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Changes in *Lupinus albus* and *Lupinus angustifolius* Alkaloid Profiles in Response to Mechanical Damage

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The aim of this work was to evaluate chemical responses to biomass removal mimicking large herbivore action in *Lupinus albus* and *Lupinus angustifolius* aerial parts. GC and GC-MS were used to determine total alkaloid content (TAC) and alkaloid relative abundances in bitter and sweet varieties of each species. Bitter genotypes Vila vehla (Vv; 3.95 ± 0.26 mg/g of DM) and El Harrach (EH; 3.99 ± 0.19 mg/g of DM) showed after damage 22 and 32.8% TAC increases, respectively. Even sweet varieties, with very low alkaloid contents, Gungurru (Gu; 0.51 ± 0.09 mg/g of DM) and Rumbo (Ru; 0.53 ± 0.09 mg/g of DM) exhibited higher induced responses of 58.8 and 67.9%, respectively, and their final TAC values remained low, distinctly apart from those corresponding to bitter species. Moreover, minor components such as ammodendrine, reported to exhibit teratogenic potential, showed no significant changes in their relative abundances in response to biomass removal in these genotypes.

KEYWORDS: ***Lupinus angustifolius*; *Lupinus albus*; quinolizidine alkaloids; mechanical damage**

INTRODUCTION

Chemical defenses are mostly associated with plant protection against biotic stress, providing advantages that lead to successful survival of plant species against invasive organisms and other environmental stresses. Herbivory, disease, drought, extreme temperatures, low soil quality, and/or the presence of herbicides and pathogens are now common features in intensively cultivated agroecosystems. Most of them can enhance secondary metabolism pathways involved in defense strategies, including those related to alkaloid biosynthesis. Because production of constitutive chemical defenses is costly for plants, especially in the absence of deleterious organisms, they often rely on induced chemical responses to many such stresses. Induced resistance is defined as changes in plant chemistry or physiology with measurable effect on herbivore performance via behavior or biology (*1*). Results previously reported by our laboratory showed that different kinds of environmental stresses could affect secondary metabolite production, quinolizidine alkaloids among them (*2*−*5*). Effects of mechanical wounding on *Lupinus* species have been earlier reported by Wink (*6*).

Although constitutive alkaloids have been mostly associated with defense strategies against herbivores and particularly against insects, several of them are also known as antibacterial, antiviral, or allelopathic compounds (*7*−*9*). The role of alkaloids in plants has been thoroughly discussed during the past century. These compounds, which were once thought to be nitrogenous wastes (analogous to urea and uric acid in animals), may have roles such as nitrogen storage or growth regulators (*10*).

The *Lupinus* genus includes different species, most of them exhibiting high nutritive value, especially related to their high protein content (*11*). *L. albus* and *L. angustifolius* are cultivated for nutrition purposes not only for their high protein content (almost 40%) but also for their oil content. These legumes, which contribute to improve soil structure and characteristics increasing nitrogen, phosphorus, and organic matter contents, are also cultivated as ruminant feed either as green forage in the areas of traditional cultivation or as grains introduced as protein supplements in the diets of livestock. Lupine is considered an emerging alternative crop to soybean, with the advantage of being adapted to cooler environments and drier soils. A total of 55000 acres were harvested in Chile in 2007, with grain yields of around 1000 kg/acre (*12*). Although currently lupines are not widely cultivated in Argentina, considerable gains may be obtained by introducing the crop in many regions due to its adaptation capacity. Several field trials have been conducted to evaluate different genotypes, and most tested materials were shown to fulfill crop requirements and had good yields (*13*).

Lupinus species are also known by their grain bitterness, which has been related to their alkaloid content. Quinolizidine alkaloids represent an important group of secondary metabolites produced by several genera within the Fabaceae family, *Lupinus* among them (**Figure 1**).

