Phytochemical Differences between *Calia secundiflora* (Leguminosae) Growing at Two Sites in Mexico

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The ecology and quinolizidine alkaloid chemistry of *Calia secundiflora* (Ortega) Yakovlev growing at two sites in Mexico were compared. At one site (Hidalgo) the vegetation was dominated by *Flourensia resinosa* and *C. secundiflora*, at the other site (Queretaro) *C. secundiflora* and *Dodanaea viscosa* were dominant. The Hidalgo site had shallower soils with less organic matter, N, P, and $CaCO_3$. Seeds of *C. secundiflora* from each site accumulated a similar range of quinolizidine alkaloids, but the profile of alkaloids in the leaves and roots were different. The leaves and roots of plants at Hidalgo accumulated a similar range of alkaloids to the seeds with cytisine and/or *N*-methylcytisine being most abundant, whereas at Queretaro the leaves and roots accumulated lupinine, with other alkaloids being relatively minor constituents. The latter profile has not been reported previously for *C. secundiflora.*

Key words: Calia secundiflora, Quinolizidine, Alkaloids, Leguminosae

Introduction

Calia secundiflora (Ortega) Yakovlev [syn. *Sophora secundiflora* (Ortega) Lag. ex DC] is a woody member of the Leguminosae distributed in Africa, America and Asia. On the American continent its range extends from the southwest United States (Hatfiled *et al.*, 1977) to the mountains of Oaxaca and Puebla in southern Mexico (García *et al.*, 1994) and it is relatively abundant in canyons and on slopes in hot, arid and semiarid climates. In Mexico it is recorded in the states of Nuevo Leon, Coahuila, Sonora, Chihuahua, Tamaulipas, San Luis Potosi, Veracruz, Queretaro, and Hidalgo (Aguilar and Zolla, 1982). In the last two states it forms part of juniper (*Juniperus* spp.) and pinion pine (*Pinus cembroides* Zucc.) forests, and is given the common names *colorín*, *patol*, and *pitol.* In other states of Mexico it is also called *coca*, *colorín*, *chocolón*, *frijolillo* and *frijolito* (Martínez, 1978) while the English name is mescal bean. It is considered a medicinal plant in Mexico but has scarcely been used due to its toxicity (Aguilar and Zolla, 1982).

The phytochemical aspects of *C. secundiflora* in North America and Asia have been widely studied, and its medicinal and toxic properties have been documented since the 1970s. The main compounds reported are carbohydrates, flavonoids and alkaloids, specifically the toxic alkaloid cytisine and other derivatives of quinolizidine (Hatfield *et al.*, 1977). In Mexico, it is known that goats grazing in pinion pine forests in some areas of Hidalgo and Queretaro are often poisoned when they consume the leaves and seeds of *C. secundiflora* (data not published), and Aguilar and Zolla (1982) mention that intoxications due to *C. secundiflora* are infrequent in humans but common in cattle, goats, sheep and horses. However, the phytochemistry of *C. secundiflora* growing in Mexico has not been studied and it is not known whether the chemistry of Mexican populations differ from those of other regions or vary in response to ecological conditions. The ecology of *C. secundiflora* in Mexico has also not been studied specifically, although it is often mentioned in Floras or works on vegetation.

Thus, the objectives of this study were to compare general ecological characteristics and quinolizidine alkaloid chemistry of *C. secundiflora* at two sites in Mexico in the states of Hidalgo and Queretaro.

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Materials and Methods

Field work

The site at Hidalgo was on the Cardonal-Santuario Highway in the municipality of Cardonal. The site had secondary vegetation, possibly derived from the disturbance of the *Pinus cembroides* forest, with evidence of intensive grazing principally by goats resulting in large areas without vegetation. The site was at an altitude of 2130 m on a slope facing SSE and SE with a gradient of about 40°. The site in Queretaro was on the Vizarron-San Joaquin Highway in the municipality of Cadereyta. This was a disturbed pinion forest (*Pinus cembroides*, *P. pinceana* with *Juniperus flaccida*) with evidence grazing mainly by cattle and there were few areas with no vegetation. This site has an altitude of 2070 m on a slope facing SE with a gradient of about 45°.

Data of the communities were taken using the point-centered quarted method. The sites were transected along the distribution of *C. secundiflora* and 60 stations were located in each transection. At each station the density, frequency and dominance of each species was recorded from which their index of importance was calculated (Mueller-Dombois and Ellenberg, 1974). The two sites were compared using this index as well as the richness of species and the similarity indexes of Sorensen to deduce differences between habitats and the sites in general. Soil samples of the upper 30 cm were taken for analysis at the Soils Department of the University of Chapingo.

At each site, ten *C. secundiflora* individuals spaced at least 50 m apart along a transect were selected and marked. From each individual, samples of leaves, roots and seeds were collected. Environmental data (vegetation, disturbance, and soil) were taken from the communities where the plant grows to estimate density values and make comparisons between the two sites. The comparisons were performed using the variables mentioned and the indexes of similarity of Sorensen (Mueller-Dombois and Ellenberg, 1974).

