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Reproduction and Pollination Biology of *Centaurea* and *Acroptilon* Species, with Emphasis on *C. diffusa*

Abstract

Reproduction and pollination of *Centaurea diffusa*, *C. maculosa*, *Acroptilon repens*, and *C. solstitialis* were examined. Based on controlled pollination, *Centaurea diffusa*, *C. maculosa*, and *A. repens* were found to be obligate outcrossers; whereas *C. solstitialis* proved to be facultatively xenogamous. The importance of floral visitors was determined by visitation frequency along established transects and by pollen load analyses. Principal visitors to at least three of the four species were *Bombus* spp., *Anthophora* sp., *Apis mellifera*, and *Megachile* sp. None of the important flower visitors of *C. diffusa* discriminated between white and purple flower heads. Time of day did not significantly influence visitation, except for *Anthophora* sp. which visited primarily in the morning. Nectar analyses showed sucrose and fructose in a ratio of approximately 2:1 to be the major sugars in three of the species. In *A. repens*, glucose was also present (sucrose: fructose: glucose: 2:1:1). The open recombination systems of *Centaurea diffusa*, *C. maculosa*, and *A. repens* promotes genetic diversity allowing invasion of natural habitats. The annual habit and versatile breeding system of *C. solstitialis* contributes to its success as a noxious weed. *Apis mellifera* may increase the reproductive capacities of knapweeds.

Introduction

The knapweeds (three *Centaurea* species and *Acroptilon repens*) examined in this study are of great importance in western North America because of their status as noxious weeds. They have expanded rapidly on rangelands of the Northern Intermountain Region since their introduction from Eurasia during the early 1900's (Cranston 1980, Roché *et al.* 1986, Roché and Talbott 1986). The knapweeds are competitive and often trigger ecosystem degradation by displacing native vegetation, decreasing biodiversity, contributing to soil erosion (Maddox 1979, Cranston 1980, Tyser and Key 1988), reducing the economic value of ranges (Strang *et al.* 1979) and decreasing recreation and property values (Talbott Roché and Roché 1988).

As is true of other successful weeds, the knapweeds combine many morphological and physiological characteristics which allow them to quickly occupy and persist in disturbed habitats. Such characteristics include high seed production (Watson and Renney 1974, Schirman 1981, Roché *et al.* 1986, Callihan *et al.* 1989); successful seed dispersal (Watson and Renney 1974, Roché 1992); discontinuous seed germination and germination over a broad range of temperatures (Watson and Renney 1974, Watson 1980, Callihan *et al.* 1989, Taylor and Harrod, unpublished study); and allelopathy (Watson and Renney 1974, Muir and Majak 1983).

Other characteristics of knapweeds which may or may not relate to their success as weeds fall in the general category of reproductive and pollination biology. The knapweeds are discussed frequently in the literature, but their pollination biology has received little attention. Watson and Renney (1974) noted that the knapweeds are entomophilous and self-compatible, but they did not go into further detail about specific floral visitors nor did they provide supporting data. Proctor and Yeo (1979, and references cited therein) refer to visitation of several insects to *Centaurea* species but provide no quantitative data.

Our study was designed to answer the following questions: Are these four knapweed species self-compatible, and if so, can they be self-pollinated in the absence of insect visitors? What are the important insect visitors? What is the visitation constancy of the representative insects? Is insect visitation influenced by time of day (see McCall and Primack 1992)? What floral rewards are being collected by the various insect visitors? What are the types and ratio of sugars present in nectar rewards of the species and does there appear to be a relationship between these sugars and insect visitors along the lines suggested by Percival 1960, Baker and Baker 1975, Faegri and van der Pijl 1979?

Flower color varies in some *Centaurea* species, especially *C. diffusa* which has both white and

purple forms. Pollinator preference for a particular color morph could lead to greater reproductive success of that form (Kay 1978, Stanton 1987). Thus, in the context of the questions stated above, we wanted to know if flower visitors discriminate between white- and purple-flowered forms of *C. diffusa*.

Methods

Study Sites

The study sites were located in Okanogan National Forest, near Winthrop, Washington (diffuse knapweed *Centaurea diffusa* Lam.); in Lolo National Forest, near Missoula, Montana (spotted knapweed *C. maculosa* Lam.); on private land near La Grande, Oregon (yellow starthistle (*C. solstitialis* L.)); and on private land near Haines, Oregon (Russian knapweed (*Acroptilon repens* (L.) DC.)). For more precise locations, see Figure 1. At each study site, the species studied was the dominant plant. The populations were extensive, although the areas studied were approximately 2500 m².