They are biosynthesized in green tissues, transported via phloem, and stored in all organs, seeds included. Biosynthesis of lysine-derived quinolizidine alkaloids seems to occur within certain chloroplasts in green leaves. Further modifications are

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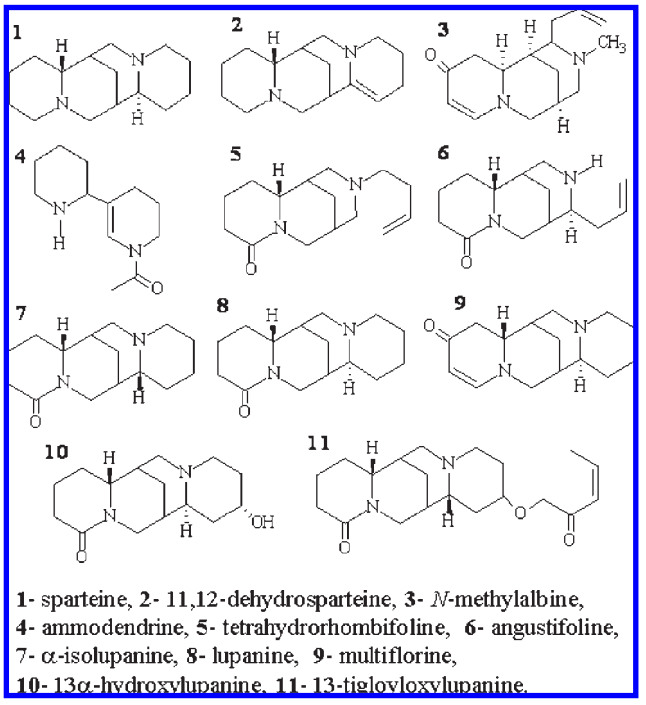

Figure 1. Chemical structures of *L. angustifolius* and *L. albus* alkaloids.

reported to occur on the quinolizidine skeleton after it enters the cell. Final products are thought to accumulate within vacuoles of epidermis cells, where their defensive role is effectively displayed (*14*, *15*).

Quinolizidine alkaloids show a wide range of biological activities including allelopathic effects inhibiting other plants (*8*) and the proliferation of viruses, bacteria, and fungi (*9*, *16*). They can also deter herbivores (nematodes, caterpillars, beetles, aphids, locusts, snails, rabbits, and cows) and pollinators such as bees (*17*). Deterrent or toxic effects of quinolizidine alkaloids such as sparteine, lupanine, and 13-tigloyloxylupanine against phytophagy have been evaluated on different insects, that is, some Lepidoptera (*18*).

In a previous study we reported the chemical response of two *Lupinus* species to *Anticarsia gemmatalis* attack (*4*), also describing its effect on subsequent herbivory. Chemical changes in plant tissues following herbivory increased plant resistance to subsequent caterpillar attack, confirming their active role as plant defenses (*19*).

Piperidine and quinolizidine alkaloids vary in toxicity, particularly when referred to mammals. LD_{50} corresponding to oral administration of a mixture of alkaloids from *L. angustifolius* seed to rats was found to be 2279 mg/kg; in the case of lupanine LD_{50} was reported to be 1464 mg/kg (*20*).

Several studies in the past three decades point to anagyrine and ammodendrine, alkaloids frequently present in *Lupinus* species, as teratogens (*21*−*24*). Ingestion of lupine by cattle was first reported to cause congenital birth defects in calves (crooked calf disease) in the late 1950s. "Crooked calf disease" was described as a condition in which calves were born with deformities such as arthrogryposis, scoliosis, kyposis, torticollis, and cleft palate. Quinolizidine alkaloid anagyrine and piperidine alkaloid ammodendrine were shown to reduce fetal movement during the critical period of gestation (40−100 days) causing the spine and limbs to develop in contracted or misaligned positions. It was demonstrated that anagyrine doses in the range of 2−30 mg/kg induced moderate-to-severe calf defects resulting in crooked animals (*22*). Cattle losses due to lupine-induced crooked calf disease continue to occur in several countries (*23*, *24*). Congenital malformations in Hereford calves were reported in Argentina (Chubut) more than two decades ago (*25*). It was suggested that diseases were related to the presence of alkaloids in species growing in forage lands.

In the past decades plant breeders developed the so-called sweet lupines with low alkaloid contents, below 0.05%, to exploit them for animal and human consumption without negative consequences on health. "Sweet" varieties are more palatable but, at the same time, more vulnerable to herbivores (*26*); nevertheless, some of them were demonstrated to deter subsequent herbivory after being attacked (*4*). However, their chemical responses upon predation by large herbivores have not yet been studied.

