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ELEVATIONAL VARIATION OF QUINOLIZIDINE ALKALOID CONTENTS IN A LUPINE (*Lupinus argenteus*) OF THE ROCKY MOUNTAINS

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Abstract—Quinolizidine alkaloid contents of leaves and seeds of *Lupinus argenteus* (Fabaceae) collected from seven different localities near Gothic, Colorado were determined by capillary GLC. Differences in alkaloid levels between sites are substantial and alkaloid quantity decreases as elevation increases. Leaves at the lowest elevation, for example, contain six times the alkaloid levels of leaves at the highest elevation. Seeds from plants of low- and high-elevation sites were grown under identical conditions in the greenhouse. Alkaloid levels of leaves of seedlings were significantly higher in those seedlings derived from populations of low elevations than those of high elevations, indicating that the observed differences in the field are at least partly genetic and not environmental. To determine whether predation rates were responsible for these genetic differences, data on seed predation rates and observations on herbivory were collected.

Key Words—Seed predation, lupine alkaloids, flower production, elevational gradient, quinolizidine alkaloids, *Lupinus argenteus*.

INTRODUCTION

Much has been made of the observation that the quantity and quality of stored secondary chemicals vary tremendously between plant species (Ehrlich and Raven, 1964; Feeny, 1976; Rhoades and Cates, 1976; Coley et al., 1985). However, allelochemical variation within species and even between organs of

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the same plant seems also to be the rule (Bowers and Puttick, 1988; Dirzo and Harper, 1982a; Dolinger et al., 1973; Langenheim et al., 1986; Macedo and Langenheim, 1989; Gould, 1988; Wink, 1987a,b, 1990, 1992, 1993a,b), and much of our insight into the mechanistic causes and consequences of such variation comes from intraspecific studies (Berenbaum, 1981; Berenbaum et al., 1989; Cates and Redak, 1988; Dirzo and Harper, 1982b; Vrieling et al., 1991).

Species within the genus *Lupinus* are infamous for variation both in morphology and allelochemical quantity and quality (Dolinger et al., 1973; Meissner and Wink, 1992; Wink, 1984, 1993a). The allelochemicals characteristic of *Lupinus* are the quinolizidine alkaloids (QAs), a number of which are known to be toxic to a variety of herbivores (Bentley et al., 1984; Cantot and Papineau, 1983; Keeler, 1969, 1976; and, for review, see Wink, 1987a,b, 1988, 1992, 1993a,b; Wink and Witte, 1991; Szentesi and Wink, 1991).

Our goals with this research were twofold. First, we wanted to evaluate the relationship between elevation and quinolizidine alkaloid levels in *L. argenteus* growing in the Rocky Mountains. Second, if a relationship was found, we wanted to explore whether these differences were environmentally or genetically based.

Natural History of Lupinus argenteus. L. argenteus is an herbaceous perennial that occurs at high elevations throughout the western Rocky Mountains and the Great Basin and is considered to be toxic to livestock (Dunn, 1956; Keller and Zelenski, 1978). On the western slope of the Colorado Rockies, near the Rocky Mountain Biological Laboratory, *Lupinus argenteus* (Fabaceae) can be found from 2775 to 3665 m. The growing season (no snow) at the lower elevations is approximately double that at the higher elevations: approximately April–October and July–September, respectively; and at the higher elevations, snow and freezing temperatures may occur at any time during the summer.

METHODS AND MATERIALS

Plants. Seven sites were established in the summer of 1990. All sites were on west-facing slopes and were chosen for accessibility and elevational range, which was between 2775 and 3665 m. The Belleview site (3599 m) was on the western slope of Mt. Belleview between the peak and the 401 trail. Site 401 (3355 m) was along the 401 trail on a west-facing section of the southern slope of Mt. Belleview. Paradise (3416 m) was on the southern side of Paradise Pass. Site 403B (3294 m) was 0.5 km NE of the 403 trailhead just above the Washington Gulch road, and 403T (3477 m) was just north of the highest point on the 403 trail. The Gothic site (2867 m) was near the workshop in Gothic. Between these upper six sites, *L. argenteus* was common, and one could find patches of this lupine at least every 100 m. Two pairs of sites (Belleview and

401, 403B, and 403T) were at different elevations on the same hillside and part of huge, nearly contiguous patches of *L. argenteus*. The last site, Upper Loop (2776 m) was quite isolated, however. It was 1.5 km up the Upper Loop trail from Grant Lake and the only location where *L. argenteus* was found within several kilometers in any direction.

Patch Size. At each site, the number of *L. argenteus* in the immediate patch were counted or estimated. Ten meters was arbitrarily chosen as the distance necessary to separate two patches (i.e., clusters of lupine plants more than 10 m apart were considered separate patches).

Flower Production. In each patch, 20 plants were selected along a linear transect, and for each plant, the number of flowers produced for the year were counted. Where flowers had fallen off, peduncle scars were counted. Occasionally, inflorescence buds were too young to count individual flowers, and in these instances, flower numbers were a crude estimate.

