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GENDER DIMORPHISM AND ALTITUDINAL VARIATION OF SECONDARY COMPOUNDS IN LEAVES OF THE GYNODIOECIOUS SHRUB *Daphne laureola*

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Abstract—In this article, we analyzed the concentration of coumarins in leaves of female and hermaphrodite individuals of the gynodioecious shrub *Daphne laureola*, along an elevational gradient in southern Spain. Combining HPLC and NMR techniques, we identified three different glycosides of 7-methoxycoumarin in leaves of this species. Total coumarin concentration averaged between 60 and 120 mg/g dry weight for mature summer leaves of *D. laureola* growing at six different populations. As predicted by optimal theory, females tended to have a higher concentration of coumarins than hermaphrodites, thus upholding the idea that male reproductive function is costly for hermaphrodites. Furthermore, concentrations in females but not hermaphrodites were positively correlated with increasing population altitude, and the magnitude of gender divergence in coumarin concentration varied among populations, suggesting that the cost of the male function may be context dependent. To our knowledge, this is the first evidence of gender differences in chemical defenses of a gynodioecious species in the field.

Key Words—Coumarins, *Daphne laureola*, elevation, gynodioecy, plant–animal interactions, plant defense.

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INTRODUCTION

In this study, we analyze the relationship between allocation to reproduction and chemical defenses, and its natural variation along an altitudinal gradient, in a long-lived perennial shrub. According to optimal theory, organisms allocate resources to survival, growth, and reproduction, such that their fitness will be maximized (Maynard-Smith, 1978). Plant defenses aim to decrease pathogen and herbivore attacks that may eventually reduce plant survival, growth, and reproduction and, thus, plant allocation to defenses can also be interpreted within optimal theory (Herms and Mattson, 1992). Within this framework, trade-offs (or opportunity costs) between growth, defense, and reproduction should exist whenever resources are limited. However, field measurements of trade-offs and other physiological costs in plant allocation to reproduction and defenses are not straightforward (see Obeso, 2002; Cipollini et al., 2003 for a review of each topic, respectively). Gender dimorphic species split the range of individual variance in reproductive allocation into categories, thus facilitating the assessment of consequences in terms of survival, growth, and defense of a higher reproductive allocation under natural conditions.

In dioecious species, allocation to reproduction in males is commonly higher than in females at flowering, though the opposite is true at fruiting time when the sink of resources to reproduction is maximum (Delph, 1990). Therefore, both the nature and magnitude of differences between genders in resource allocation may change with time (Ågren et al., 1999). Overall, herbivores usually distinguish and prefer males over females of dioecious plant species (Ågren et al., 1999, and references therein) suggesting that differences in reproductive allocation modify other plant features that in turn decrease quality of females as food for herbivores (e.g., Boecklen et al., 1990; Jing and Coley, 1990). Gynodioecy consists of populations having both hermaphrodite and female pollenless individuals. Thus, gender difference in allocation to reproduction is in principle lower and due to the male function only. The costs of male function are apparently more subtle, but still relevant (Eckhart and Seger, 1999), and there is also evidence for male-biased flower and seed predation favoring the maintenance of females in gynodioecious populations (Uno, 1982; Marshall and Ganders, 2001; Collin et al., 2002; reviewed in Ashman, 2002). To our knowledge there are no data available comparing physical or chemical defenses in gynodioecious species (but see Gouyon and Vernet, 1980) and, thus, whether gender differential consumption is mediated through distinct defenses or otherwise, for example through differential attractive properties, is unknown.