The aim of this work was to evaluate chemical responses of *L. albus* and *L. angustifolius* green aerial parts to levels of mechanical damage trying to mimic the action of large herbivores. GC and GC-MS were used to determine total alkaloid content and their individual relative abundances in bitter and sweet varieties of each species. Changes in individual alkaloid relative abundances are particularly important in sweet genotypes used for nutritive purposes, because of their potential toxicity and teratogenic effects.

MATERIALS AND METHODS

General. Analytical solvents were purchased from Sintorgan (Chemical Center SRL, Argentina).

Bradyrhizobium spp. inoculant was provided by Rizobacter Argentine S.A. (Pergamino, Buenos Aires, Argentina).

Kieselgel 60 F_{254} TLC aluminum sheets for thin layer chromatography were purchased from Merck (Research AG, Buenos Aires, Argentina).

Plant Material and Experimental Design. Bioassay was performed in a field located in Buenos Aires, Argentina (34° 37′ S, 58° 20′ W), characterized by silty clay loam soil (Argiudoll, pH 6.5). Plant material included sweet and bitter genotypes of two *Lupinus* species: *L. albus*, Rumbo (Ru, sweet variety) and El Harrach (EH, bitter variety); *L. angustifolius*, Gungurru (Gu, sweet variety) and Vila velha (Vv, bitter variety).

Bioassay was performed with a split-plot design and four replications. Each species main plot included varieties and subplots cutting treatments. Each subplot consisted of four rows, 3 m long with 0.5 m inter-row spacing. Treatments were randomly assigned within each plot. Seeds were inoculated with *Bradyrhizobium* spp. strains used for *Lupinus* species.

Plant densities were 15 and 20 plants/m^2 for *L. albus* and *L. angustifolius*, respectively (differences in density are related to their different plant sizes). Weeds were hand-controlled, and no pesticides were used to avoid any interference. Plants were watered when needed, according to visual criteria. Plants evidencing insect attack were excluded from chemical analysis.

Treatments. Treatments resulted from the factorial combination of four genotypes and three levels of biomass removal: C (control), C_1, and C_2, corresponding to 0, 25, and 50% shoot length removal, respectively.

Five plants were initially (T_0) harvested within each subplot, and their total alkaloid content and alkaloid relative abundances were determined, providing chemical data for each genotype at the time the bioassay started $C(T_0)$.

Some *Lupinus* species are commonly used as dual-purpose crops, for green biomass as annual forage and for grain yield. In the present work cutting levels mimicked that of biomass loss due to large herbivores (sheep, cattle) attack. Treatments were performed on 20 plants within each subplot at the beginning of flowering (T_0). Removed biomass corresponded to the main apex, stems, and leaves from the upper shoot. Plants surrounding treated samples were submitted to the same degree of biomass removal to avoid competition for light with intact neighbors. Five plants corresponding to each level of mechanical damage (C_1 and C_2) and five control plants C were randomly harvested 10 days (T_1) after cutting treatments. Plant material was oven-dried at 40 °C under ventilation until constant weight.

Phytochemical Analysis. Dry aerial material (10 g) was milled and submitted to continuous extraction with light petroleum ether (6 h) using a Soxhlet apparatus, in order to separate the lipid fraction. Remaining plant material was then extracted in the same way with methanol (6 h), and methanol crude extract was evaporated to dryness under vacuum conditions at 40 °C, dried in a vacuum desiccator until constant weight, and further processed to separate alkaloids. Methanolic dry extract was dissolved in water, acidified with 5% HCl to pH 2, and extracted with chloroform (three times). Defatted aqueous solution was alkalinized to pH 14 with NH_4OH and then extracted with chloroform (three times) to obtain the alkaloid extract (QA), which was dried and weighed. Alkaloids were analyzed by chromatography on Kieselgel 60 F_{254} TLC aluminum sheets using $MeOH/CHCl_3/NH_4OH$ (88:12:1, v/v/v) as mobile phase. Chromatograms were visualized by UV light and/or by a chromogenic reaction with Draggendorff reagent.

