Chemoecology16: 145-151(2006)
0937-7409/06/010145-7

DOI 10.1007/s00049-006-0340-1

CHEMOECOLOGY

Patterns of chemical defences in plants: an analysis of the vascular flora of Chile

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Summary. The plant apparency hypothesis predicts that apparent plants invest in broadly effective defences such as tannins while unapparent plants invest in specific toxins such as alkaloids. The stress hypothesis states that plants invest in cheaper defences if they have evolved in habitats that impose abiotic limitations to plant fitness. We tested these hypotheses by determining the concentrations of alkaloids and tannins in a representative sample of the vascular plants of continental Chile (with exclusion of Pteridophyta, Cactaceae, and Poaceae) consisting of 396 species. In a subsample of 166 species which contained both alkaloids and tannins, we constructed the A/T index (A/T = [alkaloids]/[tannins]). We discarded the presumed effect of phylogeny (as estimated by taxonomy) on the variation observed in the data because no correlation of A/T with taxonomic relationships among species either at family or genus levels was found in a nested ANOVA with genera nested in families. Concentration of alkaloids was negatively correlated with that of tannins. We compared the value of A/T among species differing in life form (herbs, shrubs or trees), herb longevity (annual or perennial), leaf-shedding manner of woody plants (deciduous or evergreen), latitudinal range, and level of water stress typical in their natural habitat. Unapparent plants (herbs, annual) exhibited higher mean A/T index than apparent plants (shrubs and trees, perennial). A/T did not correlate with latitudinal range. Mean A/T values decreased from deserts to deciduous forests. The comparisons were not always significant due to the inevitable unbalance of the data set which lowers the power of the statistical tests employed. The results suggest that chemical defences are indeed distributed in a non-random manner among plants, and that to a large extent the predictions derived from the apparency and stress hypotheses are sustained.

Key words. Herbivory – chemical defences – stress – plant apparency – co-evolution – native flora of Chile – alkaloid quantification

Introduction

Among the central concerns of studies dealing with the chemistry of plant-animal interactions is to elucidate the extent to which chemicals derived from secondary metabolism are differentially distributed among plants, the proximal and ultimate causes responsible for distribution patterns, and the ecological roles these chemicals play (Ehrlich & Raven 1964; Levin 1976, Silvertown & Dodd 1996; Hoy *et al*. 1998). Several hypotheses have addressed these issues. The "apparency hypothesis" (*sensu* Feeny 1976; Feeny 1991, Rhoades & Cates 1976) states that the risk of discovery by herbivores is the major factor influencing the extent of commitment by a plant to defence and also the type of defence accumulated by the plant. Thus, certain plant species which are more vulnerable to attack simply because they are more abundant, larger, or conspicuous in some way (apparent plants) will invest more in chemical defenses relative to less apparent plants. More specifically, apparent plant species will invest predominantly in broadly effective defences (quantitative defences, such as tannins) because they are exposed to a wide array of herbivores. In contrast, unapparent plants - which are likely to escape grazing – will invest comparatively less in chemical defences than apparent plants. Furthermore, they will employ relatively low concentrations of specific toxins (qualitative defences, such as alkaloids) against specialist herbivores which are able to discover them.

Another hypothesis, classically named the "stress hypothesis" (Price 1997) states that the evolution of chemical defences may also be modulated by the stress to which plants are exposed. In this case, stress is defined as any abiotic factor (e.g., amount of water or light) that constrains plant fitness. Thus, plants exposed to stressing habitats must invest energy to overcome stress and consequently they invest comparatively more in cheaper defences (e.g., alkaloids). The opposite pattern is expected in plants growing in habitats producing lower levels of stress: they invest energy in more expensive defences such as tannins. Cost in this context is defined in terms of size of the molecules and the dosage at which they are active against herbivores (Stamp 2003, for an experimental study see Ohnmeiss & Baldwin 2000).

A recent review which examined the most important hypotheses dealing with the evolution of chemical defences (e.g., optimal defence, carbon:nutrient balance, growth rate, and growth differentiation balance) (Stamp 2003) detected a confusion about the clarity and testability of these hypotheses and consequently about the existence of a tangible and mature theory of plant defence (Berenbaum 1995,

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Hartley & Jones 1997, Stamp 2003, 2004). A remarkable feature of these hypotheses is that they are hierarchically organised, *i.e.* there are sub-hypotheses nested in higher order hypotheses. This fact is not always recognised by researchers and obscures the level of analysis of studies (genetic/phenotypic, local/regional, within/between species) and therefore the specific underlying mechanisms responsible for the defensive patterns observed in plants (Stamp 2003). For example, the apparency and stress hypotheses are sub-hypotheses derived from a more general one, the optimal defence hypothesis (Ohnmeiss & Baldwin 2000; Stamp 2003). This hypothesis, based in turn on the resource allocation principle, justifies theoretically the examination of a trade-off between qualitative and quantitative chemical defences. If plants are faced with the need to invest in qualitative or quantitative defences, natural selection will favor those individuals which optimise the amounts of such defences, *i.e.* alkaloids and tannins, under conditions of resource limitation (Zangerl & Bazzaz 1992). Consequently, a negative correlation is expected between the concentration of alkaloids and the concentration of tannins in plants (for a general treatment of evolutionary trade-offs in organisms, see Roff 1992).

