Sex-mediated herbivory by galling insects on *Baccharis concinna* (Asteraceae)

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ABSTRACT. Sex-mediated herbivory by galling insects on *Baccharis concinna* (Asteraceae). The interaction patterns between the dioecious shrub *Baccharis concinna* Barroso (Asteraceae) and its speciose galling insect community were studied in southeastern Brazil. Two hypotheses were tested in this study: "the differential reproduction and growth hypothesis" that predicts that male plants present fewer reproductive structures and are larger than female plants; and the 'sex-biased herbivory hypothesis' that predicts that male plants support a larger abundance of insect galls than female plants. Plants did not show sexual dimorphism in growth (= mean leaf number). However, male plants had longer shoots and a lower average number of inflorescences than female plants. These results corroborate the hypothesis that male plants grow more and reproduce less than female plants. No statistically significant difference was found in the number of galls between male and female plants, but a sex by environmental effect on gall number was detected. When each species of galling insect was individually analyzed per population of the host plant, the rates of attack varied between sex and population of the host plant, and they were highly variable among the species of galling insects. These results highlight the importance of the interaction between sex and environment in the community structure of galling insects and indicate that other variables besides host sex may influence the patterns of attack by galling herbivores.

KEYWORDS. Dioecy; community structure; insect galls; plant gender; plant-animal interactions.

RESUMO. Herbivoria por insetos galhadores mediada pelo sexo em *Baccharis concinna* (Asteraceae). Os padrões de interação entre o arbusto dióico *Baccharis concinna* Barroso (Asteraceae) e sua diversa comunidade de insetos galhadores foram estudados na região sudeste do Brasil. Duas hipóteses foram testadas neste estudo: "a hipótese do crescimento e reprodução diferenciais", que prevê que plantas masculinas apresentam menos estruturas reprodutivas e são maiores do que plantas femininas; e a "hipótese da herbivoria mediada pelo sexo" que prevê que plantas masculinas sustentam uma maior abundância de insetos galhadores do que plantas femininas. Plantas não apresentaram dimorfismo sexual em relação ao crescimento (= número médio de folhas). Entretanto, plantas masculinas apresentaram ramos maiores e menor número de inflorescências do que plantas femininas. Estes resultados corroboram a hipótese que plantas masculinas crescem mais e se reproduzem menos do que plantas femininas. Nenhuma diferença estatisticamente significativa foi encontrada no número de galhas de insetos entre plantas masculinas e femininas, mas um efeito do sexo via meio ambiente sobre o número de galhas foi detectado. Quando cada espécie de inseto galhador em cada população da planta hospedeira foi analisada individualmente, as taxas de ataque variaram entre o sexo e a população da planta hospedeira, e estas taxas foram altamente variáveis entre as espécies de insetos galhadores. Estes resultados destacam a importância da interação entre o sexo e o meio ambiente na estrutura da comunidade de insetos galhadores e indicam que outras variáveis além do sexo da planta hospedeira podem influenciar os padrões de ataque por insetos galhadores.

PALAVRAS-CHAVE. Estrutura de comunidades; dioeicismo; insetos galhadores; interações animal-planta; sexo de plantas.

Interactions between plants and insect herbivores are highly influenced by the nutritional quality of the host plants (Herms & Mattson 1992). In dioecious plants, female plants allocate more energy and nutrients to reproduction than male plants because the production of reproductive structures is more expensive than the production of male reproductive structures (Wallace & Rundel 1979; Cipollini & Whigham 1994). Due to these functional sex -mediated differences, female plants have their growth reduced (Wallace & Rundel 1979; Bullock & Bawa 1981; Kraft & Denno 1982; Bullock *et al.* 1983; Bullock 1984; Hoffmann & Alliende 1984; Krischik & Denno 1990; Cipollini & Whigham 1994), have differential distribution along gradients or between habitats (Freeman *et al*. 1976), low nutritional status and high production of chemical defenses (Ågren 1987; 1988; Jing & Coley 1990).