Gas Chromatography Analysis. A weighted fraction of the alkaloids dry extract was dissolved in CH_2Cl_2 and analyzed by gas chromatography (GC) with a GC 6890N Agilent Technologies (Agilent Technologies Inc., Wilmington, DE) using an HP-5 (30 m × 0.25 mm i.d., 0.25 μm) capillary column (Agilent Technologies Inc.). Helium was used as carrier gas with a 1:20 split ratio and 1 mL/min flow rate. The injector temperature was 250 °C and the detector temperature, 280 °C. The temperature program was as follows: isothermal at 120 °C for 2 min, from 120 to 300 °C at a rate of 6 °C/min; then 10 min isothermal. Hexadecane (1 μL) was used as internal standard. Total alkaloid content (TAC) was calculated on the basis of the sum of individual alkaloids peak areas in the GC chromatogram, and that value was related to weighted dry matter (DM).

Gas Chromatography−Mass Spectrometry Analysis. Alkaloid GC-MS analysis was performed on a GC 6890N Agilent coupled with a MS 5973 (Agilent Technologies, Inc.) with the same column and conditions used to perform GC analysis. Electron impact mass spectra were recorded at 70 eV, scan (40−560 amu). Alkaloid structures were tentatively identified according to their mass fragmentation through library search (Wiley GC-MS library databank) and by their Kovats indices, which were determined by cochromatography with a mixture of linear alkanes.

Data Analysis. QA extracts exhibited different chromatographic profiles through TLC analysis, depending on varieties and treatments. Spots exhibiting chromogenic reaction with Dragendorff spray solution were located at R_f values between 0.19 and 0.93.

Alkaloid concentrations for C, C_1, and C_2 were statistically analyzed on the basis of GC and GC-MS data by factorial ANOVA.

RESULTS

Total Alkaloid Content. Total alkaloid content (TAC) in control samples (C) did not show significant differences when T_0 and T_1 were compared; hence, relative TAC = $TAC(C(T_1))/TAC(C(T_0))$ was 1 for all genotype control samples.

Figure 2 shows relative TAC values corresponding to treatments C, C_1, and C_2, because increases in TAC have been calculated relative to their value at T_0. Bitter varieties contained, as expected, higher total alkaloid contents than sweet genotypes: Vila vehla (Vv), 3.95 ± 0.26 mg/g of DM; El Harrach (EH), 3.99 ± 0.19 mg/g of DM; Gungurru (Gu), 0.51 ± 0.09 mg/g of DM; and Rumbo (Ru), 0.53 ± 0.09 mg/g of DM.

Mechanical damage (treatments C_1 and C_2) resulted in significant changes in all genotypes' total alkaloid contents, with higher increases in sweet varieties. Whereas Vv and EH showed 22 and 32.8% TAC increases, respectively, under C_1 treatment, sweet varieties Gu and Ru exhibited higher inductive responses, 58.8 and 67.9%, respectively.

The relative TAC increases exhibited by Vv in treatments C_1 and C_2 were 22 and 21%, respectively, whereas 32.8 and 33.5% were the corresponding values for EH.

Sweet genotype Gu increased its TAC by 58.8 and 60.7%, as induced response to treatments C_1 and C_2, respectively. The same treatments enhanced 67.9 and 66%, respectively, Ru relative TAC.

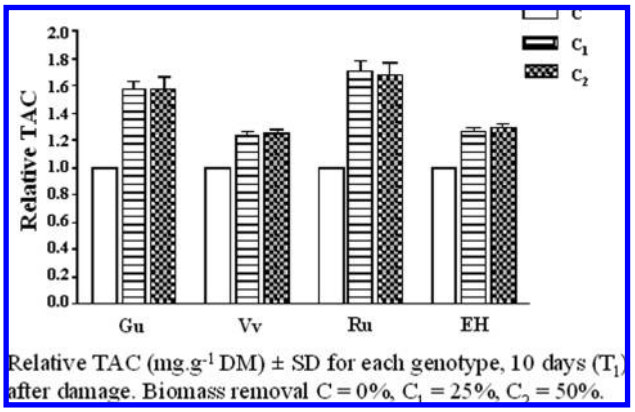

Figure 2. Changes in relative total alkaloid content (relative TAC) in *Lupinus* genotypes in response to biomass removal.

These data show that the studied *Lupinus* genotypes did not exhibit significant differences in response to different levels of damage in their inductive responses.

Alkaloid Profiles. GC analysis showed qualitative and quantitative differences in alkaloid contents between sweet and bitter varieties of *L. angustifolius* and *L. albus*.

