pure diffuse-like (Table 4). Such hybrid swarms were not encountered in North America or elsewhere in Europe. Six of the diffuse knapweed sites lacking spotted knapweed individuals in Ukraine still contained hybrid-like plants, as seen at most sites in North America.

Overall at the site level, more North American diffuse knapweed sites contained hybrid-like plants than all of the diffuse knapweed sites in Europe ($G = 11.2$, $P < 0.001$; North America = 0.98; Europe = 0.58). However, when North America was compared to just Ukraine, where hybridization appeared to be on-going, a similar proportion of diffuse knapweed sites contained hybrid-like plants ($G = 2.84$, $P = 0.09$; North America = 0.98; Ukraine = 0.83).

Interestingly, at the within-site level, 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). When Ukraine was separated from the rest of Europe, a different outcome was found. North American

Table 4. Patterns of hybridization across the global distribution of spotted and diffuse knapweed. The number of sites is denoted by *n*; the percentage indicates the percent of the *n* sites with plants of hybrid morphology.

Pattern of hybridization	North America	Europe (including Turkey, minus Ukraine)	Ukraine
Spotted knapweed	No hybrid-like plants detected (*n* = 22, 0%)	No hybrid-like plants detected (*n* = 6, 0%)	Hybrid swarms with diffuse knapweed, hybrid-like plants detected (*n* = 6, 67%)
Diffuse knapweed	Hybrid-like individuals detected at nearly all sites (*n* = 40, 98%)	< 5% hybrid-like detected at one site (*n* = 7, 14%)	Hybrid swarms with spotted knapweed, hybrid-like plants detected (*n* = 12, 83%)

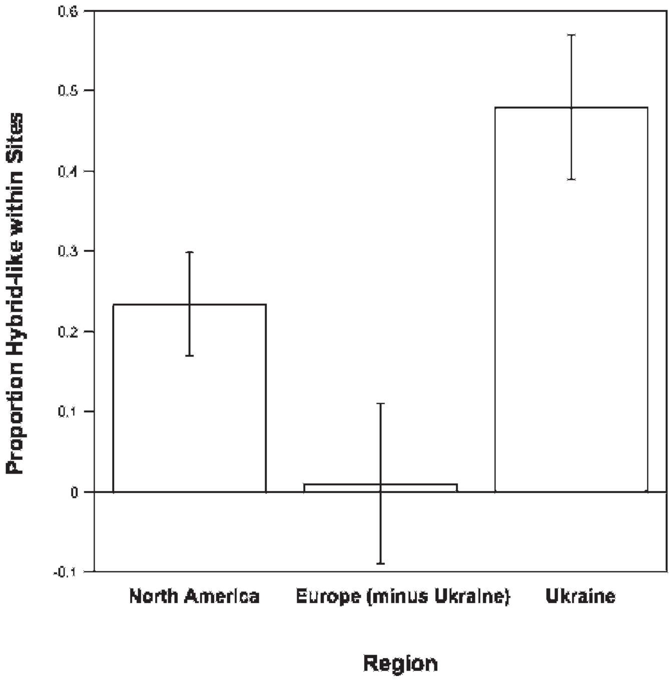

Figure 3. The proportion of hybrid-like plants found within diffuse knapweed sites in North America, Europe (minus Ukraine), and Ukraine. Values are least square means ± 1 SE.

diffuse knapweed sites contained a greater proportion of hybrid-like plants than Europe minus Ukraine ($F_{1,27}$ = 3.66, P = 0.06), whereas a smaller proportion of hybrid-like plants was found in North American sites when compared to only Ukraine ($F_{1,34}$ = 4.54, P = 0.04) (Figure 3). Clearly, the outcome is dependent upon the region of comparison within Europe.

3) Do Plants with Hybrid Morphology Exhibit Heterosis in the Field? Are Heterotic Patterns Similar between North America and Europe? We wanted to determine 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 their maintenance. 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, whereas plant height was significantly different ($F_{1,421}$ = 6.09, P = 0.014); hybrid-like plants were approximately 7% taller than diffuse-like plants (back-transformed mean [cm] [± back-transformed SE]: hybrid-like = 50.1 cm [46.2, 54.4]; diffuse-like = 46.8 cm [43.1, 50.8]). 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) (Table 2).