Because of these differences in allocation patterns, male and female plants could differ in attack by herbivores and pathogens (Cornelissen & Stiling 2005). Female plants are also less attacked by herbivorous insects than male plants (Bawa & Opler 1977; Polhemus 1988; Boecklen *et al.* 1990; Jing & Coley 1990; Krischik & Denno 1990; Muenchow & Desesalle 1992; Boecklen & Hoffman 1993; Wolf 1997), as well as by other invertebrates (Elmqvist *et al.* 1991), by mammals (Elmqvist *et al*. 1988; Elmqvist & Gardfjell 1988; Danell *et al.* 1991; Hjältén 1992), by fungus (Ågren 1988). Otherwise, generalizations can not be attempted as the vast majority of these studies have been primarily done on temperate systems where only a single or a couple of herbivore species were studied. Further, the sex-mediated herbivory hypothesis has not been corroborated (Espirito-Santo & Fernandes 1998; Faria & Fernandes 2001) in some studies.

Baccharis concinna Barroso (Asteraceae) is an endemic and restricted dioecious shrub that inhabits the rupestrian fields of Serra do Cipó in southeastern Brazil. Despite its small distribution or geographical range, *B. concinna* has one of the most diverse faunas of galling insects in the Neotropical region (Fernandes *et al.* 1996). Therefore, it provides an interesting system where the influence of plant gender can be evaluated on a wide range of taxa of herbivores.

Our aim was to explore the relationships between dioecy and a community of galling insects on *B. concinna*. The following hypotheses were separately tested: i) "the differential reproduction and growth hypothesis"; which predicts that male plants are larger and produce fewer reproductive structures than female plants; and the ii) "Sex-biased herbivory hypothesis"; which predicts that male plants support a higher abundance of galls than female plants.

MATERIALS AND METHODS

***Baccharis concinna*-galling insects.** Species in the genus *Baccharis* (Asteraceae) are native to the New World (Barroso 1976) and present the greatest diversity of galling insects in the Neotropical region (Fernandes *et al*. 1996). *Baccharis concinna* is a threatened dioecious plant endemic to the Serra do Cipó and Diamantina, southeastern Brazil (Barroso 1976). This species has galls induced by 9 different species of Cecidomyiidae (Diptera), two lepidopterans, two Curculionidae (Coleoptera), and by one psylloid *Baccharopelma concinnae* Burkhardt *et al*. (2004). All galling insect species on *B. concinna* are new to science and are being studied by specialists. The two other galls found on *B. concinna* are induced by an unknown fungus, and by mistletoe, respectively. The morphological description and illustration of the galls can be found in Fernandes *et al*. (1996). The present study considered only galls induced by insects.

Study area and sampling. The study was done in Serra do Cipó, in the southern portion of Espinhaço Mountain Range in southeastern Brazil. Four populations of the host plant were studied along highway MG 010. *B. concinna* is a locally rare species that occurred in patches at all sampled sites (900, 1000, 1100, and 1300 m above sea level, referred as populations 1, 2, 3 e 4, respectively). Habitats were typically xeric, with rocky soils, dominated by cerrado and rupestrian field vegetation (Marques *et al.* 2002).

Fifty plants of similar size, belonging to each sex, were randomly chosen in each of the four xeric habitats along the altitudinal gradient, except in population 1 where we only found 29 males and 21 females. Hence, a total of 350 plants were sampled. Galls were sampled by direct counting on the plants during the rainy season of 1993 (Fernandes & Price 1988). The number of all galls refers to (i) total number of galls induced by the community of insects, while the number of cecidomyiid galls refers to (ii) number of galls induced by galling cecidomyiids alone. Each of these was used as y-variables in separate analyses. The abundance of galls of each species was compared individually. We chose to analyze the cecidomyiids separately because they are possibly the only taxon which could furnish sound comparative data worldwide (Gagné 1994) and are responsible for the majority of galls induced on *B. concinna* (Fernandes *et al.* 1996). Ten branches were taken haphazardly around the crown of each plant. Five shoots were then randomly chosen from each branch and from these we measured the length and number of leaves and inflorescences. Data were reduced to means per plant before statistical analysis, and plants were used as replicates.

Statistical analysis. The hypotheses of sex-mediated growth and reproduction and sex-biased herbivory were tested using ANOVAs (Zar 1984). Two-factor analysis of variance was used to determine if sex (factor fixed) and altitude (factor random) influenced the distribution of the abundance of galling insects (y-variable) or the growth and reproduction of *B. concinna* (y-variable) (Zar 1984). Finally one-way ANOVAs were used to determine if sex influenced the distribution of the abundance of each species of galling insects individually at each population. On each population, we observed if the abundance of galls induced by each galling species was influenced by sex. We provide only significant variations in the results in an attempt to make easier the presentation of the results. All analyses were followed by inspection of residuals to check the models used (Crawley 1993).