We explore the relationship between allocation to reproduction and defense in a gynodioecious species. Hermaphrodite and female *Daphne laureola* plants produce a similar number of flowers and fruits, and fruit size is also similar between genders (Alonso and Herrera, 2001). However, female flowers are smaller and pollenless, thus leading to a lower allocation to reproduction of female individuals. In southern Spain, *D. laureola* is consumed by several species of Noctuid caterpillars. Defoliation has been related to plant architecture (Alonso and Herrera, 1996) and leaf nutrient composition (Alonso and Herrera, 2003), and caterpillars are able to distinguish among several plant structures (Alonso and Herrera, 2000). The role of allelochemicals in this plant-herbivore interaction remained unstudied. Coumarins are effective chemical defenses against herbivores in other plant species (Berenbaum, 2001 and references therein), and were known to exist in the *Daphne* genus (Hegnauer, 1973; Ulubelen et al., 1986; Zobel and Brown, 1988). Our expectation was that, if the increased reproductive allocation associated with the male function were costly, *D. laureola* females should have a higher concentration of coumarins than hermaphrodite conspecifics. Thus, we evaluated the concentration of the three most abundant coumarin glycosides found in leaves of female and hermaphrodite *D. laureola* individuals.

Furthermore, since the natural concentration of chemical defenses may vary geographically (Johnson and Scriber, 1994), the study was conducted in six different populations selected along an altitudinal gradient, that ranged from 950 to 1800 m asl. Increased exposure to UV radiation and low temperatures at higher altitudes may select for different chemical profiles in plants that in turn can affect herbivores (Johnson and Scriber, 1994; Stratmann, 2003). In particular, contents of UV-B absorbing compounds tend to increase at higher elevation sites in several plant species (Rozema et al., 1997). Coumarins are able to absorb UV radiation (Murray et al., 1982). Thus, we also expected an increase of coumarin concentrations in plants at higher elevation sites.

METHODS AND MATERIALS

Plant Species and Study Area. Daphne laureola L. (Thymelaeaceae) is a long-lived evergreen shrub distributed throughout the Palearctic region and generally found in the understory of coniferous and mixed montane forests in the Mediterranean area. In the Natural Park of Sierras de Cazorla, Segura y Las Villas (Jaén province, south-eastern Spain), where this study was conducted, the species is gynodioecious, and the proportion of female plants varies with site altitude (Alonso and Herrera, 2001).

In June 2002, we collected undamaged leaves from female and hermaphrodite individuals in six different populations comprising the entire altitudinal range of the species at our study area. Study locations were Coto del Valle (950 m elevation), Roblehondo (1235 m), Cañada del Espino (1575 m), Nava de las Correhuelas (1615 m), Cabeza del Tejo (1640 m), and Puerto Llano (1800 m), hereafter referred to as CV, RH, CÑE, NC, CT, and PLL, respectively. Aiming to have three replicates for each combination of gender per population, leaves of each individual ($N = 7$–20) were collected independently and later split into three different sets. Each replicate had 8–17 g fresh mass. Leaves were collected when plants were bearing mature fruits and all plants were at the same phenological stage despite the fact that the different populations were collected on different dates due to altitudinal variation in plant phenology.

Chemical Analyses. Leaves were washed, dried with filter paper, and stored at −80°C prior to analysis. Frozen samples were weighed, deep-frozen with liquid nitrogen and ground in a coffee mill. As an internal control, 1.5 mg of esculetin (6,7 dihydroxy-coumarin, Aldrich) were added to each sample to evaluate potential process errors. Leaf powder was extracted 2 × 24 hr with methanol (80%), and the combined extract was filtered and concentrated to dryness under reduced pressure. The residue was dissolved in water and cleaned by sequential decantation with chloroform. Coumarins were detected as pale-blue spots on C_{18}-TLC (Alugram® RP-18W/UV_{254}) only in the aqueous phase, that was subsequently concentrated to dryness under reduced pressure to record the weight of the final residue. The residue was suspended in 15 mg of double-distilled water to obtain a 10% concentration of the internal control, esculetin. Three 750 μl aliquots were taken from this solution and centrifuged for 10 min at 12,000 rpm. The supernatant contained the target coumarins. For every aliquot, solid phase extraction was conducted on packed MFE C_{18} 3/500 columns (Análisis Vínicos S.L.) prior to HPLC analysis. On each occasion, a new packed column was gently washed with distilled water. The sample supernatant (250 μl) was loaded and elution started with 750 μl of methanol (85%) that was discarded. A mixture of methanol–water–glacial acetic acid (60:40:1, v/v; 1500 μl) was collected directly into an HPLC vial. Two injections of 10 μl from each vial were analyzed by HPLC, and the average peak area for each compound was used for further statistical analyses (see below).