Phytochemical work

Preparation of crude alkaloid extracts

Extracts were prepared following the method described by Games *et al.* (1974). Powdered dry samples were defatted with hexane and the dried residue was extracted with methanol in a Soxhlet apparatus for 48 h. The alkaloidal fraction was then prepared as described by Games *et al.* (1974).

Analysis and identification of alkaloids

Thin-layer chromatography (TLC) was used for the preliminary confirmation of the presence of alkaloids. TLC was undertaken using silica gel 60 GF_{254} aluminium plates (Merck) and a mobile phase of 8:2 dichloromethane/methanol. The plates were observed under ultraviolet light and then developed with the Dragendorff reagent. Alkaloids were identified by gas chromatography-mass spectrometry (GC-MS). The system used was a Perkin-Elmer TurboMass quadrupole GC-MS instrument fitted with a 30 m × 0.25 mm (i.d.) × 0.25 um DB1-MS (J & W Scientific) capillary column. 1 μl injections (split 1:10) were vaporised at 250 °C and chromatography was undertaken using a temperature gradient of 120–320 °C at 6 °C/min with 1 ml/min helium carrier gas. Mass spectra following electron ionization (70 eV; source temperature 180 °C) were recorded at 0.75 s/scan in the range *m/z* 38–600. To identify the quinolizidine alkaloids, the mass spectra and retention times were compared with published data (Wink, 1993; Wink *et al.*, 1995).

Results and Discussion

In the two sites, 33 species from 26 genera of 14 families were recorded. Along the transection in the Hidalgo site, 17 bushy and herbaceous plant species were recorded. According to the data of value of importance (V.I.), the vegetation was dominated by *Flourencia resinosa* (25% dominance) and *Calia secundiflora* (21% dominance). In the Queretaro transection, 21 bushy and herbaceous species were recorded with the dominant species being *C. secundiflora* (V.I. = 30%) and *Dodonaea viscosa* (V.I. = 12%). Several authors (Muller, 1947, cited by Rzedowski, 1978; Hiriart and González, 1983; Argüelles *et al.*, 1991; Zamudio *et al.*, 1992; Fernández and Colmenero, 1997) have reported that there are at least 41 species from 35 genera of plants with which *C. secundiflora* coexists in Mexico, sharing or not sharing dominance.

The composition of species at the two sites was significantly different (Sorensen index of similarity 36%) with only four species, in addition to *C. secundiflora*, being common to both sites: *Boubardia ternifolia*, *Dodonaea viscosa*, *Jatropha dioica*,

Table I. Chemical analysis of soils collected from *Calia secundiflora* sites in Cardonal, Hidalgo and Vizarron, Cadereyta, Queretaro.

Hidalgo
Texture classification: clay to clay loam (27.4% sand, 33.8% silt, 38.8% clay)

pH	CE [dS m^{-1}]	MO (%)	N [mg kg^{-1}]	P [mg kg^{-1}]	K [mg kg^{-1}]	Ca [mg kg^{-1}]	Fe [mg kg^{-1}]	Cu [mg kg^{-1}]	Zn [mg kg^{-1}]	Mn [mg kg^{-1}]	$CaCO_3$ (%)
7.5	1.6	8.9	32.2	6.1	742	10484	10.8	0.4	19.4	7.8	1.3

Queretaro
Texture classification: sandy loam (65.2% sand, 25.4% silt, 9.4% clay)

pH	CE [dS m^{-1}]	MO (%)	N [mg kg^{-1}]	P [mg kg^{-1}]	K [mg kg^{-1}]	Ca [mg kg^{-1}]	Fe [mg kg^{-1}]	Cu [mg kg^{-1}]	Zn [mg kg^{-1}]	Mn [mg kg^{-1}]	$CaCO_3$ (%)
7.7	1.1	21.5	64.4	19.2	349	17819	6.1	0.6	2.3	4.6	7.1

CE, electric conductivity; MO, organic matter.

Montanoa tomentosa. This difference is probably due, principally, to the greater disturbance in Hidalgo and the earlier succession as well as to edaphic characteristics. The clayey soils at the Hidalgo site were shallower, with less organic matter, nitrogen, calcium and total phosphorus than the soils of Queretaro, which were sandy (Table I).

The mean yields of alkaloids (% dry weight) obtained from *C. secundiflora* at Hildago were 0.04% for both seeds and leaves and 1.33% for roots, while the mean yields from Queretaro were 0.03% for both seeds and roots and 0.12% for leaves. According to Robinson (1979), a plant can be considered a source of alkaloids when it contains more than 0.05% (dry weight); Hegnauer (1963) states a lower limit of 0.01%. The levels of alkaloids in seeds and pericarp of immature pods of *C. secundiflora* from Queretaro were also studied. The content of alkaloids in the pericarp was higher (0.31% dry weight) than in the immature seeds (0.13%). The greater alkaloid content in immature seeds compared to mature seeds (0.03%) contrasts with another Mexican legume, *Erythrina americana*, for which it was found that the alkaloid content is higher in mature seeds (García-Mateos *et al.*, 1996).