The basic assertion that quantitative defences (tannins) are expensive relative to qualitative defences (alkaloids) lies at the heart of the apparency and stress hypotheses. This fact is not always recognised in studies of chemical defences in plants. Recent investigations have unraveled that the benefits and costs to produce chemical defences is a more complex issue than originally thought. In fact, the costs and benefits of chemical defences need to be evaluated in the context of plastic responses of plants such as defence induction, plant tolerance, within plant defence mobilisation, plant compensation (McKey 1979, Coley *et al*. 1985) and environmental variability (Hartley & Jones 1997). Recognising that these processes are crucial to the understanding of plant herbivory, they apply perfectly to individual and population levels and ought to be considered at this scale. However, for large scale studies, *i.e.* community or biome/flora level (the original focus of these hypotheses), they become impractical to address. Here, the use of the dichotomy expensive (tannins) – cheap (alkaloid) chemical defences as suggested by the apparency and stress hypotheses, is more appropriate because of its conceptual simplicity and because it allows a rapid assessment of a large number of species.

In order to test the apparency and the stress hypotheses, most research has analysed taxonomic groups (families) by evaluating the frequency of species (nested within families) that produce (or not) tannins or alkaloids (Levin 1976; Silvertown & Dodd 1996). Relatively few studies have tested these hypotheses by simultaneously quantifying the concentrations of tannins and alkaloids (Gartlan *et al*. 1980). Such an integrative approach is justified in the context of the optimal defence hypothesis. In this study, we tested the apparency and the stress hypotheses using a representative sample of the vascular native flora of continental Chile (*i.e.*, the distribution of studied species within families or genera did not differ from the distribution of species within families or genera belonging to the whole flora of continental Chile, excluding Pteridophyta, Cactaceae and Poaceae) (family level: $X^2 = 1.45$, df = 165, $P > 0.9$; genus level: $X^2 = 1.86$, df = 835, $P > 0.9$). First, we quantified qualitative and quantitative chemical defences (alkaloids and tannins, respectively), and explored their distribution among taxa and life forms. We then defined, in a subset of species which contained both alkaloids (A) and tannins (T), the ratio A/T ([alkaloids]/[tannins]); the use of this algorithm is justified because of the presumed existence of a trade-off between qualitative and quantitative defences. Then, we explored whether A, T and A/T values for species (nested within genera and within family) constituted true replicates, *i.e.*, whether the phylogenetic history of species affected the variation of these attributes among plant species. Then, we tested the existence of a trade-off between the concentrations of alkaloids and tannins. Finally, we tested the apparency and the stress hypotheses by addressing the following predictions: (i) A/T is lowest in trees, followed by shrubs and then by herbs; the rationale is that there exists an apparency gradient from trees (more apparent) to herbs (less apparent); (ii) A/T is lower in perennial than in annual herbs; the rationale is that perennials are more apparent than annuals; (iii) A/T is lower in evergreen than in deciduous plants; the rationale is that plants which loose their leaves synchronously are less apparent than evergreen plants, even if these latter are continuously shedding their leaves; (iv) there exists a negative correlation between A/T and the latitudinal range of species estimated from herbarium data and regional floras, the rationale is that plants with a broader latitudinal range are more apparent than those with a narrower latitudinal range; and (v) A/T is higher in plants living in stressing (more xeric) habitats; the rationale is that in these environments plants are constrained to invest relatively more in cheaper chemical defences.

Materials and methods

Plant material.

Aerial parts of herbs and young twigs of shrubs and trees were collected unpremeditatedly in six of the eight vegetational zones of Chile (Gajardo 1994), except for the exclusion of Pteridophyta, Cactaceae, and Poaceae. The number of individuals sampled ranged from *ca.* five in the case of trees, to *ca.* 100 in the case of small herbs, depending on availability. Material collected from each species was pooled before chemical analyses. The samples were air-dried in the shade, ground, and stored in plastic containers at 15 °C ± 5 °C. Voucher specimens were lodged at herbaria at the Universidad de Concepción, Museo Nacional de Historia Natural, or Universidad de Chile. Information on the herbarium sheets included the date of collection, precise details of the locality at which the plants were found, and the name of the person identifying the material. Chemical analyses were performed on 396 species belonging to 200 genera and 76 families.