HPLC was conducted on a Waters 2690 separation module with a Waters 996 PDA detector (Waters Cromatografía S. A., Barcelona, Spain) that allowed coumarin identification by way of their characteristic UV spectrum with two maxima absorption lengths around 260 and 320 nm (Murray et al., 1982). A Waters ODS2-3 μm RP-HPLC column (4.6 mm i.d. × 15 cm length) was used for quantification. Analyses were conducted in isocratic mode at a flow-rate of 1 ml/min, using a mixture of water–methanol–glacial acetic acid (84.8:14.2:1, v/v) as mobile phase (modified from Thompson and Brown, 1984). Double distilled water and HPLC quality solvents were used for the analyses. A calibration regression line was obtained for esculetin by varying the volume injected of two different solutions w/w in methanol. Regression of peak area on amount of esculetin injected explained 99.8% of peak area variation. Analyses of esculetin recovery based on this calibration showed that on average 76% of the esculetin initially added to leaf samples was lost during sample processing. Thus, error for each individual sample was calculated as the ratio between the expected and observed area of esculetin peak.

Each coumarin was purified by liquid chromatography, and the ^{1}H and ^{13}C NMR (DMSO-d_6) obtained on a Bruker AVANCE 500 spectrometer were compared to literature data for molecule identification.

Data Analyses. Statistical analyses were performed using the SAS statistical package (SAS Institute, 1996). Peak areas were transformed into coumarin concentrations assuming that for each sample coumarin quantification had the same recovery error as that observed for esculetin, and using esculetin regression to transform peak areas into quantities. Results obtained for each aliquot were averaged by sample, and concentration was referred to total leaf dry weight of the sample. Differences between genders and populations on concentration of coumarins were analyzed by General Linear Models (Procedure GLM). Gender, population, and their interaction were treated as fixed effects.

RESULTS

The methanolic extract of *D. laureola* leaves contained three major components (Figure 1) that were identified as three different glycosides of 7-methoxycoumarin. The observed molecular structures based on ^{1}H and ^{13}C NMR, and the references where these compounds were previously reported (Konishi et al., 1993;

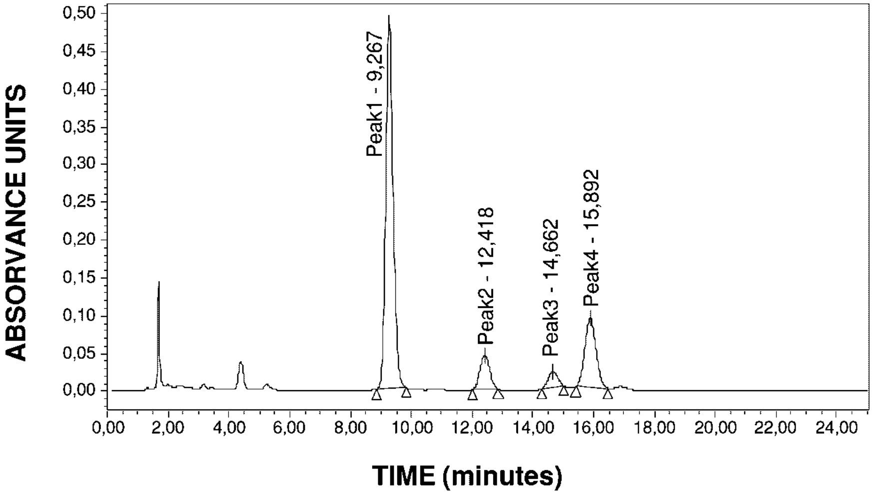


FIG. 1. HPLC chromatogram of the methanolic extract of *D. laureola* leaves with esculetin added, recorded at 320 nm wave length. Key to peak identity: Peak 1: 5-O-β—D-glucosyl-7-methoxy-8-hydroxy coumarin (**1**); Peak 2: 5-O-β—D-glucosyl-(6←1)-β-glucosyl)-7-methoxy-8-hydroxy coumarin (**2**); Peak 3: esculetin; Peak 4: 5-hydroxy-7-methoxy-8-O-β—D-glucosyl coumarin (**3**).