RESULTS

Sex-mediated growth and reproduction. Male *B. concinna* plants were larger than female plants as measured by shoot length at all populations studied. Male plants had longer shoots (average per plant = 17.8 cm) than female plants (average per plant = 10.9 cm) (Fig. 1, Table 1). Mean shoot length varied among the studied populations, but no interaction was observed between plant gender and population (Fig. 1, Table 1). Although the average number of leaves per shoot varied among populations, plant sex had no effect on it (Fig. 1, Table 1). A larger number of inflorescence per shoot was found in female plants compared to male plants (Fig. 1, Table 1). Mean inflorescence number varied among the studied populations, but no interaction was observed between plant gender and population (Fig. 1, Table 1).

Sex-biased herbivory. Host plant sex did not influence the abundance of galls induced by the entire community of galling insects on *B. concinna* or even the more restricted community of galling cecidomyiids (Fig. 2, Table 2). The only effect observed was that of population, where *B. concinna* plants supported a higher abundance of galls induced by all galling insects at population 3 (Fig. 2, Table 2). The abundance of galls induced by the cecidomyiids was also solely influenced by population; and was higher at populations 1 and 3 (Fig. 2, Table 2).

We further tested the effect of host plant gender on the galling community on *B. concinna* by observing trends in abundance of each individual galling species. Again, the effects of host plant sex were absent in the majority of the cases. Only