Pollination Studies and Fertility Analyses

At each site, 1 x 1 x 1 m cages were used to exclude insects from selected plants. The cages consisted of wooden frames covered by 1 x 1 mm mesh window screens. At the Haines, La Grande, and Missoula sites, four cages were set up, each enclosing a single plant. Criteria used in selecting plants included: (1) plant size—large enough to have numerous heads but small enough to fit in the cage; (2) location—well within the population, avoiding “edge effect”; (3) pre-anthesis developmental stage—opened heads, if any, were removed; and (4) sufficient spatial separation from other caged plants to ensure independent samples. At the Washington (*C. diffusa*) site, eight white- and eight purple-flowered plants were randomly selected and caged prior to opening of flower heads. The field work was carried out from mid June through August, 1989, at the Oregon and Montana sites, and from mid June through September, 1990, at the Washington site.

At the Oregon (*A. repens* and *C. solstitialis*) and Montana (*C. maculosa*) sites, all controlled pollinations involved caged plants. In each of three cages, ten heads were selfed via geitonogamy (pollination of flowers on the same plant) and ten outcrossed (xenogamy), by picking flowering heads of

non-caged plants and gently rubbing these over the florets of selected heads of caged plants. To avoid pollen contamination, geitonogamy and xenogamy were carried out on different days. All pollinated heads were appropriately tagged. The fourth cage at each of the three sites, in addition to untreated heads on the other three caged plants, was used to test autogamy (pollination in the same flower), i.e. the plants or heads were left untouched.

At the Washington (*C. diffusa*) site, autogamy, geitonogamy and xenogamy were compared by randomly selecting eight white-flowered plants and eight purple-flowered plants for each reproductive treatment. To test for autogamy, exclusion cages were placed around the sixteen selected plants, as noted above, and left untouched. Exclusion cages were not used in other tests. To test for geitonogamy, small cotton bags were placed over two unopened but similarly developed flower heads of each of the sixteen selected plants. Bags were briefly removed to hand pollinate flowers when anthers were dehiscing and stigmas were presumed to be receptive, i.e. the heads were gently rubbed together. Bags were left on until after the heads had closed and the corollas had become senescent. Two non-bagged open pollinated heads were selected and tagged from each of the additional sixteen plants, providing the opportunity for xenogamy.

Heads from all treatments were collected when mature and the seeds were counted. From each of the *C. maculosa*, *A. repens*, and *C. solstitialis* populations, 120 heads were collected: 30 representing geitonogamy; 60 representing xenogamy (30 from caged plants and five randomly collected from each of six non-caged plants); and 30 representing autogamy (15 from the single, “autogamous” caged plant, and five non-manipulated heads from each of the other three caged plants, all randomly collected). Seed count data of each population were subjected to a 1 x 4 factorial analysis of variance (ANOVA), factor 1 being species and the four levels of factor 2 being autogamy, geitonogamy, open-pollination, and xenogamy (bagged heads). If the probability of a Type I error was 5% or less, the result was considered to be significant.

From the *C. diffusa* population, 96 seed heads were chosen at random, two from each of the 48 plants selected for autogamy, open pollination, and geitonogamy (16 plus 16 plus 16). The 32 open-pollinated heads were selected randomly prior to