Jung et al., 1994) are shown in Table 1. The major component (**1**) averaged 54,938 (±17,646) ppm leaf dry weight, whereas average concentration of the other two compounds were 10,403 (±4182) ppm and 17,940 (±5787) ppm for (**2**) and (**3**), respectively. Concentrations of all three *D. laureola* coumarins into a sample were positively correlated ($N = 42$; $0.78 < r < 0.91$; $P < 0.001$ in all cases).

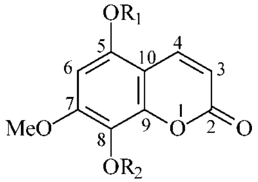
Populations differed in the concentration of the major coumarin glycoside (**1**) ($F_{5,30} = 4.56$, $P = 0.003$; Figure 2). Population × gender interaction was not statistically significant ($F_{5,30} = 1.09$, $P = 0.38$). Females tended to have greater concentrations of compound (**1**) than hermaphrodites, except in the CV population (Figure 2), when samples from this population were excluded from the analysis, differences between genders were statistically significant ($F_{1,24} = 5.76$, $P = 0.025$). Although the patterns were similar, gender differences were less apparent for concentrations of compounds (**2**) and (**3**) (analyses not shown). Populations also differed in the average concentration of compounds (**2**) and (**3**). Plants of the CT population had the highest concentration of both coumarin glycosides (14,471.0 ± 3986.0 ppm and 22,763.8 ± 7203.7 ppm for (**2**) and (**3**), respectively), whereas plants of the RH population showed the lowest concentrations of both (7614.9 ± 1818.4 ppm, and 14,903.7 ± 2355.6 ppm, respectively).

Since the concentration of the three coumarin glycosides was positively correlated, we calculated total concentration of coumarins in *D. laureola* leaves by adding them. The average concentration of total coumarins in female individuals of different populations was positively correlated to site altitude ($N = 6, r = 0.90$, $P = 0.01$; Figure 3). However, such a relationship was not found for hermaphrodite individuals ($N = 6, r = 0.27$, $P = 0.60$; Figure 3).

DISCUSSION

The three most abundant coumarin glycosides found in leaves of *D. laureola* were previously reported from other natural sources. Strangely, compounds (**1**) and (**2**) were isolated from mosses (Jung et al., 1994), compound (**1**) was found in *Polytricum formossum* and *Atrichum undulatum*, and compound (**2**) only in *P. formossum*. Compound (**3**) was previously isolated from leaves of the congeneric *Daphne pseudo-mezereum* (Konishi et al., 1993). No evidence for a similar coumarin daphnetin (7,8-dihydroxy coumarin) was detected in *D. laureola* leaves, despite a former report on its presence in the bark of this species (cf. Murray et al., 1982). This highlights that further analyses are needed to determine the identity and abundance of coumarins in other plant structures. The three compounds found in leaves are 5,7,8 trioxygenated coumarins. Apparently, all coumarins with an oxygen-containing substituent at the 7-position seem to be biosynthetically distinct from those that lack such a function, and derived from *p*-coumaric acid, an intermediate in lignin biosynthesis (Brown, 1970). It is also remarkable that all *D. laureola* coumarins share a 7-methoxy function, differing only in the nature and

Table 1. Chemical Structure and ^{1}H (J Hz) and ^{13}C NMR Data (500 MHz, DMSO-d_6) of the Three Coumarins Obtained From the Methanolic Extract of *Daphne laureola* Leaves, and the References Where These Compounds Were previously Reported