Vegetative morphological traits were similar among spotted-like, diffuse-like, and hybrid-like plants in Ukraine. Plant height ($F_{2,403}$ = 0.24, P = 0.79), plant diameter ($F_{2,403}$ = 0.33, P = 0.72), and number of stems ($F_{2,403}$ = 1.72, P = 0.18) did not significantly differ among the plant groups. Plant groups differed in the proportion of polycarpy ($F_{2,398}$ = 5.53, P = 0.0043) (Table 2). Diffuse-like and hybrid-like plants were less often polycarpic than spotted-like plants.

We compared vegetative data from North American diffuse knapweed sites containing hybrid-like plants to the apparently pure European diffuse knapweed sites lacking hybrids to see if the plants from the admixed North American sites were larger. North American plants were larger than European plants in both plant diameter ($F_{1,20}$ = 12.4, P = 0.0021) and height ($F_{1,20}$ = 10.50, P = 0.0041), and number of stems was marginally higher for North American plants as well ($F_{1,20}$ = 3.35, P = 0.0823) (Table 3). To evaluate whether hybrid-like plants drive this size difference, we removed them from the North American data set and reran the analyses. Even without hybrid-like individuals, North American plants still were significantly taller than European plants ($F_{1,20}$ = 9.49, P = 0.0059). Overall, more North American diffuse knapweed 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).

Discussion

Global Patterns of Hybridization. The frequency and distributions of plants with hybrid morphology revealed patterns of hybridization between spotted knapweed and diffuse knapweed that differ in Europe and North America. In the introduced range, hybrid-like plants were not detected within spotted knapweed sites, but instead were found in almost every diffuse knapweed site sampled. In the native range, it is clear that in some regions (i.e., Ukraine), hybridization is currently ongoing and likely results from an overlap in the distributions of diploid spotted and diploid diffuse knapweed (recall that the hybrids result from crosses between the diploid variants). In other places within Europe, hybridization was not detected, which suggests that the diploid species do not overlap within those regions. 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 only tetraploid individuals (H. Müller-Schärer, unpublished data). Regarding the introduced range, two scenarios are plausible: (1) hybridization is frequent and ongoing, but back-crossing occurs only in the direction of diffuse knapweed, or (2) hybrids were introduced along with diffuse knapweed. We argue that the latter scenario matches the evidence most parsimoniously for two main reasons.

First, it is highly likely that spotted and diffuse knapweed in North America are cytologically incompatible. 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, and both individuals were tetraploid. Chromosome number is the only unambiguous character to discern between the diploid *C. stoebe* subsp. *stoebe* and tetraploid *C. stoebe* subsp. *micranthos* (Ochsmann 2001b). Recently, more extensive surveys of spotted knapweed ploidy across North America detected only *C. stoebe* subsp. *micranthos* (H. Müller-Schärer, personal communication). Additionally, it recently has been found that North American spotted knapweed individuals have up to four alleles at microsatellite loci, which strongly suggests they are the tetraploid *C. stoebe* subsp. *micranthos* (Marrs et al. 2008b). Diffuse knapweed has received less attention, but cytological analyses (A. C. Blair, unpublished data; Moore and Frankton 1954) and microsatellite variation (Marrs et al. 2008a) reveal only diploids in North America. F1 hybrids between tetraploids and diploids should produce triploids, yet triploids have never been encountered in North America and instead, plants with hybrid morphology that were assayed for ploidy were exclusively diploid (A. C. Blair, unpublished data; Moore and Frankton 1954; Ochsmann 2001a). Thus, ongoing hybridization seems unlikely. Also, despite conducting many successful hand-pollinations within cytotypes of both species, hand-pollinations between spotted and diffuse knapweed from across North America never set seed (A. C. Blair, unpublished data), which fits with genetic incompatibilities resulting from spotted knapweed being tetraploid and diffuse knapweed being diploid.

Second, the distributions of spotted and diffuse knapweed rarely overlapped in North America, and when we found areas of co-occurrence, we did not detect different patterns of hybridization than when we surveyed isolated diffuse knapweed sites (A. C. Blair, personal observation). It therefore seems unlikely that the hybridization detected in the field stems from recent events; instead, it is probable that diffuse knapweed was introduced one or multiple times (Hufbauer and Sforza 2008; Marrs et al. 2008a) with individuals containing introgression from diploid spotted knapweed.

Patterns of Heterosis. The North American plants that clustered as hybrid-like were slightly taller and exhibited polycarpy more often than plants that clustered as diffuse-like. Thus, there might be some fitness advantages to plants that display hybrid floral characters in North America, although a common garden study is needed to evaluate this possibility rigorously. In Ukraine we found that plants from the parental-like and hybrid-like classes did not differ for size-related vegetative morphological traits, and hybrid-like and diffuse-like plants both exhibited less polycarpy than spotted-like plants.