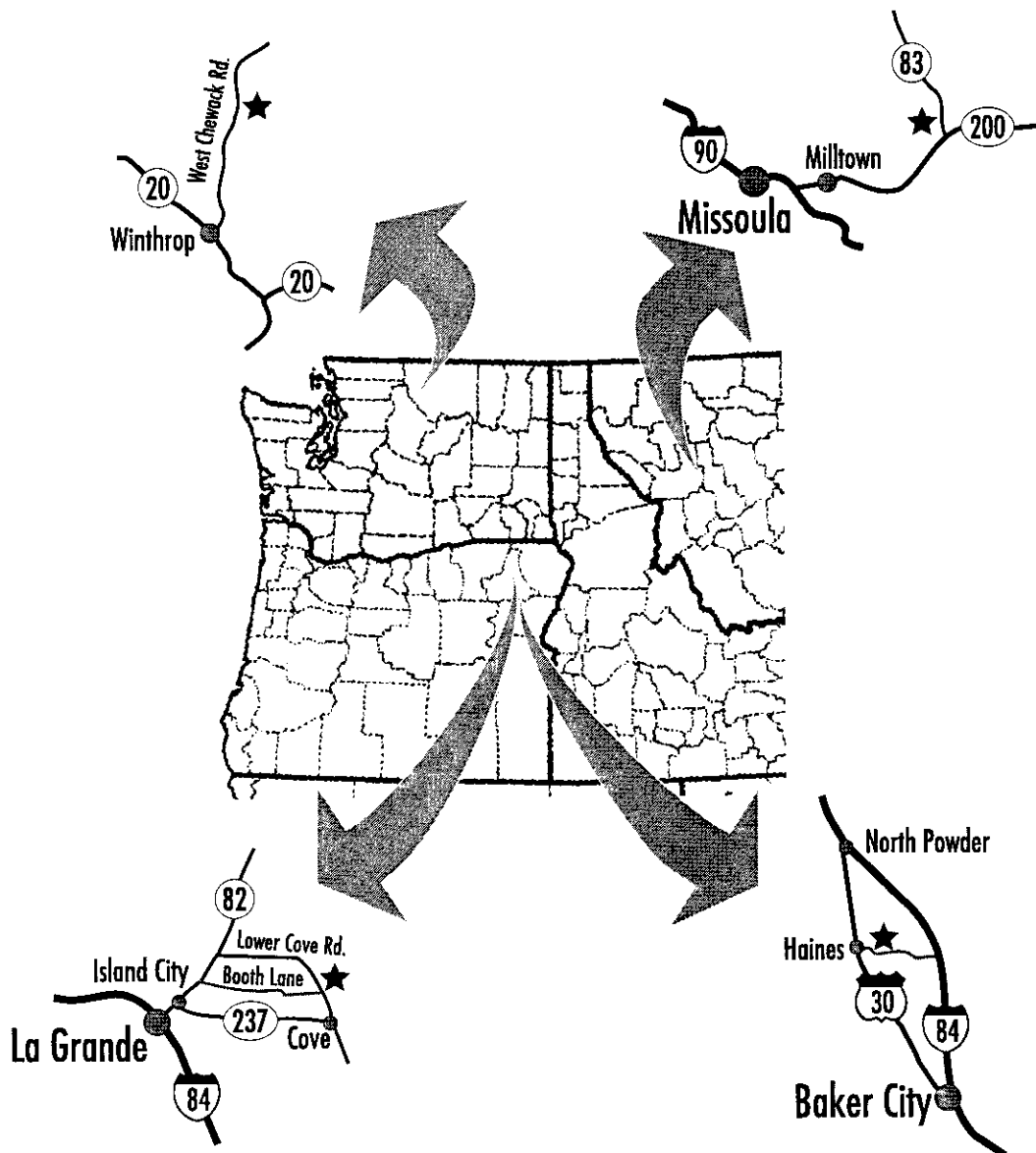


Figure 1. Location of study sites (★). The “diffusa” site is located on Forest Service land 8 miles north of Winthrop, Washington, on West Chewack Road. The “maculosa” site is located on state land northeast of Milltown, Montana, on Highway 83 approximately 2 miles north of Highway 200. The “repens” site is located on private land by following 3rd Street 5 miles east of Haines, Oregon. The “solstitialis” site is located on private land by following Booth Lane 4 miles east of Island City, Oregon, to Lower Cove Road.

pollination. Seed count data from this population were subjected to a 2 x 3 factorial ANOVA with subsampling ($\alpha = .05$). The two levels of factor 1 were flower head color, white, and purple. The three levels of factor 2 were reproductive treatments, autogamy, geitonogamy, and open pollination.

To test for the possibility of anemophily (wind pollination), a pollen sampler was operated for six hours (from 0900 to 1500) for each of three days during peak flowering (late July, early August) in the *C. diffusa* population. The pollen sampler was attached to a vacuum pump which was calibrated

at 486.5 L of air/hour. Pollen was deposited on a clear petroleum grease coated microscope slide which advanced every hour. At each hour, wind speed was recorded at canopy height using a turbine digital anemometer and was averaged for a five minute period.

Determination of Flower Visitor Frequency

Frequency of insect visits was measured along a 50m transect roughly through the center of each population. The transects were traversed once in the morning (ca. 0900) and once in the afternoon (ca. 1500) for three consecutive days in the *C. maculosa*, *A. repens*, and *C. solstitialis* populations, and for a total of ten times at various dates (late July, early August) during flowering in the *C. diffusa* population. One minute was spent at each meter along the transect, counting the number of visits to capitula (heads) by insect species. Visits were counted only if they occurred within an area approximately 200 x 20 cm, centered on and perpendicular to the transect line. A visit involved physical contact by the insect with any flower of the capitulum. For *C. diffusa*, numbers of visits per meter per minute, morning and afternoon, were analyzed using a 2 x 9 (*C. diffusa*, *C. maculosa*, *C. solstitialis*) and a 2 x 12 (*A. repens*) factorial ANOVA ($\alpha = .05$). Morning and afternoon were the two levels of factor 1 and the nine or twelve levels of factor 2 represented different insect species.