Chemical assay of alkaloids: validation of methodology. Although a large number of families of plant secondary metabolites may be classified as qualitative defences (Rosenthal & Berenbaum 1991), alkaloids are one of the most ubiquitous and best studied examples (Cordell 2003), and thus constitute a good proxy for qualitative defences. A common method to detect alkaloids employs the Dragendorff reagent, and large scale studies have

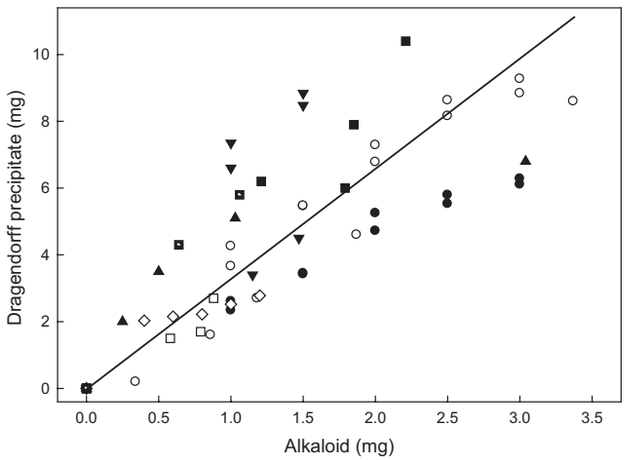

Fig. 1 Amount of Dragendorff precipitate obtained by applying the analytical method described to different alkaloids. Symbols: • = tomatidine (glycoalkaloid aglucone), ○ = tomatine (glycoalkaloid), ▼ = tropinone (tropane alkaloid), ◊ = demisidine (glycoalkaloid aglucone), □ = ergonovine (ergot alkaloid), ■ = tropine (tropane alkaloid), ▲ = boldine (benzylisoquinoline alkaloid), ◘ = isotetrandine (*bis*-benzylisoquinoline alkaloid)

demonstrated the usefulness of the reagent in predicting alkaloid presence or absence in plants (Raffauf 1996). This reagent occasionally gives false positives and negatives in thin layer chromatography (TLC) tests (Sthal 1965; Anderson et al. 1977). In the method described below, we incorporated an initial acidic extraction of plant material to reduce the chances of interferences, and have used the reagent as an alkaloid precipitation agent. The validity of the method for alkaloid quantification (see details below) was assessed in three ways: i) by testing its selectivity, ii) by applying it to different amounts of pure alkaloids belonging to different structural families, and iii) by examining its reproducibility. Scopoletin, a coumarin giving positive Dragendorff test in TLC, did not produce a precipitate, while senecionine, a pyrrolizidine alkaloid giving a weak Dragendorff colour reaction and boldine, an isoquinolinic alkaloid giving a good Dragendorff colour reaction, did. On the other hand, the data from the quantification of pure alkaloids generated a highly significant correlation between amount of pure alkaloid and amount of Dragendorff precipitate [Fig. 1; regression model: Precipitate (mg) = (3.37 ± 0.18; average ± 1 s.e.) * Alkaloid (mg), r = 0.74, $F_{1, 50} = 73.93$, $P < 0.001$]. Thus, the amount of precipitate is able to accurately predict the amount of alkaloid present, independent of the structure of the alkaloid. The reproducibility of the method was tested by subjecting 15 replicates of each of three species showing different alkaloid levels (*Cistanthe grandiflora*, 0.15 mg/g dry tissue; *Solanum maritimum*, 0.51; *Nicotiana undulata*, 1.30), to the quantification procedure. The standard error of the determinations was less than 3% in each of the three cases.

Chemical assay of alkaloids: methodology. Two grams of dry plant material were extracted with 100 ml of 4N HCl at room temperature for one hour. Ten drops of Dragendorff reagent (Wagner *et al*. 1984) were added and after 3 min the mixture was centrifuged for 3 min at 10,000 rpm. The solution was withdrawn with a Pasteur pipette, and the precipitate (if it occurred) was dried and weighed. A further aliquot of Dragendorff reagent was added to the solution, and the mixture again separated into precipitate (which was dried and weighed) and solution. The process was repeated until no further precipitation was observed upon addition of Dragendorff reagent. The combined weight of the precipitates was used to predict the amount of alkaloids from the regression line of figure 1.

The Dragendorff reagent was prepared by independently dissolving 8 g $Bi(NO_3)_3$. $5H_2O$ in 20 ml 30 % (w/v) HNO_3, and 27.2 g KI in 50 ml H_2O. These solutions were stored separately in amber glass bottles at 4 °C and mixed in equal volumes just before use.

Chemical assay of tannins. Precipitation of bovin serum albumin was used to quantify tannins. In spite of the wide structural variety of tannins (Hagerman & Butler 1991), the dependency of stoichiometry of tannin-protein adducts on tannin and protein structure, and the dependency of protein precipitation on environmental variables such as pH, temperature, solvent, concentrations, etc. (Hagerman *et al*. 1998), when screening for tannins a large number of taxonomically unrelated plants, exhaustive chemical analysis of each extract is impracticable (Mole & Waterman 1987a). The use of a common methodology for analyses is justified in broad surveys, particularly given that different analytical procedures for quantifying tannins have been found to yield ecologically useful information (Mole & Waterman 1987b).