Overall, plants from the North American diffuse knapweed sites with hybrid-like individuals were more robust than plants from the apparently pure European diffuse knapweed sites. North American plants had both greater diameter and height than European plants. North American plants were larger even when hybrid-like individuals were excluded from the analysis, which demonstrates that the hybrid-like individuals did not drive the pattern. Other factors, including environmental conditions, are certain to influence these traits. This pattern does support the findings that introduced organisms are often larger than their conspecifics in the native range (Blair and Wolfe 2004; Groholz and Ruiz 2003; Leger and Rice 2003; Jakobs et al. 2004; Siemann and Rogers 2001; but see Thébaud and Simberloff 2001; Willis et al. 2000). To explain this pattern, the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzold 1995) proposes that when plants are introduced 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 toward 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. Although these results are based on only four populations, it suggests that in our study, diffuse knapweed might be larger in North America because of differential herbivory or other environmental factors, rather than genetically based differences.

Hybridization has recently been posited as a mechanism that could stimulate or aid invasiveness (Ellstrand and Schierenbeck 2000). Indeed, some of the most problematic invaders have turned out to be hybrids (e.g., *Spartina* [Ayers et al. 1999]; *Tamarix* [Gaskin and Schaal 2002]; *Reynoutria* [Katerina et al. 2004]). Diffuse knapweed has attained densities that are ecologically and environmentally damaging in the introduced range (Watson and Renney 1974). Introduced diffuse knapweed individuals might 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. The introduction of hybrids between diffuse knapweed and diploid spotted knapweed could have influenced the invasion by creating more vigorous individuals and/or novel or enhanced variation. A common garden experiment is focusing on the performance of diploid hybrids relative to their diploid parental species to evaluate whether hybridization might have facilitated invasion by diffuse knapweed.

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Literature Cited

Ayers, D. R., D. Garcia-Rossi, H. G. Davis, and D. R. Strong. 1999. Extent and degree of hybridization between exotic (*Spartina alterniflora*) and native (*S. foliosa*) cordgrass (Poaceae) in California, USA determined by random amplified polymorphic DNA (RAPDs). Mol. Ecol. 8:1179–1186

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.

Blair, A. C., U. Schaffner, P. Häfliger, S. K. Meyer, and R. A. Hufbauer. 2008. How do biological control and hybridization affect enemy escape? Biol. Control 46:358–370.

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

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.

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.

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

Hufbauer, R. A. and R. Sforza. 2008. Multiple introductions of two invasive *Centaurea* taxa inferred from cpDNA haplotypes. Divers. Distrib. 14:252–261.

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. Divers. Distrib. 10:11–19.

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

Khattree, R. and D. N. Naik. 2000. Multivariate Data Reductuion and Discrimination with SAS Software. Cary, NC: SAS Institute, Inc. p. 442.

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 Technol. 3:627–631.

Leger, E. A. and K. J. Rice. 2003. Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. 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* (Asteraceae): 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. Syst. 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. 2008a. When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biol. Invasions 10:561–572.

Marrs, R. A., R. Sforza, and R. A. Hufbauer. 2008b. Evidence for multiple introductions of *Centaurea stoebe micranthos* (spotted knapweed, Asteraceae) to North America. Mol. Ecol. 17:4197–4208.

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.

Ochsmann, J. 1998. Ein bestand von *Centaurea* × *psammogena* Gáyer (*Centaurea diffusa* Lam. × *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). 242 p.

Ochsmann, J. 2001a. An overlooked hybrid in North America: *Centaurea* × *psammogena* Gáyer (diffuse knapweed × spotted knapweed). Page 76 *in* L. Smith, ed. The First International Symposium of the Twenty-First Century. Albany, CA: USDA-ARS. [Abstract]

Ochsmann, J. 2001b. 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. Albany, CA: USDA-ARS.

Ortega, Y. K., K. S. McKelvey, and D. L. Six. 2006. Invasion of an exotic forb impacts reproductive succes and site fidelity of a migratory songbird. Oecologia 149:340-351.

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.

SAS Institute. 2002. JMP Statistics and Graphics Guide. Version 5. Cary, NC: SAS Institute Inc. 707 p.

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. Corvallis, OR: Oregon State University 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 Manag. 50:39–43.

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

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

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

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.