Compound	R_1	R_2	References
1	O-β-1-glc	H	Jung et al. (1994)
2	O-β-1-glc-6←1-β-glc		Jung et al. (1994)
3	H	O-β-1-glc	Konishi et al. (1993)

^{1}H (J Hz) and ^{13}C NMR data

	1		**2**		**3**	
Aglycone	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		159.8		161.4		160.3
3	6.21 d (9,7)	110.1	6.24 d (9.8)	111.9	6.11 d (9.6)	109.4
4	8.23 d (9,7)	139.4	8.25 d (9.6)	140.7	8.04 d (9.7)	139.8
5		146.1		147.6		151.9
6	6.91 s	96.5	6.85 s	97.9	6.52 s	95.6
7		150.8		152.4		155.8
8		127.7		–		124.1
9		142.2		143.8		147.9
10		103.5		106.6		102.5
Ome	3.85 s	55.8	3.85 s	57.3	3.84 s	56.2
Glucose						
1’	4.80 m	102.2	4.86 d (7.0)	102.9	4.93 d (7.3)	102.4
2’	3.29 m	73.2	–	74.1	3.29 t (7.9)	74.1
3’	3.30 m	76.2	–	77.1	3.24 t (8.4)	76.5
4’	3.12 m	70.0	–	70.7	3.17 t (8.4)	69.9
5’	3.37 m	77.4	–	76.7	3.08 m	77.2
6’	3.39 m/3.75 m	60.8	–	70.0	3.61 m/3.37 m	60.9
1”			4.17 d (7.6)	105.3		
2”			3.03 t (10.9)	74.3		
3”			3.11 t (8.5)	77.6		
4”			–	70.8		
5”			2.95 t (7.9)	74.3		
6”			–	66.6		

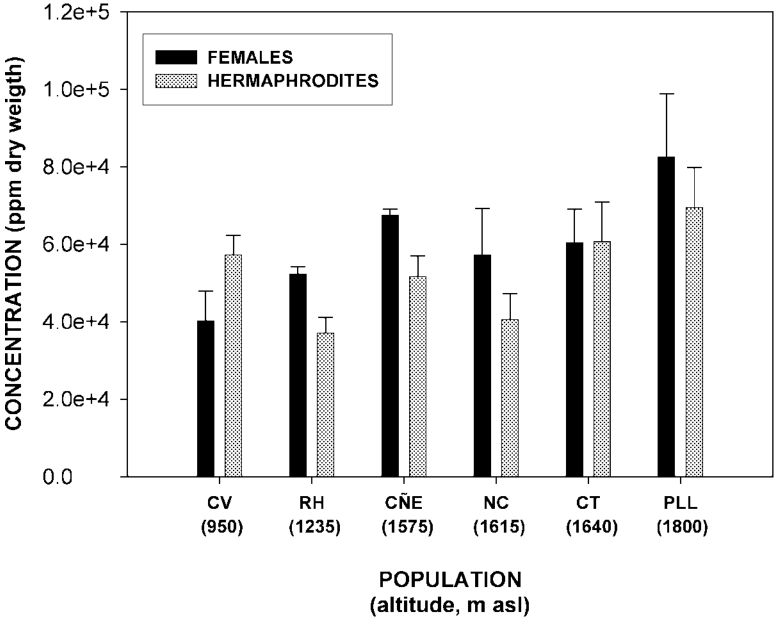


FIG. 2. Average concentration (+SE) of the most abundant coumarin, 5-O-β—D-glucosyl-7-methoxy-8-hydroxy coumarin, in leaves of female and hermaphrodite *D. laureola* individuals from six populations in southern Spain. Note that populations are ordered from lower to higher elevation.

position of the glycoside substituent, suggesting a common biosynthetical pathway. Further studies are needed to elucidate the metabolic relationships between these compounds and other plant physiological processes such as leaf maturation, since it is known that hydroxycoumarin content in *Daphne mezereum* from the Moscow region is maximal during leaf formation and at the end of the growth period (cf. Murray et al., 1982).