GC analysis of control samples revealed the presence of 10, 7, 5, and 9 alkaloids in Gu, Vv, Ru, and EH alkaloid extracts, respectively. Alkaloids were identified through library search (Wiley library databank) and confirmed by comparing their mass spectral data with literature data (*27*, *28*).

Mass spectra data corresponding to alkaloids identified in studied samples are given in **Table 1**, including retention index (RI), molecular ion (M^+), and characteristic ions.

Alkaloid relative abundances were determined by their peak area, relative to that of the internal standard, and expressed as percentage. *L. angustifolius* and *L. albus* individual alkaloid mean concentrations corresponding to different treatments (C, C_1, and C_2) are given in **Table 2**.

L. angustifolius. GC chromatograms of Gungurru (sweet variety) control samples (C) showed 13-tigloyloxylupanine (40.1%) as the major alkaloid followed by lupanine (15.2%), 11,12-dehydrosparteine (12.3%), and tetrahydrorhombifoline (10.5%) (**Figure 3**A). Two predominant alkaloids were found in Vv (bitter variety) control, 13-tigloyloxylupanine (50.5%), and lupanine (43%) (**Figure 3**B). Less abundant alkaloids corresponding to both *L. angustifolius* genotypes are also shown in **Figure 3A**,**B**.

Ammodendrine abundance was higher in the sweet variety; nevertheless, it was present at very low proportions in both of them.

L. albus. As shown in **Figure 3C**, GC data indicated that 13α-hydroxylupanine (72.2%) was the predominant alkaloid in Rumbo (sweet) control samples followed by much lower abundances of 13-tigloyloxylupanine (13%), tetrahydrorhombifoline (6.9%), and lupanine (6%). Instead, lupanine (81.2%) was the major alkaloid in El Harrach (bitter) control samples, followed by minor abundances of multiflorine (8%) and 13-tigloyloxylupanine (4.3%) (**Figure 3D**). Ammodendrine was present as a minor component in EH; it was not detected in *L. albus* sweet variety (Ru).

Anagyrine was not detected in any of the studied genotypes.

After damage, the studied genotypes exhibited different responses in their alkaloid profile, which strongly depended on the sweet or bitter character of each particular variety.

Among *L. angustifolius* genotypes (**Figure 3A**,**B**), Gu showed increases in α-isolupanine, angustifoline, 13α-hydroxylupanine, 11,12-dehydrosparteine, tetrahydrorhombifoline, and sparteine

Table 1. Mass Spectra Data of Identified *Lupinus* Alkaloids[a]

alkaloid	RI	M^+	characteristic ions (abundance percentage)
sparteine	1784	234 (22)	193 (42), 176 (10), 150 (7), 148 (7), 137 (100), 136 (44), 122 (27), 110 (12), 98 (65)
11,12-dehydrosparteine	1837	232 (51)	175 (45), 163 (17), 148 (31), 135 (26), 134 (100), 97 (18), 96 (27)
ammodendrine	1862	208 (21)	191 (100), 165 (59), 136 (39), 123 (38), 122 (28), 120 (20), 110 (62), 94 (44)
tetrahydrorhombifoline	2037	248 (1)	208 (17), 207 (100), 112 (48), 108 (31), 55 (33)
angustifoline	2069	234 (1)	193 (100), 150 (63), 112 (79), 84 (26), 55 (42)
α-isolupanine	2098	248 (36)	150 (35), 149 (62), 136 (100), 98 (28), 94 (21)
lupanine	2161	248 (41)	219 (11), 150 (33), 149 (64), 136 (100), 110 (19), 98 (29), 84 (36)
N-methylalbine	2216	246 (11)	205 (93), 149 (12), 136 (15), 110 (25), 94 (23), 58 (100)
multiflorine	2317	246 (61)	148 (17), 134 (100), 110 (35), 97(16)
13α-hydroxylupanine	2410	264 (28)	246 (67), 165 (43), 152 (100), 148 (42), 134 (78), 112 (26), 108 (24)
13-tigloyloxylupanine	2763	346 (1)	246 (100), 148 (37), 134 (95), 112 (22) (17), 98 (14)

[a] RI, retention index; M^+, molecular ion.