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Appendix 1. Spotted and diffuse knapweed sites surveyed in Europe and North America in 2005 and 2006. Species overlap refers to whether or not we 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	Lat/long	Species overlap	Survey type
2005 Europe				
Romania 3	DK	N43°54′8.76″ E28°34′26.1″	Yes	Comprehensive
Romania 4	DK	N44°23′22.8″ E28°31′35.9″	Yes	Comprehensive
Romania 5	DK	N44°94′34.3″ E28°91′4.9″	Yes	Comprehensive
Romania 6	DK	N45°11′8.8″ E28°47′8.3″	Yes	Comprehensive
Romania 7	DK	N45°29′52.3″ E27°54′42.5″	Yes	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″	Yes	Comprehensive
Romania 8	SK	N47°28′30.3″ E26°16′6.0″	Yes	Comprehensive
Romania 9	SK	N46°21′33.1″ E25°47′38.7″	Yes	Comprehensive
2005 North America				
Colorado 1	DK + H	N39°40′17.0″ W102°33′1.3″	?	Comprehensive
Colorado 2	DK + H	N39°39′21.0″ W102°33′18.6″	?	Visual
Colorado 3	DK + H	N39°40′30.5″ W102°33′22.3″	?	Comprehensive
Colorado 4	DK + H	N40°06′44.8″ W103°11′27.6″	?	Comprehensive
Colorado 43	DK + H	N39°42′10.2″ W106°40′32.8″	?	Comprehensive
Colorado 46	DK + H	N39°37′18.0″ W106°28′17.9″	?	Visual
Colorado 47	DK + H	N39°41′48.5″ W105°11′32.7″	?	Comprehensive
Colorado 44	SK	N39°39′27.0″ W106°38′13.3″	?	Visual
Colorado 45	SK	N39°39′22.3″ W106°35′59.9″	?	Comprehensive
Idaho 29	DK + H	N43°24′52.5″ W114°52′17.3″	?	Visual
Idaho 30	SK	N43°18′16.6″ W114°48′6.4″	?	Comprehensive
Idaho 27	SK	N43°38′35.5″ W116°15′19.7″	?	Comprehensive

Appendix 1. Continued.

Location	Species	Lat/long	Species overlap	Survey type
Idaho 28	SK	N43°32′55.0″ 116°09′38.3″	?	Visual
Idaho 31	SK	N46°02′37.1″ 112°48′52.2″	?	Visual
Montana 32	SK	N45°46′23.7″ W109°47′56.8″	?	Comprehensive
Montana 33	SK	N46°04′29.3″ W109°56′8.7″	?	Comprehensive
Montana 34	SK	N45°41′21.8″ W108°46′17.2″	?	Comprehensive
Montana 35	SK	N45°44′26.9″ W108°31′56.9″	?	Comprehensive
New York 48	SK	N44°16′42″ W73°31′52.6″	?	Comprehensive
New York 49	SK	N42°17′33.9″ W76°42′49.0″	?	Comprehensive
Ontario 50	SK	N43°20′58.5″ W80°06′43.6″	?	Comprehensive
Oregon 21	DK + H	N45°54′58.8″ W119°33′31.8″	?	Comprehensive
Oregon 22	DK	N45°40′26.0″ W118°49′15.5″	?	Visual
Oregon 24	DK + H	N45°14′14.6″ W120°11′6.5″	?	Visual
Oregon 25	DK + H	N45°36′17.1″ W121°11′2.3″	?	Comprehensive
Oregon 26	DK + H	N45°35′53.3″ W121°08′19.5″	?	Visual
Oregon 23	SK	N45°18′6.8″ W120°11′7.4″	?	Visual
Washington 5	DK + H	N46°34′58.9″ W102°28′15.8″	?	Visual
Washington 6	DK + H	N46°35′6.7″ W120°27′33.0″	?	Comprehensive
Washington 7	DK + H	N46°24′0.34″ W120°16′46.3″	?	Visual
Washington 8	DK + H	N46°35′6.4″ W120°28′7.0″	?	Visual
Washington 9	DK + H	N46°32′32.5″ W120°27′39.6″	?	Comprehensive
Washington 11	DK + H	N47°33′40.4″ W120°16′11.3″	?	Comprehensive
Washington 12	DK + H	N47°22′32.6″ W120°14′59.0″	?	Visual
Washington 13	DK + H	N47°28′14.4″ W120°20′11.5″	?	Comprehensive
Washington 15	DK + H	N47°35′56.2″ W120°39′4.5″	?	Visual
Washington 16	DK + H	N47°29′6.0″ W120°18′0.2″	?	Visual

Appendix 1. Continued.