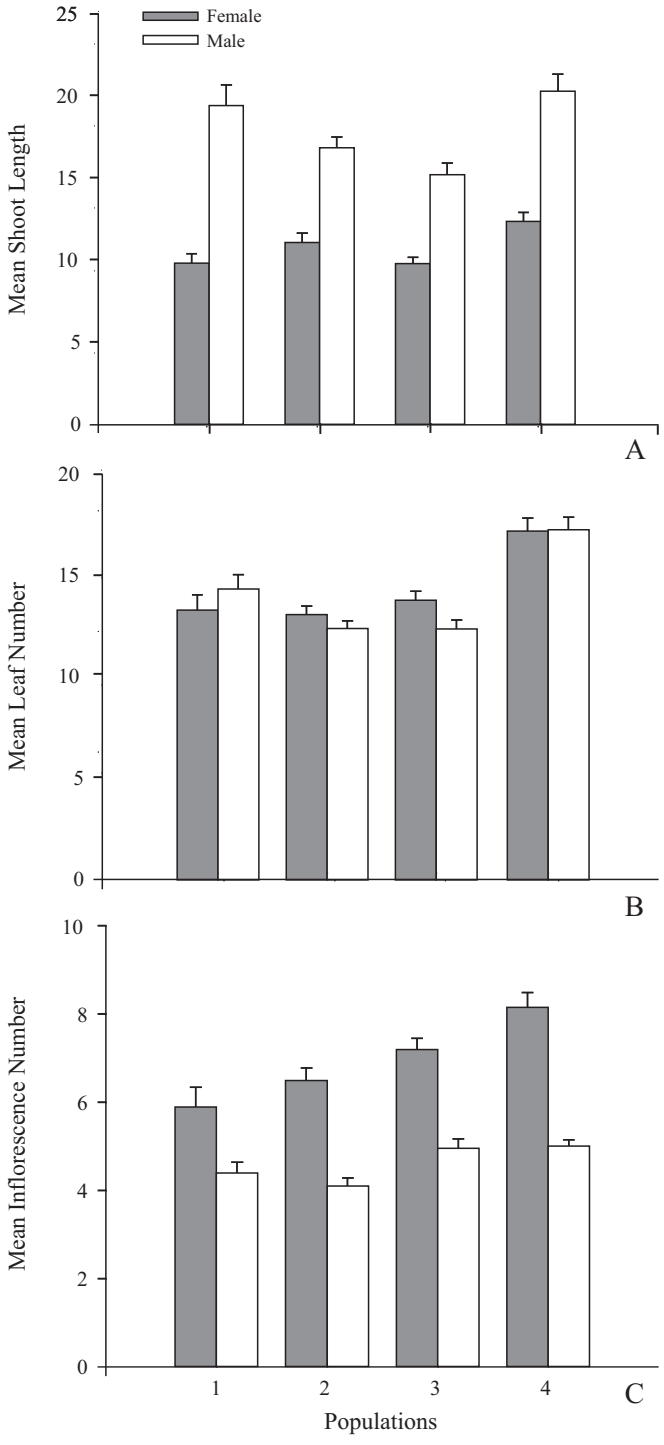

Fig. 1 Growth and reproduction of male and female plants of *Baccharis concinna* in four populations in Serra do Cipó, Brazil. (A) Mean shoot length (cm) and standard errors (SE), (B) Mean leaf number and standard errors (SE); (C) Mean inflorescence number and standard errors (SE).

in three instances gall abundance varied with host sex, but otherwise with no apparent trend. Female plants supported more galls than male plants of species 'b' ($F_{1,98}$ = 6.010, P = 0.016) in population 3, and species 'e' ($F_{1,98}$ = 12.831, P = 0.001) in population 4. On the other hand, male plants supported more galls than female plants of species 'n' ($F_{1,98}$ = 4.850, P = 0.030) in population 4.

DISCUSSION

Plant gender is argued to be an important source of variation in growth, reproduction, and resource allocation patterns (Wallace & Rundel 1979; Bullock 1982; 1984; Bullock *et al.* 1983; Hoffmann & Alliende 1984; Krischik & Denno 1990). The outcome of these qualitative and quantitative differences can be observed in their defense/growth allocation patterns and, consequently intersexual variation in herbivore attack (Wallace & Rundel 1979; Boecklen & Hoffman 1993). Accordingly, to the prediction of the hypothesis of differential growth and reproduction between the sexes, male plants had longer shoots and fewer inflorescences than female plants of *B. concinna*. Paradoxically, leaf number per shoot was not influenced by host sex. Otherwise, the variation in growth (mean shoot length and mean leaf number) and inflorescence number among populations indicates that environmental conditions differentially affect the growth and reproduction patterns of dioecious plants.

Environmental factors, including cold and nutritional stresses, which are typical of tropical highland environments, favor the evolution of a series of plant morphology types (Smith & Young 1987; Safford 1999). In shrubs, such as *B. concinna*, the leaf is often cupressoid, with leaves clustered at the stem termini. *Baccharis concinna* is an endemic species of rupestrian, water and nutritionally stressed habitats of Serra do Cipó. The greater number of clustered leaves should provide better protection against sun irradiancy and desiccation. Otherwise, if it is a plant adaptation to environmental stresses it should not be expected to vary in a large degree according to plant sex.

Despite the widespread occurrence of dioecism and resource variation caused by sexual differences (e.g., plant

Table I. Effects of sex and population on *Baccharis concinna*´s growth and reproduction.

Variables	df	MS	F	P
Mean Shoot Length				
Sex	1	3944.087	61.075	< 0.010
Population	3	242.816	10.423	< 0.001
Population*Sex	3	64.578	2.772	> 0.050
Error	326	23.296		
Mean Leaf Number				
Sex	1	4.951	0.257	> 0.500
Population	3	404.327	32.868	< 0.001
Population*Sex	3	19.277	1.567	> 0.050
Error	326	12.301		
Mean Inflorescence Number				
Sex	1	413.128	51.972	< 0.001
Population	3	36.127	12.995	< 0.001
Population*Sex	3	7.949	2.859	> 0.050
Error	326	2.780		