Table 2. Changes in Individual Alkaloid Mean Concentrations in *Lupinus* Genotypes Related to Mechanical Damage[a]

Lupinus angustifolius						
	Vv			Gu		
QA	C	C1	C2	C	C1	C2
1	0.015 ± 0.003	0.033 ± 0.004	0.030 ± 0.006	0.009 ± 0.002	0.017 ± 0.001	0.014 ± 0.003
2				0.063 ± 0.010	0.117 ± 0.003	0.113 ± 0.014
3				0.027 ± 0.003	0.025 ± 0.003	0.027 ± 0.002
4	0.014 ± 0.003	0.032 ± 0.003	0.030 ± 0.008	0.028 ± 0.004	0.031 ± 0.002	0.030 ± 0.008
5	0.026 ± 0.004	0.079 ± 0.009	0.071 ± 0.008	0.054 ± 0.006	0.112 ± 0.015	0.115 ± 0.021
6				0.005 ± 0.001	0.057 ± 0.006	0.058 ± 0.009
7	0.154 ± 0.024	0.251 ± 0.013	0.248 ± 0.035	0.001 ± 0.001	0.046 ± 0.011	0.047 ± 0.014
8	1.699 ± 0.182	1.972 ± 0.116	1.958 ± 0.091	0.078 ± 0.005	0.052 ± 0.005	0.053 ± 0.004
10	0.044 ± 0.018	0.068 ± 0.005	0.067 ± 0.006	0.042 ± 0.004	0.145 ± 0.008	0.148 ± 0.009
11	1.995 ± 0.292	2.392 ± 0.192	2.37 ± 0.109	0.205 ± 0.028	0.211 ± 0.031	0.214 ± 0.037
TAC	3.95 ± 0.26	4.82 ± 0.38	4.78 ± 0.23	0.51 ± 0.09	0.81 ± 0.13	0.82 ± 0.18
Lupinus albus						
	EH			Ru		
QA	C	C1	C2	C	C1	C2
1	0.011 ± 0.003	0.147 ± 0.004	0.144 ± 0.006			
2	0.051 ± 0.008	0.077 ± 0.010	0.082 ± 0.009			
3	0.029 ± 0.008	0.036 ± 0.007	0.041 ± 0.009	0.010 ± 0.004	0.013 ± 0.006	0.011 ± 0.005
4	0.041 ± 0.011	0.084 ± 0.009	0.083 ± 0.011			
5	0.082 ± 0.012	0.109 ± 0.013	0.103 ± 0.015	0.037 ± 0.009	0.058 ± 0.011	0.061 ± 0.009
6						
7						
8	3.243 ± 0.213	4.113 ± 0.274	4.155 ± 0.318	0.032 ± 0.008	0.295 ± 0.019	0.298 ± 0.024
9	0.320 ± 0.025	0.482 ± 0.014	0.467 ± 0.023			
10	0.043 ± 0.008	0.091 ± 0.012	0.094 ± 0.006	0.383 ± 0.018	0.421 ± 0.027	0.415 ± 0.030
11	0.172 ± 0.019	0.161 ± 0.022	0.158 ± 0.028	0.069 ± 0.008	0.107 ± 0.02	0.098 ± 0.006
TAC	3.99 ± 0.19	5.30 ± 0.27	5.33 ± 0.33	0.53 ± 0.09	0.89 ± 0.14	0.88 ± 0.13

[a] Vv, Vila vehla; Gu, Gungurru; EH, El Harrach; Ru, Rumbo. Biomass removal: C = 0%, C_1 = 25%, C_2 = 50%. Values are means ± SD (n = 4), expressed as mg g^{-1} og DM. Alkaloids: **1**, sparteine; **2**, 11,12-dehydrosparteine; **3**, *N*-methylalbine; **4**, ammodendrine; **5**, tetrahydrorhombifoline; **6**, angustifoline; **7**, α-isolupanine; **8**, lupanine; **9**, multiflorine; **10**, 13-α-hydroxylupanine; **11**, 13-tigloyloxylupanine.

relative abundances. α-Isolupanine, detected as only traces in control samples, showed a striking 46-fold increment in response to biomass removal, followed by angustifoline that increased 11-fold its concentration compared to that in control samples. Significant increases were also found for 13α-hydroxylupanine (245%), tetrahydrorhombifoline (107%), sparteine (88%), and 11,12-dehydrosparteine (85%). Minor differences were observed in ammodendrine and 13-tigloyloxylupanine relative concentrations. Lupanine was the only alkaloid showing a significantly lower concentration in damaged Gu samples compared to control ones.