Our estimates of total coumarin concentration averaged between 60 and 120 mg/g dry weight for mature summer leaves of *D. laureola*. This figure is ca. 10 times higher than the concentration of dihydroxycoumarins reported for mature summer leaves of *Daphne mezereum* (Zobel and Brown, 1988), although the difference could partially reflect differences in the accuracy of the methods applied. The use of esculetin as internal standard in all samples allowed us to estimate the actual process errors and be confident of our estimates. Moreover, the low rates of standard recovery we observed revealed that further efforts to improve our methods would be important it to detect less abundant compounds.

Female and hermaphrodite individuals of *D. laureola* growing in the same population can have differential concentrations of secondary compounds. As

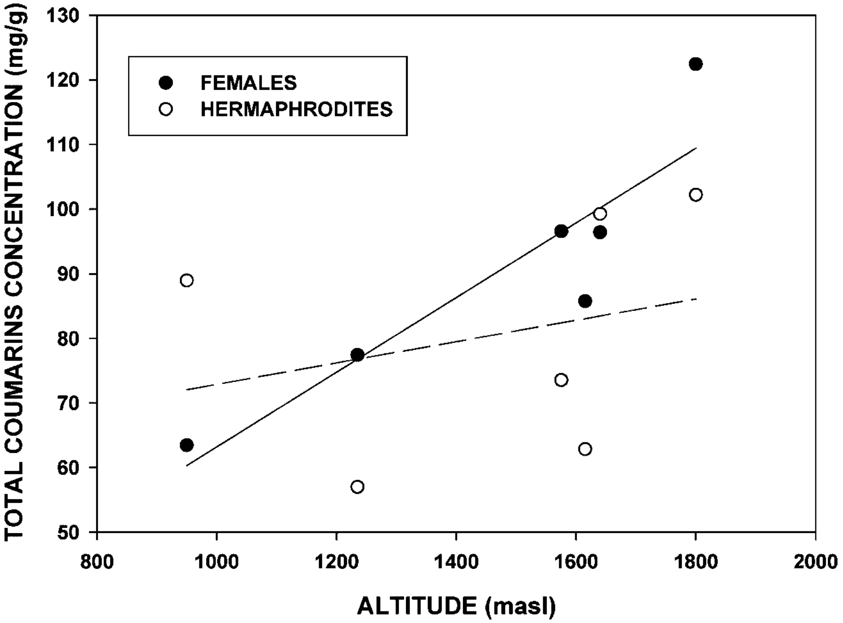


FIG. 3. Average concentration of the sum of all coumarins found in hermaphrodite and female *D. laureola* individuals at six different Spanish populations and the relationship with site altitude (solid line for females and dashed line for hermaphrodites).

expected from optimal theory, due to lower allocation to flowers, female leaves had on average higher concentrations of coumarins, upholding the idea that male function is costly for hermaphrodites (Eckhart and Seger, 1999). However, the magnitude of the difference was not constant, and even in the CV population, the one at the lowest altitude and with the highest proportion of females, the sign of the difference was reversed (Figure 2). Thus, costs of male (and likely also female) reproduction seem to be context dependent.

Finally, we found that average coumarin concentrations differed among *D. laureola* populations within a relatively small region. Heterogeneous spatial distribution of allelochemicals seems to be ubiquitous in both managed and natural systems (Hoy et al., 1998). Occurrence and concentration of plant allelochemicals may vary with latitude, elevation, sun exposure, and other environmental factors (Louda and Rodman, 1983; Dudt and Shure, 1994; Johnson and Scriber, 1994; Salmore and Hunter, 2001; Gómez et al., 2003). A negative relationship between elevation, occurrence, and concentration of some alkaloids (Salmore and Hunter, 2001) and glucosinolates (Louda and Rodman, 1983) has been found in some species, although unrelatedness and nonlinear relationships were found for different alkaloids and glucosinolates in the same species. Also the content of UV-B absorbing compounds increases with site elevation in several plant species (Rozema et al., 1997). *D. laureola* showed a gender-specific response to site altitude since concentration of coumarins in leaves of female shrubs increased with population altitude, but hermaphrodites did not show a similar altitudinal pattern. On one hand, a higher concentration of coumarins could benefit plants by increasing the UV absorbance in higher elevation sites. Apparently, mostly females would be able to benefit from this advantage, once more supporting the existence of a cost of the male function in this species. Interestingly, some field experiments have shown that plants exposed to ambient solar UV-B radiation are more resistant to herbivorous insects than plants grown under filters that excluded the UV-B component of solar radiation (Stratmann, 2003). In addition to the flavonoids, isoflavonoids, and tannins quoted by Stratmann (2003), coumarins could be also associated with the observed overlapping between plant physiological responses to UV radiation and herbivory. Ongoing studies aimed at specifically evaluating the defensive role of *D. laureola* coumarins against insect defoliation will help to clarify the consequences of gender and altitudinal variation in coumarin concentrations in leaves herein reported.