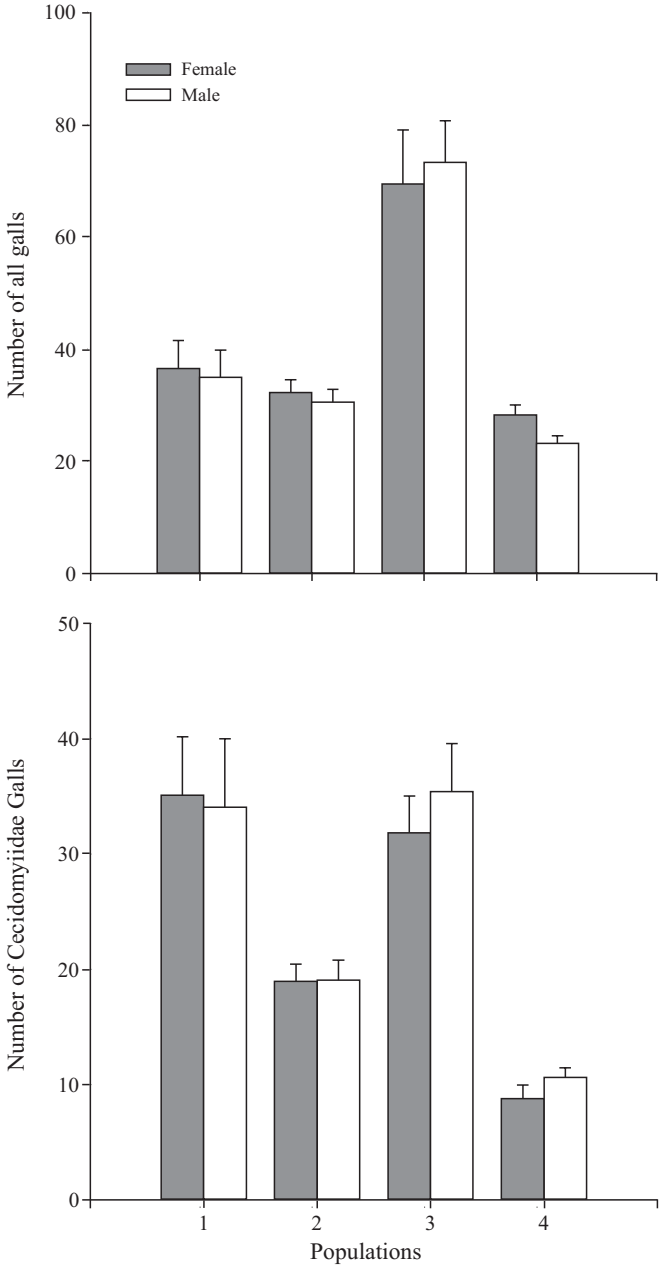

Fig. 2 Abundance of galls on male and female plants of *Baccharis concinna* in four populations in Serra do Cipó, Brazil. (A) Mean number and standard errors (SE) of all galls per plant (B) Mean number and standard errors (SE) of galls induced by Cecidomyiidae per plant.

quality, defenses, and even differential distribution of the sexes), there are still few studies on herbivory in dioecious plants (e.g., Espirito-Santo & Fernandes 1998; Faria & Fernandes 2001; Ribeiro-Mendes *et al.* 2002). In a congeneric dioecious shrub, *B. dracunculifolia* DC, in the Espinhaço mountain range, plant gender did not influence *Baccharopelma bacharidis* gall abundance (Faria & Fernandes 2001). In a broader study along the distribution of the host plant species in Brazil, the abundance of galls was not influenced by host sex (Ribeiro-Mendes *et al.* 2002). Other exceptions to the pattern of increased herbivory on male plants

Table II. Effect of plant gender and altitude on the number of galls induced by all galling insects and those by the cecidomyiids.

Variables	df	MS	F	P
Number of All Galls				
Sex	1	103.464	0.303	0.500
Altitude	3	41812.925	33.155	<0.001
Altitude*Sex	3	342.004	0.271	0.500
Error	342	1261.136		
Number of Cecidomyiidae Galls				
Sex	1	99.030	1.262	0.500
Altitude	3	12359.850	36.440	<0.001
Altitude*Sex	3	78.444	0.231	0.500
Error	342	339.180		

are that of *Rhacchiptera limbata* Bigot (Tephritidae) on *Baccharis lineares* (R. et Pav.) Pers. (Aljaro *et al.* 1984), and herbivores and pathogens on *Rubus chamaemorus* L. (Ågren 1987).

The sex-mediated herbivory hypothesis is based on the assumption that differential rates of growth between plants influence the quality and quantity of defenses (Jing & Coley 1990; Boecklen & Hoffman 1993). Although, the consistent differences in growth and reproduction were found between plant genders, male plants did not support larger number of galls than female plants of *B. concinna*. Our results on individual responses of gallers to plant gender support the results that plant gender is a weak predictor or even an irrelevant variable influencing gall abundance in this host plant species, and they shed some light on the importance of the environment in gall abundance. Some galling species (sp. b, e and sp. n) presented higher abundance on one host plant sex than on the other. Otherwise, the trend was not consistent as it varied between populations. This could indicate that the acceptance by one galling species to one plant sex varies among populations, implying that factors other than host sex (e.g., nutrient concentration in host tissue, meristem dynamics) may influence herbivore abundance. Likewise, other studies have shown similar results in which different herbivore species respond differentially to host plant sex (Boecklen & Hoffman 1993; Boecklen *et al.* 1994). On the other hand, long term studies would be necessary to better elucidate these trends.