A representative sample of insects visiting the respective knapweed species was collected from each population. The insects were killed using ethyl acetate and allowed to dry. The pollen was later scraped from the head, abdomen, and legs, and placed on a microscope slide in a small amount of 30 percent glycerol in distilled water, with two crystals of thymol (following Puris *et al.* 1964). We counted up to 100 pollen grains at 100x or 400x from a few random locations on the slide; or when fewer than 100 grains were present, all were counted. Pollen grains were identified using permanent reference pollen mounts of knapweed and associated species. Differences among visitors based on pollen load purity for each species were analyzed using a complete randomized design, ANOVA ($\alpha = .05$).

In the *C. diffusa* population, we determined if insects discriminated between white- and purple-flowered plants. Ten equally spaced (ca. 1 m apart)

combinations of two white-flowered plants and one purple-flowered plant, and ten combinations of two purple-flowered plants and one white-flowered plant were selected for a total of 20 experimental sets. For each experimental set, one plant, white- or purple-flowered, served as a "subject" plant and two plants, one of each flower color type, served as "choice" plants. Color preference was recorded only when an insect would visit the subject plant and then select one of the choice plants. Insect observations were made for one hour both in the morning (ca. 0900) and afternoon (ca. 1500). Differences among insects based on color preference and time of day were analyzed using a 2 x 2 x 2 x 2 factorial ANOVA ($\alpha = .05$). The levels of factor 1 were color of the "subject" plant (white or purple), levels of factor 2 were colors of "choice" plants (white or purple), levels of factor 3 were morning and afternoon, and levels of factor 4 were insect species.

Nectar Analysis

The sugar constituents of nectar were determined by paper chromatography. Eight mature plants of each species and flower color (*C. diffusa*) were selected at random and four to six flowering head of each plant were used per nectar sample. To access the nectar, the upper part of the corolla tubes, along with the anthers and styles, were cut away with a sharp knife. The nectar was then squeezed out into a spot on Whatman #1 chromatography paper. Spotting was done both in the morning (ca. 0900) and afternoon (ca. 1500) of the same day for a total of 16 nectar samples from each population and floral color. Standards of approximately 2% glucose, fructose, and sucrose were later spotted alongside nectar samples. Ascending chromatography was used to separate sugars (Markham 1982). Nectar sugars were identified by comparing spot colors (following treatment with a reagent (Markham 1982)) and rate of flow to the standard sugars. Color intensities were used to determine the relative concentration of the nectar sugars.

Results

Wind-borne Pollen

A mean of 22 (sd, ± 15.9) pollen grains of *C. diffusa* was fixed on the slides of the pollen sampler per hour of operation. There was no apparent correlation between wind speed or time of day and pollen numbers.

TABLE 1. Mean numbers¹ of seeds per head for the four knapweed species, with *C. diffusa* separated into white and purple color forms, in response to different pollination treatment.

Species	Treatment			
	Autogamy	Geitonogamy	Outcrossed	Open pollinated
<i>C. diffusa</i>				
white	<u>1.0</u>	<u>0.2</u>	n/a	7.1
purple	<u>1.2</u>	<u>0.2</u>	n/a	10.6
<i>C. maculosa</i>	<u>1.1</u>	<u>1.0</u>	<u>22.0</u>	<u>24.5</u>
<i>C. solstitialis</i>	<u>10.4</u>	<u>18.9</u>	<u>34.6</u>	<u>56.5</u>
<i>A. repens</i>	<u>1.8</u>	<u>1.1</u>	<u>10.3</u>	<u>20.1</u>

¹For each species, underlined values are not significantly different from one another.

Seed Production

Based on seed production in response to the various pollination treatments, *C. diffusa*, *C. maculosa*, and *A. repens* are obligately xenogamous (Table 1). The few seeds that were produced autogamously and geitonogamously could have resulted from anemophily since the presence of wind-blown pollen was confirmed. The fewer seeds produced in bagged vs. caged capitula of *C. diffusa* also suggests this, although the differences were not significant. In *C. maculosa* and *A. repens*, open pollinated heads produced significantly more seeds than caged, crossed heads, probably because hand-pollinated plants were pollinated once, whereas open-pollinated plants could have received numerous visits.

Centaurea solstitialis produced significantly more seeds by xenogamy than by other treatments, but it must be concluded that the species is facultatively autogamous, and the greater number of seeds set by geitonogamy than by autogamy can probably be attributed to more pollen deposited on the stigmas and thus greater opportunity for self-compatibility (Table 1).

Open pollinated purple-flowered plants of *C. diffusa* set significantly more seed than white-flowered plants, even though white-flowered plants outnumbered purple-flowered plants by a ratio of 3.2:1 and major insect visitors did not discriminate according to color (see below).