Location	Species	Lat/long	Species overlap	Survey type
Washington 20	DK + H	N46°43′16.9″ W117°09′50.5″	?	Comprehensive
Washington 10	SK + DK + H	N46°32′7.4″ W120°52′2.6″	?	Visual
Washington 14	SK	N47°36′31.3″ W120°52′46.5″	?	Visual
Washington 17	SK	N46°44′47.9″ W117°10′30.3″	?	Comprehensive
Washington 18	SK	N46°44′14.4″ W117°03′4.0″	?	Visual
Washington 19	SK	N46°44′27.3″ W117°11′42.4″	?	Visual
Wyoming 39	DK + H	N43°50′13.7″ W106°52′28.6″	?	Comprehensive
Wyoming 40	DK + H	N42°53′11.5″ W106°26′24.8″	?	Visual
Wyoming 41	DK + H	N43°23′7.9″ W107°03′45.6″	?	Comprehensive
Wyoming 36	SK	N44°30′3.7″ W109°11′27.9″	?	Visual
Wyoming 37	SK	N44°29′59.9″ W109°12′44.3″	?	Comprehensive
Wyoming 38	SK	N44°22′38.7″ W106°42′37.2″	?	Comprehensive
Wyoming 42	SK	N42°54′14.6″ W106°27′9.7″	?	Comprehensive
2006 Europe				
Ukraine 2	DK + H	N48°38′45.7″ E30°46′30.3″	Yes	Comprehensive
Ukraine 3	DK + H	N48°52′18.2″ E30°42′40.3″	Yes	Comprehensive
Ukraine 5	DK	N48°48′22.8″ E30°33′54.9″	Yes	Comprehensive
Ukraine 7	DK + H	N48°30′42.7″ E37°44′10.3″	Yes	Comprehensive
Ukraine 9	DK	N47°51′43.2″ E38°27′38.5″	Yes	Comprehensive
Ukraine 10	DK + H	N48°06′02.4″ E37°48′58.0″	Yes	Comprehensive
Ukraine 11	DK + H	N48°09′8.4″ E37°50′26.1″	Yes	Comprehensive
Ukraine 12	DK + H	N48°00′43.0″ E37°47′16.5″	Yes	Comprehensive
Ukraine 4	SK + DK + H	N48°53′31.2″ E30°40′33.2″	Yes	Comprehensive
Ukraine 6	SK + DK + H	N48°34′51.9″ E37°54′36.9″	Yes	Comprehensive
Ukraine 13	SK + DK + H	N50°29′1.2″ E30°30′25.4″	Yes	Comprehensive
Ukraine 14	SK + DK + H	N50°28′50.7″ E30°29′10.7″	Yes	Comprehensive

Appendix 1. Continued.

Location	Species	Lat/long	Species overlap	Survey type
Ukraine 1	SK	N48°38′45.7″ E30°46′30.3″	Yes	Comprehensive
Ukraine 8	SK	N47°48′24.7″ E38°33′10.0″	Yes	Comprehensive
Switzerland	SK	N47°16′58.5″ E8°08′51.9″	No	Comprehensive
Istanbul, Turkey 1	DK	N/A	No	Visual
Istanbul, Turkey 2	DK	N/A	No	Visual
2006 North America				
Colorado 1	DK + H	N39°56′31.1″ W104°51′43.1″	?	
Colorado 2	DK + H	N39°52′02.5″ W104°55′30.8″	?	Visual
Colorado 3	DK + H	N39°24′36.7″ W104°52′12.0″	?	Visual
Colorado 4	DK + H	N39°20′23.8″ W104°49′53.3″	?	Visual
Colorado 5	DK + H	N40°22′09.5″ W105°31′54.2″	?	Visual
Oregon 1	DK + H	N44°50′34.6″ W120°54′01.7″	?	Visual
Oregon 2	DK + H	N45°14′35.7″ W120°10′55.5″	?	Visual
Oregon 3	DK + H	N45°20′34.4″ W119°32′58.1″	?	Visual
Oregon 4	DK + H	N45°47′28.2″ W120°01′51.8″	?	Visual
Oregon 5	DK + H	N45°41′01.9″ W121°24′08.3″	?	Visual
Oregon 6	DK + H	N45°38′08.8″ W122°45′24.4″	?	Visual
Oregon 7	DK + H	N45°15′14.9″ W121°09′05.8″	?	Visual
Wyoming 1	DK + H	N41°04′55.1″ W105°29′06.2″	?	Visual