There have been few studies aiming to establish a pattern with male plants bearing a greater number of herbivores than female plants. Besides, most studies have centered on one, or just a few species (see Boecklen & Hoffman 1993). Our study showed that abundance patterns may vary among species and sites and hence, studies done with only a few species can lead to erroneous generalizations about attack patterns at the community level.

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REFERENCES

Ågren, J. 1987. Intersexual differences in phenology & damage by herbivores and pathogens in the dioecious *Rubus chamaemorus* (L.). **Oecologia 72**: 161–169.

Ågren, J. 1988. Sexual differences in biomass and nutrient allocation in the dioecious *Rubus chamaemorus*. **Ecology 69**: 962–973.

Aljaro, M. E.; D. Frias & G. Montenegro. 1984. Life cycle of *Rhachiptera limbata* (Diptera, Tephritidae) and its relationship with *Baccharis lineares* (Compositae). **Revista Chilena Historia Natural 57**: 123–129.

Bawa, K. S. & P. A. Opler. 1977. Why are pistilate inflorescences of *Simarouba glauca* eaten less than staminate inflorescences? **Evolution 32**: 673–676.

Barroso, G. 1976. Compositae-subtribo Baccharidinae Hoffmann estudo das espécies ocorrentes no Brasil. **Rodriguésia 40**: 7–273.

Boecklen, W. J. & M. T. Hoffman. 1993. Sex-biased herbivory in *Ephedra trifurca*: the importance of sex-by-environment interactions. **Oecologia 96**: 49–55.

Boecklen, W. J.; P. W. Price & S. Mopper. 1990. Sex and drugs and herbivores: sex-biased herbivory in arroyo willow (*Salix lasiolepis*). **Ecology 71**: 581–588.

Boecklen, W. J.; S. Mopper & P. W. Price. 1994. Sex-biased herbivory in arroyo willow: are there general patterns among herbivores? **Oikos 71**: 267–272.

Bullock, S. H. 1982. Population structure and reproduction in the neotropical dioecious tree *Compsoneura sprucei*. **Oecologia 55**: 238–242.

Bullock, S. H. 1984. Biomass and nutrient allocation in a neotropical dioecious palm. **Oecologia 63**: 426–428.

Bullock, S. H. & K. S. Bawa. 1981. Sexual dimorphism and the annual flowering pattern in *Jacaratia dolichaula* (D. Smith) Woodson (Caricaceae) in a Costa Rican rain forest. **Ecology 62**: 1494–1504.

Bullock, S. H.; J. H. Beach & K. S. Bawa. 1983. Episodic flowering and sexual dimorphism in *Guarea rhopalocarpa* in a Costa Rican rain forest. **Ecology 64**: 851–861.

Burckhardt, D.; M. M. Espírito-Santo; G. W. Fernandes & I. Malenovský. 2004. Gall-inducing jumping plant-lice of the Neotropical genus *Baccharopelma* (Hemiptera, Psylloidea) associated with *Baccharis* (Asteraceae). **Journal of Natural History 38**: 2051–2071.

Cipollini, M. L. & D. F. Whigham. 1994. Sexual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). **American Journal of Botany 81**: 65–75.

Cornelissen, T. & P. Stiling. 2005. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. **Oikos 111**: 488–500.

Crawley, M. L. 1993. **GLIM for ecologists**. Oxford, Blackwell Scientific Publication, 379p.

Danell, K.; J. Hjältén; L. Ericson & T. Elmqvist. 1991. Vole feeding on male and female willow shoots along a gradient of plant productivity. **Oikos 62**: 145–152.

Elmqvist, T.; L. Ericson; K. Danell & A. Salomonson. 1988. Latitudinal sex ratio variation in willows, *Salix* spp., and gradients in vole herbivory. **Oikos 51**: 259–266.

Elmqvist, T. & K. Gardfjell. 1988. Differences in response to defoliation between males and females of *Silene dioica*. **Oecologia 77**: 225–230.