Floral Visitation

The various insects collected from the flowers of the four knapweed species are listed in Table 2

with the purity of pollen loads provided. The purity of pollen carried by honey bees (*Apis mellifera*), a species of digger bee (*Anthophora* sp. 1), bumble bees (*Bombus bifarius nearcticus* and *B. occidentalis*), and a species of leafcutting bee (*Megachile* sp.) was significantly greater than other species. However, there was a considerable amount of overlap in the multiple range tests and several species of *Bombus*, *Anthophora* sp. 2, various bees of the families Megachilidae and Halictidae, and some of the syrphid flies also carried relatively pure loads of knapweed pollen. Other insects were infrequent visitors or carried very little knapweed pollen, as was the case of lepidopterids. Most of the important floral visitors exhibited a high degree of constancy as noted by pure pollen loads, although there were exceptions, such as *Megachile* sp. (Table 2).

Based on transect samples (Table 3), *Apis mellifera* dominated the visitor faunas of all knapweed species except *A. repens* which was most frequently visited by *Megachile*. The second, significantly most frequent visitors were *Bombus bifarius* (at the *C. maculosa* site), *B. huntii* (at the *A. repens* site), *B. occidentalis* (at the *C. solstitialis* site), and *Anthophora* sp. 1 at the *C. diffusa* site. Among the important visitors, only *Anthophora* sp. 1 and *Bombus huntii* differed significantly in the time of their visits, both favoring morning over afternoon.

Only *Apis mellifera* and *Anthophora* sp. 1 visited *C. diffusa* in sufficient numbers to assess their color discrimination. Based on number of visits per hour, neither showed a significant preference for flower color, nor did time of day influence color choice. Based on observations made in the

TABLE 1. Insects collected from heads of knapweed species and purity of pollen loads. Data represent mean numbers based on counts up to 100 grains from each of ten individuals, or fewer in cases when specimens were limited.

Insect taxa ORDER—family—sp.	Knapweed pollen ¹ /foreign pollen/# insects ¹			
	diffusa	macul.	repens	solst.
HYMENOPTERA—Apidae				
<i>Apis mellifera</i>	100 ^a /1/10	100 ^a /2/10	-----	100 ^a /1/10
<i>Bombus bifarius</i>	86 ^{ab} /2/08	81/1/06	100 ^a /3/05	93 ^a /1/06
<i>Bombus occidentalis</i>	89 ^{ab} /1/10	58/2/05	-----	62/1/05
<i>Bombus huntii</i>	-----	-----	87 ^b /5/08	-----
<i>Bombus centralis</i>	-----	-----	-----	88/2/04
<i>Bombus fervidus</i>	91/0/03	-----	-----	-----
<i>Bombus flavifrons</i>	48/52/01	-----	-----	-----
<i>Bombus vosnesenskii</i>	-----	-----	-----	100/0/01
—Anthophoridae				
<i>Anthophora</i> sp. 1	97 ^a /3/10	100 ^a /9/10 ²	100 ^a /3/05	88 ^a /24/05 ⁴
<i>Anthophora</i> sp. 2	66 ^{b,c} /1/10	-----	100 ^a /2/02	-----
—Megachilidae				
<i>Megachile</i> sp.	88 ^{ab} /12/05	100 ^a /8/05	100 ^a /0/10	100 ^a /9/08 ⁴
<i>Osmia</i> sp.	88/12/02	-----	-----	-----
unknown sp.	50 ^c /2/05	-----	-----	-----
—Halictidae				
unknown sp. 1	-----	-----	82/5/06	51/15/04 ⁴
unknown sp. 2	-----	-----	-----	02/0/01
<i>Agopastemon</i> sp.	-----	-----	65/1/02	-----
—Colletidae				
unknown sp.	-----	-----	76/45/02 ⁵	-----
—Sphecidae				
<i>Bombix</i> sp.	78/22/01	-----	-----	-----
unknown sp.	-----	01/3/01	18/1/01	-----
<i>Sceliphron</i> sp.	-----	-----	25/5/01	-----
—Vespidae				
<i>Vespa</i> sp.	-----	03/0/01	-----	-----
DIPTERA—Syrphidae				
<i>Eristalis arbustorum</i>	-----	-----	47/15/04	63/1/03
<i>Eristalis tenax</i>	00/0/01	-----	-----	28/7/03
<i>Eristalis hirtus</i>	-----	-----	100/12/02	-----
<i>Scaeva pyrastris</i>	-----	100/7/01	-----	20/3/02
<i>Helophilus fociatus</i>	-----	-----	75/33/04 ⁵	-----
—Bombyliidae				
<i>Systoechus oreas</i>	00/0/01	00/0/03	06 ^c /0/05	25/0/02
<i>Villa</i> sp.	14/0/01	08/2/02	-----	-----
—Tachinidae				
unknown sp.	-----	13/42/02 ⁵	63/38/04 ⁶	-----
LEPIDOPTERA—Hesperidae				
<i>Ochlodes sylvanoides</i>	13/2/02	04/0/02	-----	-----
—Lycanidae				
<i>Lycæna</i> sp.	-----	-----	04 ^c /3/06	-----
<i>Plebejus melissa</i>	14 ^a /2/09	-----	-----	-----
—Pieridae				
<i>Colias philodice</i>	25 ^d /1/10	-----	-----	-----
<i>Pontia protodice</i>	-----	-----	03/1/05	-----
—Satyridae				
unknown sp.	-----	04/7/01	-----	-----