Dry plant material (0.5 g) was defatted by extracting it with ether for 15 min. Methanol (10 ml) was added to the defatted extract. The suspension was homogenised for 1 min with an Ultraturrax high speed blender (Janke & Kunkel, Ika-Werk) and allowed to stand for 15 min; thereafter, it was centrifuged for 5 min at 10,000 rpm. To 1 ml of the supernatant, 2 ml of bovine seroalbumin (BSA) solution (1 mg/ml of 0.2 M acetate buffer pH 5 with 0.17 M NaCl) were added and allowed to stand for 15 min. The suspension was centrifuged for 15 min at 5,000 rpm. To the precipitate, 4 ml of sodium dodecylsulphate solution (1% SDS and 5% triethanolamine in H_2O), and 1 ml of $FeCl_3$ reagent (0.01 M in 0.01 N HCl) were added (Hagerman & Butler 1978). After 20 min, the absorbance of the solution was read at 520 nm. The absorbance of the samples was compared with a standard curve constructed by dissolving different amounts of tannic acid (in the 0 to 6 mg range) in 1 ml methanol, adding 2 ml BSA solution, and subjecting the resulting suspension to the procedure above [calibration curve: A_{520}= (0.983 ± 0.082) Tannic acid (mg), N = 6, $r^2 = 0.959$]. Thus, the amount of tannin reported corresponds to tannic acid equivalents. The reproducibility of the method was tested by subjecting 15 replicates of each of three species showing different tannin levels (*Gnaphalium viravira*, 0.12 mg/g dry tissue; *Maytenus boaria*, 5.47; *Escallonia illinita*, 16.9), to the quantification procedure. The standard error of the determinations was less than 3% in each of the three cases.

Analysis of data. To test for possible phylogenetic effects on A, T and A/T at family and at genus levels, a nested ANOVA was performed (Sokal & Rohlf 1995). Families analysed contained at least two genera, each of which contained at least two species; hence, a sub-sample of 134 species in 8 families and 33 genera could be used for alkaloids and tannins. For testing the apparency and stress hypotheses the response variable chosen was the index A/T, defined as the ratio between the concentrations (w/w) of alkaloids and tannins. Two-hundred-thirty species were excluded because they did not contain both alkaloids and tannins, leaving 166 for these analyses. When nested ANOVAs were performed on this

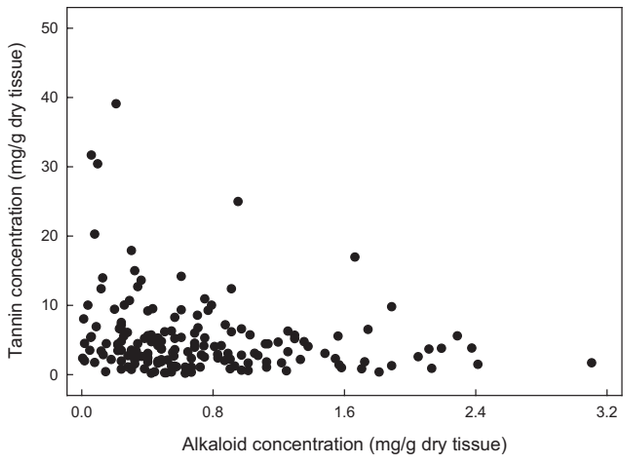

Fig. 2 Correlation between concentrations of tannins and alkaloids in 166 species of the vascular flora of continental Chile which contained both alkaloids and tannins

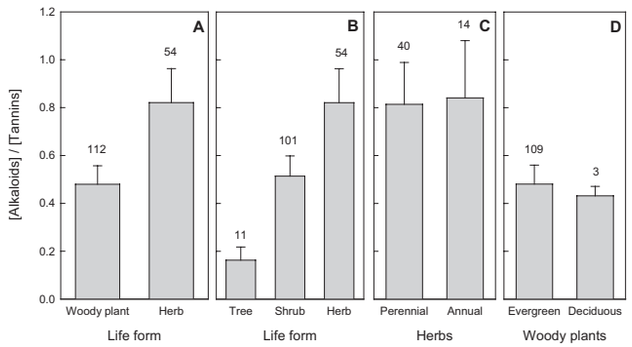

Fig. 3 Mean A/T index ([Alkaloids] / [Tannins]) for species differing in life form (A and B), herbs differing in longevity (C), and woody plants differing in leaf shedding manner (D). Number of species analysed are given above the bars

data, a sub-set of only 59 species in 5 families and 15 genera could be used.