Seed Predators. At the time that alkaloids were sampled in the summer of 1990, seed pods were also collected from the same plants as well as the ten most mature pods of 20 other plants in the immediate vicinity. These pods were all opened and examined for damage or seed predators (there are several species of dipteran and lepidopteran larvae that feed on lupine seeds; details in Carey, 1992). Any pod with damage or containing seed predators was counted as damaged. Healthy seed pods were classified as undamaged. Using the same methods and at the same localities, pods were again sampled for seed predators on August 26, 1991.

Herbivores. Other studies on lupines near Gothic (Carey, 1992) have found the most common predator of lupine herbaceous tissue to be gophers. All sites were examined for gopher activity and other evidence of herbivory both in 1990 and 1991.

Alkaloid Sampling. Plants at all seven localities were sampled within an hour of noon on August 10 and 12, 1990, in order to avoid variation due to diurnal rhythms (Wink and Witte, 1984). Both days were clear until noon. From each locality, three plants were systematically chosen at least 10 m apart, and each consisted of at least 10 stalks bearing both flowers (on secondary inflorescences) and nearly matured seeds (on primary inflorescences). Undamaged leaves attached to the fourth leaf node from the top of such stalks were snipped off, weighed in the field using a spring scale accurate to 0.1 g (all samples were about 5 g), and placed immediately in methanol. In addition, seed pods bearing full sized, but still green and moist, seeds were collected. Several hours later, pods were examined in the laboratory for seed predators (see below), and undamaged seeds were weighed and placed in methanol.

Alkaloid Analysis. All samples were analyzed with capillary GLC in Heidelberg, Germany. GLC-MS was carried out by L. Witte (Braunschweig). For extraction, samples were thoroughly ground, allowed to stand in MeOH, and

filtered with fresh MeOH. MeOH was removed with a rotavapor and the residue transferred into 15 ml 0.5 M HCl. Homogenate was then made alkaline with 6 M NaOH and subjected to solid-phase extraction using Chem elute columns (Analytichem, Frankfurt) and methylene chloride as a solvent. Solvent was evaporated with a rotavapor. For analysis, alkaloid extracts were separated on fused-silica columns (30 m × 0.32 mm, 1 μm film) with bonded methylsilicone phases (DB-1; J&W Scientific). A Varian gas chromatograph (3300), equipped with a nitrogen-specific detector and a Spectra Physics integrator, was employed. GLC conditions: carrier gas: helium 1.2 bar, split; injection 1:20; injector temperature: 250°C; detector: 300°C; oven: 150°C, 1 min isothermal, 150–300°C with 10°C/min, then 20 min isothermal. Sparteine was used as an external standard. GLC-MS consisted of a Carlo-Erba 5160 GC (equipped with a DB-1 30-m × 0.32-mm column), which was coupled to the quadrupole mass spectrometer Finnigan MAT 4515. Retention indices were calculated using cochromatographed standard hydrocarbons (Wink 1993a). Spectra were recorded at 70 eV and evaluated with the Incos Data System. Details on alkaloid identification have been reported in Meissner and Wink (1992) and Wink and Carey (1994).

Seedling Alkaloids. During the fall of 1991, dried seeds were collected from three sites: Upper Loop, 403T, and Belleview. Seeds of equivalent weight were planted in potting soil in planters and grown intermixed in a greenhouse in Tucson, Arizona. On January 12, when surviving plants were 4 months old and approximately 21 cm high, leaves were clipped, weighed immediately on a spring scale (as above), and placed in methanol. There was no obvious difference in size or appearance between seedlings from different locations. Samples were mailed to Germany and analyzed by capillary GLC.

RESULTS

A Spearman rank correlation between elevation and mean alkaloid contents at each site shows a significant decrease in alkaloid level with increasing elevation for both leaves ($R_s = 0.857$, two-tailed $P < 0.05$) and seeds ($R_s = 0.929$, two-tailed $P < 0.01$) (Figure 1). Although the elevational effect is strong, individual variation in alkaloid contents is large at most sites (note the large standard errors, Figure 1). To give one extreme example, although leaf alkaloids at the highest site, Belleview, averaged one fourth of those at a much lower site, Gothic; one Belleview individual had a greater leaf alkaloid concentration than one Gothic individual. Very little of this variation comes from the alkaloid analysis itself. Samples analyzed repeatedly always varied by less than 5%.

Pod predation was not consistent between years and bore no relationship

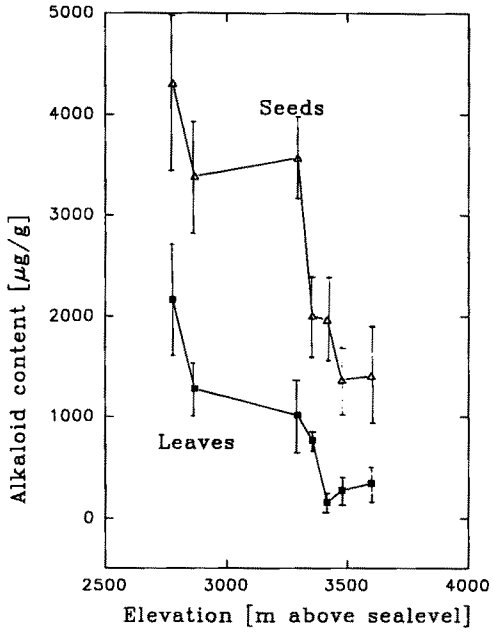

FIG. 1. Correlation between elevation and alkaloid contents of leaves and seeds. Three plants were sampled at each site. Given is the average (±SE) micrograms per gram wet weight.

to elevation, patch size, or seed alkaloid levels (Table 1). The only seemingly consistent trend was low levels of pod predation at Belleview and Upper Loop, the highest and lowest sites, respectively. Gopher activity was common at all but the Upper Loop site, but there was no other evidence of grazing at any site.