¹a,b,c,d—For each knapweed species, means are not significantly different from each other. In *C. diffusa*, overlap in the Newman-Keuls multiple range test is indicated by double lettering. Fewer than five individual insects were not included in the statistical analyses.

²One specimen had a large load of *Hieracium* sp. pollen.

³Two specimens had large loads of *Cirsium vulgare* pollen.

⁴Some specimens had large loads of *Convolvulus arvensis* pollen.

⁵Some specimens had large loads of *Chrysothamnus viscidiflorus* pollen.

⁶Both specimens had large loads of *Lactuca serriola* pollen.

TABLE 3. Mean¹ morning (0900) and afternoon (1500) visits made to transect observation areas of the four *Centaurea* populations by various insects. The number of visits represents importance values of the potential pollinators.

Insect taxa	Transect visitations per population			
	diffusa	macul.	repens	solst.
<i>Apis mellifera</i>	38.4	42.4	0.0	25.3
<i>Bombus bifarius</i>	4.9	19.7	6.2	11.8
<i>Megachile</i> sp.	1.3	2.1	27.5	10.2
<i>Anthophora</i> sp. 1	8.4	11.3	5.8	4.7
<i>Bombus occidentalis</i>	0.3	7.4	0.0	19.8
<i>Bombus huntii</i>	0.0	0.0	13.1	0.0
Syrphidae ²	0.4	0.7	5.7	3.9
<i>Lycæna</i> sp.	0.0	0.0	8.3	0.0
Halictid sp.	0.0	0.0	4.9	3.0
Tachinidae	0.0	0.1	6.6	0.0
<i>Bombus centralis</i>	0.0	0.0	0.0	5.9
<i>Pontia protodice</i>	0.0	0.0	5.0	0.0
<i>Sytoechus oreas</i>	0.0	0.4	0.8	0.3
<i>Anthophora</i> sp. 2	0.3	0.0	0.4	0.0
<i>Villa</i> sp.	0.5	0.0	0.2	0.0
<i>Bombus fervidus</i>	0.3	0.0	0.0	0.0
<i>Ochloides sylvanoides</i>	0.0	0.3	0.0	0.0

¹Mean values represent multiple a.m. and p.m. transects in respective populations. In only three cases did a.m. and p.m. values differ significantly: (1) *Anthophora* sp. 1 visited more frequently in the a.m. at the *diffusa* and *maculosa* sites; (2) *Bombus huntii* was observed more frequently in the a.m. at the *repens* site; (3) the tachinid fly was observed more frequently in the p.m. at the *repens* site.

²*Eristalis* spp., *Scaeva pyrastris* and *Helophilus fociatus* are included in this family group.

field, the same can be said for other insects visiting *C. diffusa*. The general trend for pollinators was to visit several heads of the same plant and then fly to the next closest plant, regardless of its flower color. The only exceptions were the Lepidopterids which visited sporadically.

Nectar Analysis

Chromatographic analyses showed the nectar to consist of sucrose and fructose in a 2:1 ratio in *C. diffusa*, *C. maculosa*, and *C. solstitialis*. In *A. repens*, glucose was detected in small quantities (sucrose:fructose:glucose; 2:1:1). The similarity of sugar type and concentration between morning and afternoon samples (and between flower color in *C. diffusa*) helps to explain the general lack of visitation discrimination by major visitors.

Discussion

Autogamy may function to promote population establishment and most weeds are obligately or facultatively autogamous (see Allard 1965, Baker 1965, Stebbins 1965, Mulligan and Findlay 1970). How-

ever, inbreeding restricts recombination and, accordingly, limits adaptability. For most weeds this seems not to be a serious compromise because they have broad ecological tolerances (Baker 1965, Taylor 1990) and can exploit a variety of open habitats. The knapweeds examined in this study are somewhat exceptional in that they are obligate or preferential (*C. solstitialis*) outcrossers. This open recombination system may limit reproductive success of isolates but promotes genetic diversity allowing invasion of natural habitats.