Predictions concerning A/T values derived from the apparency and stress hypotheses were evaluated using Kruskall Wallis ANOVA for ranks followed by Dunn's *a posteriori* tests (SigmaStat 3.1). The morphological attributes utilised to test the apparency hypothesis were life form, plant longevity, leaf-shedding manner of plants, and species latitudinal range. For the stress hypothesis, plant species used for comparison grew in biomes that clearly differed in precipitation levels (a proxy of water stress) as shown by differences in the range of mean annual precipitation, *i.e.*, desert and high Andean steppes (< 200 mm), matorral and sclerophyllous forests (200 – 1000 mm), deciduous forests (1000 – 2000 mm), and evergreen forests (> 2000 mm) (di Castri and Hajek 1976; Gajardo 1994; Benítez 1994).

Results

Alkaloid concentration ranged from 0 to 3 mg/g dry tissue (mean ± 1 s.e. = 0.71 ± 0.04), and tannin concentration from 0 to 39 mg/g dry tissue (mean ± 1 s.e. = 4.15 ± 0.42). One hundred and eighty species contained tannins but lacked alkaloids, 27 lacked both tannins and alkaloids, 23 lacked tannins and contained alkaloids, and 166 species contained both tannins and alkaloids. When the complete set of 396 species were grouped by life form, tannins were found in 100 % of trees, 95 % of shrubs, and 73 % of herbs. On the other hand, alkaloids were found in 27 % of trees, 51 % of shrubs, and 49 % of herbs.

Taxonomic differences in alkaloid and tannin content were detected among families (nested ANOVA, $F = 3.10$, $P = 0.005$ and $F = 3.4$, $P = 0.003$ for alkaloids and tannins, respectively) but not among genera (nested ANOVA, $F = 1.27$, $P = 0.20$ and $F = 1.03$, $P = 0.43$ for alkaloids and tannins, respectively). While alkaloids were found in every species studied in the typical alkaloid-containing families, *i.e.* Rubiaceae, Solanaceae, Iridaceae, Valerianaceae, Amaryllidaceae, Aristolochiaceae, and Berberidaceae (Frohne and Jensen 1998), tannins were found in every species studied in families containing mostly or exclusively woody species, such as Anacardiaceae, Ephedraceae, Fagaceae, Escalloniaceae, Ledocarpaceae, Monimiaceae, Podocarpaceae, and Sapindaceae (Zomlefer 1994). The large variation in the number of species in each genus might have determined an unbalanced statistical design and hence a low statistical power which prevented from finding genus effects, if they existed.

For the ratio A/T no differences were detected either among genera (nested ANOVA, $F = 1.42$, $P = 0.21$) or among families (nested ANOVA, $F = 0.93$, $P = 0.40$). Thus, for testing the apparency and stress hypotheses we used only the ratio A/T as this algoritm was not affected by phylogeny and hence is amenable to test adaptive hypotheses.

A negative and significant correlation was detected between the concentration of tannins and the concentration of alkaloids ($r_S = -0.21$, $P = 0.006$; Fig. 2), thus corroborating the occurrence of a trade-off between qualitative and quantitative chemical defences and justifying empirically the use of the ratio A/T to test the apparency and stress hypotheses.

Significant differences were detected in A/T between woody species and herbs ($T = 7.154$, $P = 0.007$, Fig. 3A), and among life forms ($H = 9.976$, d.f. = 2, $P = 0.007$, Fig. 3B). Mean A/T values were highest for herbs and lowest for trees. However, while herbs showed significantly higher A/T than trees (*a posteriori* test, $P < 0.05$), shrubs did not differ significantly from herbs ($P > 0.05$) nor from trees ($P > 0.05$) (Fig. 3B). Although the mean value of A/T for annual herbs was higher than for perennial herbs, the difference was not statistically significant ($H = 0.224$, $P = 0.64$, Fig. 3C). Finally, the difference in mean A/T value between deciduous and evergreen trees and shrubs was not statistically significant ($H = 1.111$, $P = 0.29$, Fig. 3D). The lack of statistical significance of these comparisons may be attributed to an unbalanced statistical design (see N values in Fig. 3), which leads to a low power of the statistical test (Sokal & Rohlf 1998).

A/T was not affected by the latitudinal range of plant ($r_S = 0.02$, $P = 0.82$) (Fig. 4). Furthermore, although mean A/T values decreased from deserts up to biomes with mean

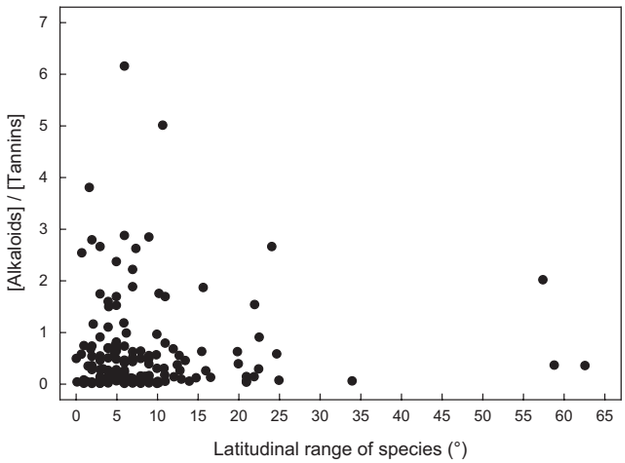

Fig. 4 Correlation between A/T index ([Alkaloids] / [Tannins]) and latitudinal range of species