Table II shows the profiles of alkaloids identified in *C. secundiflora.* All the identified alkaloids have been reported previously from *C. secundiflora* (Southon *et al.*, 1994; Kite and Pennington, 2003). While the seeds from plants growing at the two sites showed a similar range of alkaloids, the profile of alkaloids in the leaves and roots was different. The leaves and roots of plants from Hidalgo accumulated a similar range of alkaloids to the seeds, with the most abundant being cytisine

Table II. Alkaloids identified in tissues of *C. secundiflora*.

RRT	M^+	Alkaloid	Hidalgo			Queretaro		
			Seeds	Leaves	Roots	Seeds	Leaves	Roots
0.36	169	Lupinine		5.18 ± 0.04		2.61 ± 0.03	33.29 ± 0.05	21.84 ± 0.10
0.71	234	Sparteine	1.81 ± 0.01	4.60 ± 0.04	1.43 ± 0.03	2.93 ± 0.02		0.21 ± 0.01
0.81	208	Ammodendrine		0.29 ± 0.05				
0.94	204	*N*-Methylcytisine	2.05 ± 0.02	7.57 ± 0.02	89.72 ± 0.11	4.83 ± 0.04	16.32 ± 0.1	1.56 ± 0.01
1.00	190	Cytisine	14.98 ± 0.04	5.50 ± 0.03	30.00 ± 0.62	3.08 ± 0.02	0.85 ± 0.01	
1.01	246	5,6-Dehydrolupanine	0.43 ± 0.02	0.73 ± 0.05		0.56 ± 0.10		
1.10	248	Lupanine	9.85 ± 0.01	0.58 ± 0.04				
1.11	244	(not identified)		2.65 ± 0.04	5.14 ± 0.03	5.35 ± 0.02		0.82 ± 0.02
1.19	230	(not identified)	2.32 ± 0.02	4.57 ± 0.03		3.59 ± 0.03	13.72 ± 0.1	
1.30	244	Anagyrine/thermopsine	4.43 ± 0.05	4.97 ± 0.03	6.63 ± 0.05	5.03 ± 0.02	50.89 ± 0.02	
1.55	302	*N*-Acetoxyanagyrine		3.34 ± 0.04				

RRT, retention time relative to cytisine.
* mg/100 g of alkaloid fraction; average ± S.D., *n* = 3.

and/or *N*-methylcytisine. The leaves and roots of plants from Queretaro accumulated lupinine, with other alkaloids noted being minor constituents in comparison. The range of sparteine/lupanine-, bicyclic lupinine- and α-pyridone-type alkaloids identified in the Hidalgo plants and seeds of the Queretaro plants was generally similar to that reported in the literature for seeds and leaves of *C. secundiflora* collected from America but differ from the range of alkaloids found in seeds collected from plants in Pakistan in which sparteine- and lupinine-type alkaloids were not reported (Murakoshi *et al.*, 1986). In accumulating lupinine, the leaves and roots of plants from Queretaro represent another variation.

Several authors state that physiological events such as flowering, fruit growth and ripening can affect the formation and content of alkaloids (Mears and Mabry, 1971; Robinson, 1979; Waller and Nowacki (1978). In the present study it was found that the pericarp of immature pods from the Queretaro plants contained the same range of alkaloids as the mature seeds, but in the immature seeds only lupinine and sparteine were detected. Differences in the ecological conditions between the two sites may also account for the variation in alkaloid profiles, but this variation may also be controlled genetically. In order to investigate this further, plants from the two sites would need to be grown under the same environmental conditions.

Ohmiya *et al.* (1995) and Kinghorn and Balandrin (1983) reviewed the literature of the biological activity of several quinolizidine alkaloids, and that α-pyridone alkaloids such as cytisine and anagyrine are more acutely toxic than the corresponding saturated alkaloids such as sparteine and lupanine. Thus it might be anticipated that the leaves of the Hidalgo plants would be more toxic than those from Queretaro due to the greater abundance of α-pyridone alkaloids in the former. Certainly, the presence of quinolizidine alkaloids in *C. secundiflora* may explain why the plant is one of the dominant species in these heavily grazed habitats.

Hidalgo was the more disturbed of the two regions; its soil had a lower content of organic matter, nitrogen, and phosphorus. Intensive grazing was observed and there were large spaces with no vegetation. The vegetation comprised a smaller number of species, and *C. secundiflora* dominated. The leaves of *C. secundiflora* at Hidalgo accumulated a greater range of quinolizidine alkaloids than at Queretaro but the content of alkaloids in the leaves of the latter plants was higher.

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