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REFERENCES

ÅGREN, J., DANELL, K., ELMQVIST, T., ERICSON, L., and HJÄLTÉN, J. 1999. Sexual dimorphism and biotic interactions, pp. 217–246, *in* M. A. Geber, T. E. Dawson, and L. F. Delph (eds.). Gender and Sexual Dimorphism in Flowering Plants. Springer, Berlin.

ALONSO, C. and HERRERA, C. M. 1996. Variation in herbivory within and among plants of *Daphne laureola* (Thymelaeaceae): Correlation with plant size and architecture. *J. Ecol.* 84:495–502.

ALONSO, C. and HERRERA, C. M. 2000. Seasonal variation in leaf characteristics and food selection by larval noctuids on an evergreen Mediterranean shrub. *Acta Oecol.* 21:257–265.

ALONSO, C. and HERRERA, C. M. 2001. Neither vegetative nor reproductive advantages account for high frequency of male-steriles in southern Spanish gynodioecious *Daphne laureola* (Thymelaeaceae). *Am. J. Bot.* 88:1016–1024.

ALONSO, C. and HERRERA, C. M. 2003. Developmental and spatial covariation of nutrients in growing leaves of *Daphne laureola* and their relationships with herbivory. *New Phytol.* 159:645–656.

ASHMAN, T.-L. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. *Ecology* 83:1175–1184.

BERENBAUM, M. R. 2001. Chemical mediation of coevolution: phylogenetic evidence for Apiaceae and associates. *Ann. Mo. Bot. Gard.* 88:45–59.

BOECKLEN, W. J., PRICE, P. W., and MOPPER, S. 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). *Ecology* 71:581–588.

BROWN, S. A. 1970. Biosynthesis of furanocoumarins in parsnips. *Phytochemistry* 9:2471–2475.

CIPOLLINI, D., PURRINGTON, C. B., and BERGELSON, J. 2003. Costs of induced responses in plants. *Basic Appl. Ecol.* 4:79–85.

COLLIN, C. L., PENNINGS, P. S., RUEFFLER, C., WIDMER, S., and SHYKOFF, J. A. 2002. Natural enemies and sex: How seed predators and pathogens contribute to sex-differential reproductive success in a gynodioecious plant. *Oecologia* 131:94–102.

DELPH, L. F. 1990. Sex-differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology* 71:1342–1351.

DUDT, J. F. and SHURE, D. J. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* 75:86–98.

ECKHART, V. M. and SEGER, J. 1999. Phenological and developmental costs of male sex function in hermaphroditic plants, pp. 195–213, *in* T. O. Vuorisalo and P. K. Mutikainen (eds.). Life History Evolution in Plants. Kluwer, Dordrecht.

GÓMEZ, D., AZORÍN, J., BASTIDA, J., VILADOMAT, F., and CODINA, C. 2003. Seasonal and spatial variations of alkaloids in *Merendera montana* in relation to chemical defense and phenology. *J. Chem. Ecol.* 29:1117–1126.