There was a consistent relationship between elevation and the number of flowers produced per plant. Plants at lower elevations averaged more flowers (Table 1). A Spearman rank correlation shows the relationship between elevation and mean flower number to be statistically significant in 1990 ($R_s = 0.89$, two-tailed $P < 0.05$) and almost so in 1991 ($R_s = 0.73$, two-tailed $P < 0.10$).

Due to experimental problems, the number of seedlings produced from seeds of wild lupines (grown in a greenhouse under identical conditions) was comparably small. The corresponding data are therefore preliminary. Plants derived from seeds originating from high elevations (403T and Belleview) produced a significantly lower concentration of leaf alkaloids than did those from lower elevations (Upper Loop): 105.4 ± 38 ($N = 7$) and 512.1 ± 86 ($N = 6$) μg alkaloid per gram wet weight, respectively (t test; $P < 0.001$). Although

TABLE 1. CHARACTERISTICS OF SEVEN *L. argenteus* PATCHES[a]

Site	Elevation (m)	Patch size (No. of plants)	Flowers/Plant		Pods Predated (%)	
			1990	1991	1990	1991
Belleview	3,599	19,000	212	134	0	4
403T	3,477	28,000	330	324	1	28
Paradise	3,416	1,410	347	330	0.1	14
401	3,355	77,000	266	318	15	26
403B	3,294	1,990	410	312	9	32
Gothic	2,867	42	520	400	18.5	8
Upper Loop	2,776	124	683	704	2.5	1

[a] Elevation was taken from a topographical map. Patch size was determined by counting the number of plants in the patch in 1990. Both the mean number of flowers produced per plant and the mean percentage of seed pods with predators were determined for both 1990 and 1991. For determination of the latter, more than 200 pods were sampled for each year at each patch.

the absolute alkaloid contents are lower in these greenhouse plants than in corresponding lupines grown in the wild (Figure 1), the general trend is similar.

DISCUSSION

The relationship between elevation and alkaloid level is statistically significant: lupines from higher elevations accumulate lower alkaloid contents than those growing at lower elevations (Figure 1). This phenomenon persists even when seedlings from the highest and lowest elevations are grown under identical conditions in a greenhouse. This latter result indicates a genetic basis for the elevational pattern, which could explain at least some of the effects observed.

The lower alkaloid contents of greenhouse plants could be due to several reasons: Since alkaloid accumulation can be induced by herbivory (Wink, 1983; Johnson et al., 1989), it could be argued that lupines in the wild are already induced by herbivore damage, whereas greenhouse plants were unmolested and therefore not induced. Quinolizidine alkaloid biosynthesis is light-dependent (Wink, 1987a,b, 1990). Since light is a problematic factor in most greenhouse experiments, lower alkaloid accumulation may be due to insufficient light.

What could be the selection pressures leading to the genetically based elevational gradient of alkaloid accumulation? It has been shown for other lupine species that quinolizidine alkaloids provide the plants with chemical defense against herbivores (both mammals and insects) and, to some degree, against microorganisms (Wink, 1983, 1984, 1987a,b, 1988, 1992, 1993b). A preliminary field experiment at the study site (near Upper Loop) in the Rocky mountains

indicates that herbivores exert selection pressure on lupines that is correlated with alkaloid content. Alkaloid-rich seeds of *Lupinus albus* (bitter lupines) and nearly alkaloid-free seeds (sweet lupins) were grown in the field unprotected by any fence. Whereas seeds and seedlings of sweet lupines disappeared within several weeks, about 50% of the alkaloid-rich counterparts survived. Thus, alkaloid accumulation and predation are highly correlated. We expect that predation should lead to a selection of plants genetically tuned for high alkaloid accumulation.

Despite a sixfold difference in alkaloid contents between sites 1 and 7 (Figure 1), actual herbivory was almost equal (Table 1). This seems to be strong evidence that lower elevation plants have been naturally selected for higher QA levels because predation pressures at low elevations are greater, i.e., much higher alkaloid concentrations are required to maintain the same level of damage.

Alternatively, we suggest that the longer growing season at lower elevations could make rapid growth less critical—plants can afford to allocate a greater amount of resource to protection. Moreover, genetically based physiological responses to light quality or temperatures (both factors vary with altitude) on alkaloid production cannot be ruled out.

Our results clearly implicate a strong genetic component for altitude-dependent alkaloid production. Because of limited sample size, our data do not explain unequivocally which forces have been responsible for the natural selection that must have taken place, but an impact of herbivory seems most likely.

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