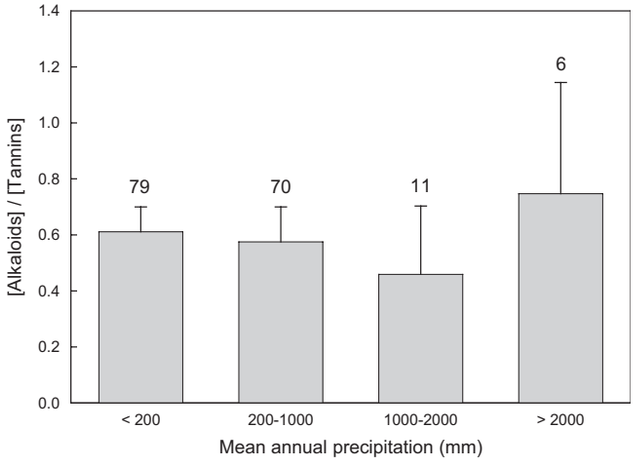

Fig. 5 Mean A/T index ([Alkaloids] / [Tannins]) for species growing in biomes differing in water stress levels. Number of species analysed are given above the bars

annual precipitation less than 2000 mm, differences were not statistically significant (H= 2.591, d.f. = 3, P = 0.46, Fig. 5).

Discussion

In order to test evolutionary hypotheses (such as the apparency and stress hypotheses) chemical defences must be compared among plant species. However, across-species comparisons have to be corrected in order to be made independent of phylogeny (Felsenstein 1985; Harvey & Pagel 1991; Silvertown & Dodd 1996). One approximation to this problem is to use taxonomy in a nested ANOVA (Read & Harvey 1989; Harvey & Pagel 1991). This method allows the evaluation of differences among taxonomic levels and also the partition of the total variance of data into components representing each of the nested levels in a taxonomy. If statistical differences among lineages are detected (at some taxonomic level), then caution must be exerted in interpreting the data in adaptive terms. On the contrary, if differences among lineages are not detected, then the phylogenetic history should not be important and one can be confident that the observed variation among species is adaptive. Results from the present study suggest that only the ratio A/T is not constrained by phylogenetic effects either at genus or family levels; hence, each A/T chemical datum obtained for a given species may be considered an independent replicate, and adaptive explanations are appropriately tested with this kind of information. This independence (or invariance) of A/T from phylogeny is a direct consequence of the trade-off between alkaloids and tannin concentration, which in turn is the basis for testing the apparency and stress hypotheses (Edwards 1989). It should be noted that a large proportion of plant species showed low amounts both of alkaloids and tannins (Fig. 2), suggesting that these species may produce other types of chemical defences or defences other than chemical, *i.e.*, morphological or physiological ones (Kennedy & Barbour 1992). Despite these particular plant responses, it is notable that there exists no species that invested in high quantities of alkaloids and tannins simultaneously.

As predicted by the apparency hypothesis, herbs expressed higher values of A/T relative to shrubs, *i.e.* herbs allocated proportionally more resources to alkaloids than to tannins, as expected from their non-apparency. Trees showed mean A/T values lower than shrubs and herbs, as expected from being the most apparent plants (Fig. 3B); however, the differences were not statistically significant, probably as a consequence of an unbalanced statistical design which led to a low statistical power of the test (Sokal & Rohlf 1998). An increase in the number of trees analysed seems to be the only way to surmount this difficulty; however, it is clear that the design will remain asymmetrical given that in the flora of Chile the number of tree species is considerably lower than the number of shrubs and herbs (a survey of the mediterranean flora of central Chile comprising ca. 60% of all native species of Chile showed the existence of 70 tree species, 426 shrub species, and 1894 herb species) (Kalin Arroyo *et al*. 1995). Similarly, although mean A/T values were lower in perennial than in annual herbs as expected from the apparency hypothesis, the difference was not significant, most likely due to an unbalanced statistical design (Fig. 3C).

The correlation between A/T and latitudinal range of the species was not statistically significant. This may be attributed to the fact that more than 80 % of species with a narrow latitudinal distribution (0 ° to 10° latitudinal range) were collected from 18 to 40 ° S, a Mediterranean area highly heterogeneous in time and space, in which plants are exposed to a wide diversity of habitat and herbivore pressures (Espinoza & Hajek 1988; Fuentes & Etchegaray 1983). Thus, a serious limitation emerges from this kind of studies: what is the appropriate spatial scale of analysis? Ideally, this scale should be such that the herbivore assemblage does not vary and plants studied are exposed to similar herbivore and abiotic pressures. Unfortunately, data on herbivores interacting with plants in Chile is very scarce.