Elmqvist, T.; R. G. Cates; J. K. Harper & H. Gardjell. 1991. Flowering in males and females of a Utah willow, *Salix rigida* and effects on growth, tannins, phenolic glycosides and sugars. **Oikos 61**: 65–72.

Espírito-Santo, M. M. & G. W. Fernandes. 1998. Abundance of *Neopelma baccharidis* (Homoptera: Psyllidae) galls on the dioecious shrub *Baccharis dracunculifolia* (Asteraceae). **Environmental Entomology 27**: 870–876.

Faria, M. L. & G. W. Fernandes. 2001. Vigour of a dioecious shrub and attack by a galling herbivore. **Ecological Entomology 26**: 37–45.

Fernandes, G. W. & P. W. Price. 1988. Biogeographical gradients in galling species richness: tests of hypotheses. **Oecologia 76**: 161–167.

Fernandes, G. W.; M. A. A. Carneiro; A. C. F. Lara; L. R. Allain; G. Julião; G. I. Andrade; T. R. Reis & I. M. Silva. 1996. Galling insects on neotropical species of Baccharis (Asteraceae). **Tropical Zoology 9**: 315–332.

Freeman, D. C.; L. G. Klikoff & K. T. Harper. 1976. Differential resource utilization by the sexes of dioecious plants. **Science 193**: 597–599.

Gagné, R. J. 1994. **The gall midges of the neotropical region**. Ithaca, Cornell University Press, 352p.

Hjältén, J. 1992. Plant sex and hare feeding preferences. **Oecologia 89**: 253–256.

Herms, D. A. & W. J. Mattson. 1992. The dilemma of plants: to grow or defend. **Quarterly Review of Biology 67**: 283–335.

Hoffmann, A. J. & M. C. Alliende. 1984. Interactions in the patterns of vegetative growth and reproduction in woody dioecious plants. **Oecologia 61**: 109–114.

Jing, S. W. & P. D. Coley. 1990. Dioecy and herbivory: the effect of growth rate on plant defense in *Acer negundo*. **Oikos 58**: 369–377.

Kraft, S. K. & R. F. Denno. 1982. Feeding responses of adapted and non-adapted insects to the defensive properties of *Baccharis halimifolia* L. (Compositae). **Oecologia 52**: 156–163.

Krischik, V. A. & R. F. Denno. 1990. Patterns of growth, reproduction, defense and herbivory in the dioecious shrub *Baccharis halimifolia* (Compositae). **Oecologia 83**: 182–190.

Marques, A. R.; G. W. Fernandes; I. A. Reis & R. A. Assunção. 2002. Distribution of adult male and female *Baccharis concinna* (Asteraceae) in the ruprestrian fields of Serra do Cipó, Brazil. **Plant Biology 4**: 94–103.

Muenchow, G. & V. A. Delesalle. 1992. Patterns of weevil herbivory on male, monoecious and female inflorescences of *Sagittaria latifolia*. **American Midland Naturalist 127**: 355–367.

Polhemus, D. A. 1988. Intersexual variation in densities of plant bugs (Hemiptera: Miridae) on *Juniperus scopulorum*. **Annals of the Entomological Society America 81**: 742–747.

Ribeiro-Mendes, H. Nt.; E. S. A. Marques; I. M. Silva & G. W. Fernandes. 2002. Influence of host-plant sex and habitat on survivorship of insect galls within the geographical range of the host-plant. **Tropical Zoology 15**: 5–15.

Safford, H. D. 1999. Brazilian páramos I. An introduction to the physical environment and vegetation of the campos de altitude. **Journal of Biogeography 26**: 693–712.

Smith, A. P. & T. P. Young. 1987. Tropical alpine plant ecology. **Annual Review of Ecology and Systematics 18**: 137–158.

Wallace, C. S. & P. W. Rundel. 1979. Sexual dimorphism and resource allocation in male and female shrubs of Simmondsia chinensis. **Oecologia 44**: 34–39.

Wolf, L. M. 1997. Differential flower herbivory and gall formation on males and females of *Neea psychotrioides*, a dioecious tree. **Biotropica 29**: 169–174.

Zar, J. H. 1996. Biostatistical analyses (Third edition). Prentice-Hall, New Jersey, 662p.

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