GOUYON, P. H. and VERNET, P. 1980. Étude la de variabilité génétique dans une population de *Thymus vulgaris* L. Observations sur le polymorphismes sexuel et chimique, effets du régime de reproduction. *Acta Oecol., Oecol. Plant.* 1:165–178.

HEGNAUER, R. 1973. Chemotaxonomie der Pflanzen. VI. Thymeleaeceae. Birkauser Verlag, Basel.

HERMS, D. A. and MATTSON, W. J. 1992. The dilemma of plants to grow or to defend. *Quart. Rev. Biol.* 67:283–334.

HOY, C. W., HEAD, G. P., and HALL, F. R. 1998. Spatial heterogeneity and insect adaptation to toxins. *Annu. Rev. Entomol.* 43:571–594.

JING, S. W. and COLEY, P. D. 1990. Dioecy and herbivory: The effect of growth rate on plant defense in *Acer negundo*. *Oikos* 58:369–377.

JOHNSON, K. and SCRIBER, J. M. 1994. Geographic variation in plant allelochemicals of significance to insect herbivores, pp. 7–31, *in* T. N. Ananthakrishnan (ed.). Functional Dynamics of Phytophagous Insects. Science Publishers, Lebanon, New Hampshire, USA.

JUNG, M., ZINSMEISTER, H. D., and GEIGER, H. 1994. New three- and tetraoxygenated coumarin glucosides from the mosses *Atrichum undulatum* and *Polytrichum formosum*. *Z. Naturforsch* 49 c:697–702.

KONISHI, T., WADA, S., and KIYOSAWA, S. 1993. Constituents of the leaves of *Daphne pseudomezereum*. *Yakugaku Zasshi J. Pharm. Soc. Jap.* 113:670–675.

LOUDA, S. M. and RODMAN, J. E. 1983. Ecological patterns in the glucosinolate content of a native mustard, *Cardamine cordifolia*, in the Rocky Mountains. *J. Chem. Ecol.* 9:397–422.

MARSHALL, M. and GANDERS, F. R. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plant. *Am. J. Bot.* 88:1437–1443.

MAYNARD-SMITH, J. 1978. Optimization theory in evolution. *Annu. Rev. Entomol.* 9:31–56.

MURRAY, R. D. H., MÉNDEZ, J., and BROWN, S. A. 1982. The Natural Coumarins. Occurrence, Chemistry and Biochemistry. Wiley, Chichester, UK.

OBESO, J. R. 2002. Tansley review no. 139. The costs of reproduction in plants. *New Phytol.* 155:321–348.

ROZEMA, J., CHARDONNENS, A., TOSSERAMS, M., HAFKENSCHEID, R. and BRUIJNZEEL, S. 1997. Leaf thickness and UV-B absorbing pigments of plants in relation to an elevational gradient along the Blue Mountains, Jamaica. *Plant Ecology* 128:151–159.

SALMORE, A. K. and HUNTER, M. D. 2001. Elevational trends in defense chemistry, vegetation, and reproduction in *Sanguinaria canadensis*. *J. Chem. Ecol.* 27:1713–1727.

SAS Institute. 1996. SAS/STAT Software: Changes and Enhancements Through Release 6.11. SAS Institute, Cary, North Carolina.

STRATMANN, J. 2003. Ultraviolet-B radiation co-opts defense signalling pathways. *Trends. Plant. Sci.* 8:526–533.

THOMPSON, H. J. and BROWN, S. A. 1984. Separations of some coumarins of higher plants by liquid chromatography. *J. Chrom.* 314:323–336.

ULUBELEN, A., TEREM, B., and TUZLACI, E. 1986. Coumarins and flavonoids from *Daphne gnidioides*. *J. Nat. Prod.*49:692–694.

UNO, G. E. 1982. Comparative reproductive biology of hermaphroditic and male-sterile *Iris douglasiana* Herb. (Iridaceae). *Am. J. Bot.* 69:818–823.

ZOBEL, A. M. and BROWN, S. A. 1988. Localization of daphnetin and umbelliferone in different tissues of *Daphne mezereum* shoots. *Can. J. Bot.* 67:1456–1459.