Allocation to chemical compounds by plants may be related to factors other than herbivores, for instance, physical stress (Hoy *et al*. 1998). The putative effect of water stress on patterns of allocation of defences was tested, and although mean A/T decreased from deserts up to biomes with mean annual precipitation less than 2000 mm (as expected from the stress hypothesis) the differences were not statistically significant. Moreover, mean A/T was highest in the biome with mean annual precipitation higher than 2000 mm (Fig. 5). Again, an unbalanced data design (low number of species studied from evergreen forests) may be behind this lack of effects.

The apparency and stress hypotheses are subject to a suite of unresolved conceptual problems (Hartley & Jones 1997; Stamp 2003). For instance, over the course of evolution, there have been changes in the functionality of chemical defences (Close & McArthur 2002), *i.e.* the same compounds can assume a variety of roles and thus obscure the causal relationships to explain the evolution of chemical compounds in plants. Despite these shortcomings, these hypotheses have made important contributions to the understanding of the evolution of plant defences and they have also stimulated the development of alternative hypotheses that have emphasised other aspects such as plant growth, physiology, and environment instead of the eventual discovery of plants by herbivores.

In summary, our results suggest that chemical defences are indeed distributed in a non-random manner among plants. Higher A/T values observed in less apparent species and lower values observed in species with a wider latitudinal range conform to the predictions derived from the apparency hypothesis. Furthermore, comparisons of A/T values between woody plants and herbs, herbs of different longevity, and woody plants with different leaf shedding manners, and between plants subjected to different levels of stress showed the tendencies expected from the apparency and stress hypotheses, respectively; however, some of the comparisons were obscured by the low power of statistical tests used in the analysis. This problem can not be resolved as the data base used in this study reflects the distribution of life forms found in the Chilean flora, *i.e.* tree species are less abundant compared with shrubs and herbs. Thus, although the apparency and the stress hypotheses constitute a good starting point to explain the distribution and abundance of chemical defences in an ample set of species, additional studies will be needed to unravel the ecological and historical origin of patterns of chemical defences in plants at the community level.

Acknowledgements

We thank Mrs. Paola Bravo and Ms. Alejandra Mendizával for chemical analyses, and Dr. Alejandro Urzúa for helpful chemical advice. We also thank two anonymous reviewers of the original manuscript for their generous constructive suggestions. The financial support of Cátedra Presidencial en Ciencias awarded to HMN is gratefully acknowledged.

References

Anderson LA, Doggett NS Ross MSF (1977) The non-specificity of Dragendorff reagent in thin layer chromatography. Planta Medica 32: 125 –129

Benítez A (1994) Características climáticas y geológicas de Chile. Pp 19-37 *in* Espinoza G, Pisan P & Contreras L (eds) Perfil Ambiental de Chile. Comisión Nacional del Medio Ambiente. Chile-Santiago: Alfabeta Impresores

Berenbaum MR (1995) The chemistry of defense: theory and practice. Proc Nat Acad Sci USA 92: 2 –8

Close DC, McArthur M (2002) Rethinking the role of many plant phenolics – protection from photodamage not herbivores? Oikos 99: 166 –172

Coley PD, JP Bryant, Chapin III FS (1985) Resource availability and plant anti-herbivore defense. Science 230: 895 –899

Cordell GA (2003) The Alkaloids: Chemistry and Biology. Vol. 61. New York: Academic Press

di Castri F, Hajek ER (1976) Bioclimatología de Chile. Imprenta-Editorial de la Universidad Católica de Chile. Santiago. 129 pp

Edwards PJ (1989) Insect herbivory and plant defense theory. Pp 275 – 297 *in* Grubb PJ, Whittaker JB (eds) Toward a More Exact Ecology. The 30th Symposium of the British Ecological Society London 1988. GB-Cambridge: University Press

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in plant coevolution. Evolution 18: 586 – 608

Espinoza GA, Hajek ER (1988) Riesgos climáticos: evidencias en Chile Central. Pp 41-52 *in* Fuentes E, Penafreta S (eds) Ecología del Paisaje en Chile Central. Estudios Sobre sus Espacios Montañosos. Chile-Santiago: Ediciones Universidad Católica de Chile

Feeny P (1976) Plant apparency and chemical defense. Rec Adv Phytochem 10: 1 –41

Feeny P (1991) The evolution of chemical ecology: contributions from the study of herbivorous insects. Pp 1 – 44 *in* Rosenthal GA, Berenbaum MR (eds) Herbivores: Their Interactions with Secondary Plant Metabolites, Volume II: Evolutionary and Ecological Processes. Second edition. London: Academic Press

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125: 1–15

Frohne D, Jensen U (1998) Systematik des Pflanzenreichs: unter besonderer Berücksichtigung chemischer nerkmale und pflanzicher Droge. Wissenschaftliche Verlagsgesselschaft mbH. Stuttgart, Germany