L. angustifolius bitter variety Vv increased all of its alkaloid relative abundances after damage, with different intensities depending on each particular alkaloid. Tetrahydrorhombifoline, a minor alkaloid in this genotype, showed the highest increment (3-fold). Other alkaloids also increased their relative concentrations dramatically; ammodendrine present at very low concentration in control samples increased 128%; sparteine, 120%; α-isolupanine, 63%; and 13α-hydroxylupanine, 55%. The main alkaloids in control samples, lupanine and 13-tigloyloxylupanine, showed lower induced increments.

El Harrach, a *L. albus* bitter variety (**Figure 3D**), increased all individual alkaloid relative abundances, except for 13-tigloyloxylupanine, which did not show significant differences. Sparteine, present as only traces in control samples, showed the highest

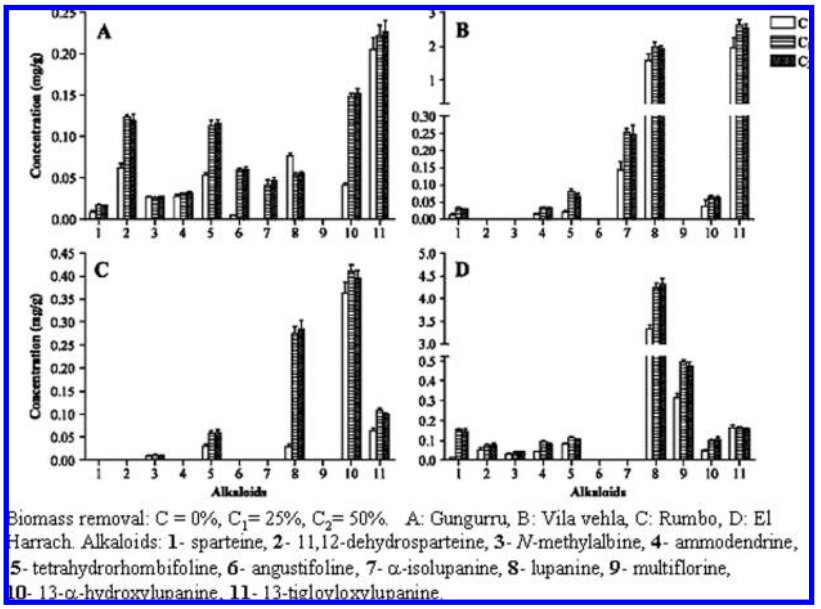

Figure 3. Changes in alkaloid abundances in *L. angustifolius* and *L. albus* genotypes in response to mechanical damage.

induced response (13-fold), whereas 13α-hydroxylupanine and ammodendrine were increased almost 100%. A sweet *L. albus* variety Rumbo (**Figure 3D**) responded to mechanical damage with a striking increase in lupanine relative abundance (almost 8-fold), being the alkaloid with higher induced response in this variety. Relative concentrations of other components were also increased, tetrahydrorhombifoline (57%) and 13-tigloyloxylupanine (55%), as a result of biomass removal. No significant differences were observed in 13α-hydroxylupanine and *N*-methylalbine relative abundances.

Our results suggest that studied levels of biomass removal corresponding to different intensities of mechanical damage did not trigger de novo synthesis of individual alkaloids.

DISCUSSION

Most studies about inductive responses to herbivory in *Lupinus* species have been related to insect attack; however, chemical induction might depend on level of damage.

The present work studies changes in QA abundances in response to higher biomass removal levels (25 and 50%), trying to mimic the action of large herbivores. Inductive responses were similar for both levels of damage in all genotypes. Although biomass removal triggers the chemical response, there were no significant differences in TAC between treatments with different removal levels (C_1 and C_2), suggesting that when levels of damage are higher than those in treatment C_1, these species do not allocate further resources in enhancing chemical defenses.