Although self-compatibility had been reported in *Centaurea* species (Watson and Renney 1974), many species are nearly completely self-incompatible (Marsden-Jones and Turrill 1954, Lack 1976, 1982). Roché (personal communication) noted that *C. diffusa*, *C. maculosa*, and *A. repens* appear to be self-sterile and *C. solstitialis* nearly so. The potential for selfing in *C. solstitialis* suggested by our study, may be correlated with its annual habit. According to Grant (1981), autogamy is a particularly common and adaptive trait of annuals. In any case, the versatile breeding system

of *C. solstitialis* must contribute to its success as a noxious weed in the Pacific Northwest.

Most weeds have a generalist pollination strategy, that is, they can be successfully pollinated by a variety of insect types, an adaptive trait of colonizing species (Allard 1965, Baker 1965). In knapweed species, the pollen is openly presented and readily accessible to all pollen-foraging Coleoptera, Diptera, and Hymenoptera. On the other hand, nectar is concealed in short, narrow corolla tubes which also attracts "specialist" pollinators known for their high degree of floral constancy. In their comprehensive treatment of *Centaurea*, Marsden-Jones and Turrill (1954) list numerous floral visitors from several Orders, including "generalists" and "specialists," many of the same genera collected by us. Finally, the fact that knapweed species flower later in the summer than most associated species probably reduces competition for pollinators and increases constancy and reproductive success.

The importance of the respective insect visitors concentrating on the *Centaurea* and *Acroptilon* populations of our study can be judged by a combination of: (1) visitation frequency—as measured in transect observation areas; and (2) constancy—as determined by purity of knapweed pollen. It should be noted that our data do not provide direct evidence for the relative success of floral visitors as pollinators. It is known that frequent visitors are sometimes poor pollinators (Faegri and van der Pijl 1979). However, the open flower structure of the knapweeds likely promotes effective pollination by any visitor.

In three of the four populations (*A. repens* excluded), *Apis mellifera* was the most frequent visitor by all criteria. This species was present throughout the flowering season (at least at the *C. diffusa* site) and exhibited slow, deliberate manipulation and probing of florets. This behavior likely makes the species an important pollinator. It foraged throughout the day, primarily for nectar but some individuals collected pollen. *Apis mellifera* is an important pollinator of other *Centaurea* species including *C. jacea* and *C. nemoralis* (Marsden-Jones and Turrill 1954), *C. nigra* (Lack 1976), and *C. solstitialis* (Gary *et al.* 1980).

Considering all *Centaurea* and *Acroptilon* populations, bumblebees (*Bombus* spp.) were the second most important frequent visitors. These bees foraged on nectar, apparently exclusively, and exhibited a high degree of constancy suggesting that they are also important pollinators. The nectar of the *Centaurea* and *Acroptilon* species was found to be sucrose dominant, typical of flowers visited by bumblebees (Baker and Baker 1975, Faegri and van der Pijl 1979).

Other frequent visitors, listed in apparent order of importance, were solitary bees (*Anthophora*, *Megachile*, and colletids), halictids and syrphid flies, all pollen foragers. Butterflies were frequent visitors, feeding on nectar, but they carried little pollen and were probably of minor importance as pollinators.

Finally, there was the question of color preference among pollinators in *C. diffusa*. Observation and statistical analysis indicated that at least the major visitors did not discriminate by color. In the absence of favoritism for the white flower color and the significantly greater seed production of the purple form, it would seem that the population structure would shift toward the purple flower color. Perhaps it does but the greater numbers of white capitula in this population (3.2 white/1 purple) and in others observed but not analyzed suggests not. This apparent anomaly could relate to allele frequency of colonizers; greater fitness of white flowered plants (white color alleles linked with genes promoting fitness); greater predation of white flowers by fly larvae (a statistical analysis suggests not); or inadvertent sampling error. Further work is needed to distinguish among these possibilities.