Fuentes E, Etchegaray J (1983) Defoliation patterns in matorral ecosystems. Pp 525–542 *in* Krug F, Mitchell D, Jarvis J (eds) Mediterranean-Type Ecosystems: the Role of Nutrients. Berlin: Springer-Verlag

Gajardo R (1994) La Vegetación Nativa de Chile. Clasificación y Biogeografía. Chile-Santiago: Editorial Universitaria

Gartlan JS, McKey DB, Waterman PG, Mbi CN, Struhsaker TT (1980) A comparative study of the phytochemistry of two African rain forests. Biochem Syst Ecol 8: 401–422

Hagerman AE, Butler LG (1978) Protein precipitation method for the quantitative determination of tannins. J Agric Food Chem 26: 809 –812

Hagerman AE, Butler LG (1991) Tannins and Lignins. Pp. 355-388 *in* G.A. Rosenthal GA, Berenbaum MR (eds) Herbivores.

Their Interactions with Secondary Plant Metabolites, Volume I, The Chemical Participants. Second edition. London: Academic Press

Hagerman AE, Rice ME, Ritchard NT (1998) Mechanisms of protein precipitation for two tannins, pentagalloyl glucose and epichatechin$_{16}$ (4→ 8) catechin (procyanidin). J Agric Food Chem 46: 2590–2595

Hartley SE, Jones CG (1997) Plant chemistry and herbivory or why the world is green. Pp. 284 – 324 *in* Crawley MJ (ed) Plant Ecology. Second Edition. Backwell Science Ltd: Oxford, England

Harvey PH, Pagel MD (1991) The Comparative Method in Evolutionary Biology. New York: Oxford University Press

Hoy CW, Head GP, Hall FR (1998) Spatial heterogeneity and insect adaptation to toxins. Ann Rev Entomol 43: 571 –594

Kalin Arroyo M, Cavieres L, Marticorena C, Muñoz-Schick M (1995) Convergence of the mediterranean floras in central Chile and California: insights from comparative biogeography. Pp. 47 – 88 in Kalin Arroyo M, Zedler PH, Fox MD (eds) Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia. New York, Berlin, Heidelberg: Springer-Verlag

Kennedy GG, Barbour JD (1992) Resistance variation in natural and managed systems. Pp 13 – 41 *in* Fritz RS, Simms EL (eds) Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics. Chicago and London: The University Chicago Press

Levin DA (1976) Alkaloid-bearing plants: an ecogeographical perspective. Am Nat 110: 261 –284

McKey D (1979) The distribution of secondary compounds within plants. Pp. 55 – 133 *in* Rosenthal GA and DH Janzen (eds) Herbivores: Their Interactions With Secondary Plant Metabolites. New York: Academic Press

Mole S, Waterman PG (1987a) A critical analysis of techniques for measuring tannins in ecological studies. I. Techniques for chemically defining tannins. Oecologia 72: 137–147

Mole S, Waterman PG (1987b) A critical analysis of techniques for measuring tannins in ecological studies. II. Techniques for biochemically defining tannins. Oecologia 72: 148–156

Ohnmeiss TE, Baldwin IT (2000) Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology 81: 1765–1783

Price PW (1997) Insect ecology. Third Edition. New York: John Wiley & Sons Inc

Raffauf RF (1996) Plant Alkaloids: A Guide to Their Discovery and Distribution. Binghampton: Products Press

Read AF, Harvey PH (1989) Life history differences among the eutherian radiation. J Zool 219: 329–353

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. Rec Adv Phytochem 10: 168–213

Roff DA (1992) The Evolution of Life Histories. Theory and Analysis. New York: Chapman & Hall

Rosenthal GA, Berenbaum MR (1991) Herbivores: Their Interactions with Secondary Plant Metabolites, Volume II: Evolutionary and Ecological Processes. Second edition. London: Academic Press

Silvertown J, Dodd M (1996) Comparing plants and connecting traits. Phil. Trans. R. Soc. London 351: 1233–1239

Sokal RR, Rohlf FL (1995) Biometry. Third Edition. New York: Freeman, W.H. & Company

Stamp N (2003) Out of the quagmire of plants defense hypotheses. Quart Rev Biol 78: 23–55

Stamp N (2004) Can the growth-differentiation balance hypothesis be tested rigorously? Oikos 107: 439–448

Sthal E (1965) Thin Layer Chromatography: A Laboratory Handbook. New York: Academic Press

Wagner H, Bladt S, Zgainski EM (1984) Plant Drug Analysis. New York: Springer-Verlag

Zangerl AR, Bazzaz FA (1992) Theory and pattern in plant defense allocation. Pp 363-391 *in* Fritz RS, Simms EL (eds) Plant Resistance to Herbivores and Pathogens. Ecology, Evolution and Genetics. Chicago and London: The University of Chicago Press

Zomlefer WB (1994) Guide to Flowering Plant Families. Chapel Hill and London: The University of North Carolina Press

Received 7 October 2005; accepted April 13, 2006.
Published Online First 14 July 2006.