When species are compared, *L. albus* varieties showed higher chemical induction than *L. angustifolius* ones. Responses to mechanical damage are in partial agreement with those we found before in a bioassay with *A. gemmatalis* caterpillars, already reported (*4*). Previous work suggested that *L. albus* higher response to herbivory could be related to a difference in strategies against it between the species. *L. albus* and *L. angustifolius* exhibited different behaviors related to induced resistance, particularly with reference to the ratio between chemical induction and regrowth. Whereas the first mainly allocated resources in enhancing chemical defenses, the latter exhibited remarkable regrowth levels (*13*).

It was reported (*4*) that *L. albus* genotypes responded to herbivory by *A. gemmatalis* with significant chemical induction. Vilariño and Ravetta also demonstrated that 2 weeks after cutting, there was lack of significant compensatory ability (regrowth) in these species genotypes (*13*). This behavior suggests that the higher alkaloid abundances we found in *L. albus* genotypes in response to mechanical damage in the present bioassay seem also to be related to chemical induction.

L. angustifolius behavior was quite the opposite, its genotypes showing high levels of tolerance (*13*). Alkaloid concentration was also significantly enhanced during this bioassay in narrow-leaf lupine genotypes (Vv, 22%; and Gu, 58.8%), with remarkable differences in alkaloid relative abundances, suggesting that besides TAC changes related to young leaves during regrowth, chemical induction may also be involved modulating the alkaloid profile after mechanical damage.

Our results suggest that both *Lupinus* species responded to mechanical damage through chemical induction, with higher responses in sweet varieties, in agreement with the idea that higher levels of constitutive chemical defenses will trigger lower induced responses (*1*). Optimal defense theory predicts that genotypes with high constitutive defense levels should be less inducible than those with low levels (*29*, *30*).

A higher induction level in sweet varieties could represent an advantage for plants that use its resources to produce chemical defenses only when they are needed (*31*). A fast alkaloid induction can imply changes in palatability that could curb further damage, as was proved with *L. albus* sweet variety (Ru).

In a previous work we have reported that *A. gemmatalis* consumed three times more when fed undamaged Rumbo leaves, compared to its behavior toward samples foraged by the same species 72 h before, demonstrating that chemical induction resulted in an effective way to avoid further damage. We also described the chemical responses of these *Lupinus* genotypes to herbivory by *A. gemmatalis* (*4*). Only *L. albus* varieties showed chemical induction after herbivory, while no such response was detected in *L. angustifolius*.

Considering the present findings, one of the reasons for the discrepancies found between both bioassays could be differential timing for the chemical response to take place in both species.

There were quantitative changes in quinolizidine alkaloid profiles after damage, but no new alkaloids were found even when individual alkaloid relative abundances changed.

Lupanine is a common precursor in many quinolizidine alkaloid biosynthetic pathways, 13α-hydroxylupanine and isolupanine among them (*15*, *32*). Lupanine lower relative concentration in damaged Gu samples might be partially related to the enhancement of 13α-hydroxylupanine and isolupanine abundances.

Quinolizidine alkaloid levels are known to depend on many factors such as organ, development stage, leaf age, and time, among others (*6*, *15*). The fact that senescent lupine leaves hardly have alkaloids would agree with theories suggesting transient use of these metabolites, lupanine among them, as nutrient resources. Lower lupanine concentrations in damaged samples might be related in part to this possibility. Biosynthesis rate, traslocation ability, and metabolic transformation rates to structurally related compounds, all affected by the time elapsed after damage, are some other factors modulating relative abundances of quinolizidine alkaloids, including lupanine, in different tissues. Further studies with labeled precursors would be needed determine time course changes in ratio between lupanine and its metabolic products.

Changes in alkaloid abundances may not only affect these species' palatability and quality, affecting herbivory by insects, but also increase the potential harm associated with its ingestion. Quinolizidine alkaloids have, individually, differential effects as deterrents or on herbivore health, some of them becoming lethal over certain thresholds (*24*, *33*). Even when sweet genotypes with low alkaloid levels, which have been developed in part to overcome this threat, showed higher levels of chemical induction than bitter ones, only the latter exhibited in this bioassay significant differences in ammodendrine relative concentrations in response to mechanical wounding.

Our results demonstrate that even when the TAC of sweet varieties (Gu and Rumbo) was increased 58.8 and 67.9%, respectively, in response to mechanical damage, the final TAC values were still remarkably low compared to those of bitter species.

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