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Literature Cited

- Allard, R. W. 1965. Genetic systems associated with colonizing ability in predominantly self-pollinated species. In H. G. Baker and G. L. Stebbins (eds.) *The genetics of colonizing species*. Academic Press, New York. Pp. 50-76.
- Baker, H. G. 1965. Characteristics and modes of origin of weeds. In H. G. Baker and G. L. Stebbins (eds.) *The genetics of colonizing species*. Academic Press, New York. Pp. 147-172.
- Baker, H. G. and I. Baker. 1975. Studies of nectar-constitution and pollinator-plant coevolution. In L. E. Gilbert and P. H. Raven (eds.) *Coevolution of animals and plants*. University of Texas Press, Austin. Pp. 100-140.
- Callihan, R. H., F. E. Northam, J. B. Johnson, E. L. Michalson, and T. S. Praher. 1989. Yellow starthistle—biology and management in pasture and rangeland. University of Idaho, Cooperative Extension Service, CIS No. 634.
- Cranston, R. 1980. Knapweed, its cause and effect in British Columbia. Knapweed Action Committee, Ministry of Agriculture, British Columbia.
- Faegri, K. and L. van der Pijl. 1979. *Pollination ecology* (3rd ed). Pergamon Press, Oxford.
- Gary, N. E., P. C. Withcreek, and K. Lorenzen. 1980. Distribution of foraging honey bees to multiple, small floral plots of various species. *Envir. Entom.* 9: 43-46.
- Grant, V. 1981. *Plant speciation*. Columbia University Press, New York.
- Kay, Q. O. N. 1978. The role of preferential and assortative pollination in the maintenance of flower colour polymorphisms. In A. J. Richards (ed.) *Pollination of flowers by insects*. Academic Press, New York. Pp. 175-190.
- Lack, A. J. 1976. Competition for pollinators and evolution in *Centaurea*. *New Phytol.* 77: 787-792.
- . 1982. Competition for pollinators in the ecology of *Centaurea scabiosa* L. and *Centaurea nigra* L. III. Insect visits and the number of successful pollinations. *New Phytol.* 91: 321-339.
- Maddox, D. M. 1979. The knapweeds—their economics and biological control in the western states, U.S.A. *Rangelands* 1(4): 139-141.
- Markham, K. R. 1982. *Techniques of flavonoid identification*. Academic Press, New York.
- Marsden-Jones, F. M. and W. B. Turrill. 1954. *British knapweeds; a study in synthetic taxonomy*. Ray Society, London.
- McCall, C. and R. B. Primack. 1992. Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *Amer. J. Bot.* 79(4): 434-442.
- Muir, A. D. and W. Majak. 1983. Allelopathic potential of diffuse knapweed (*Centaurea diffusa*) extracts. *Can. J. Plant Sci.* 63: 989-996.
- Mulligan, G. A. and J. N. Findlay. 1970. Reproductive systems and colonization in Canadian weeds. *Can. J. Bot.* 48: 859-860.
- Perceival, M. 1950. Pollen presentation and pollen collection. *New Phytol.* 49(1): 40-63.
- Powell, R. D. 1990. The role of spatial pattern in the population biology of *Centaurea diffusa*. *J. Ecology* 78: 374-378.
- Proctor, M. and P. Yeo. 1979. *The pollination of flowers*. Collins Publ., London.
- Puris, M. J., D. C. Collier and D. Walls. 1964. *Laboratory techniques in botany*. Butterworths, London.
- Roché, B. F., Jr. 1992. Achene dispersal in yellow starthistle (*Centaurea solstitialis* L.). *Northw. Sci.* 66(2): 62-65.
- Roché, B. F., Jr. and C. J. Talbott. 1986. The collection history of *Centaureas* found in Washington state. *Agric. Research Center, Res. Bull. XB0978*. Wash. State Univ., Pullman.
- Roché, B. F., Jr., G. L. Piper, and C. J. Talbott. 1986. *Knapweeds of Washington*. *Coop. Ext. Bull. 1393*. Washington State University, Pullman.
- Schirman, R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. *J. Range Manag.* 34: 45-47.
- Stanton, M. L. 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: I. Pollinator response to color morphs. *Amer. J. Bot.* 74(2): 178-1987.
- Stebbins, G. L. 1965. Colonizing species of the native California flora. In H. G. Baker and G. L. Stebbins (eds.) *The genetics of colonizing species*. Academic Press, New York. Pp. 173-192.
- . 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: Pollination mechanism. *Ann. Rev. Ecol. Syst.* 1: 307-326.
- Strang, R. M., K. M. Lindsay, and R. S. Price. 1979. Knapweeds: British Columbia's undesirable aliens. *Rangelands* 1: 141-143.
- Talbott, C. J. 1987. Distribution and ecologic amplitude of selected *Centaurea* species in eastern Washington. Wash. State Univ. Pullman. M.S. Thesis.
- Talbott Roché, C. J. and B. F. Roché, Jr. 1988. Distribution and amount of four knapweed (*Centaurea* L.) species in Eastern Washington. *Northw. Sci.* 62(5): 242-253.
- Taylor, R. J. 1990. *Northwest weeds*. Mountain Press Publ. Co., Missoula, Mt.
- Tyser, R. W. and C. H. Key. 1988. Spotted knapweed in natural area fescue grasslands: an ecological assessment. *Northw. Sci.* 62(4): 151-160.
- Watson, A. K. 1980. The biology of Canadian weeds. 43. *Acropilton* (*Centaurea*) *repens*. *Can. J. Plant Sci.* 60: 993-1004.
- Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds. 6. *Centaurea diffusa* and *C. maculosa*. *Can. J. Plant. Sci.* 54: 687-701.

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