

Bract Liquid as a Herbivore Defense Mechanism for *Heliconia wagneriana* Inflorescences¹

J. Timothy Wootton

Department of Zoology, NJ-15, University of Washington, Seattle, Washington 98195, U.S.A.

and

I-Fang Sun²

Department of Botany, University of Iowa, Iowa City, Iowa 52242, U.S.A.

ABSTRACT

The evolution of inflorescence morphology is usually considered to have been shaped by natural selection to enhance the chances of pollination or control gene flow. However, herbivores can affect plant fitness by damaging seeds and reproductive parts, or by nectar robbing, which reduces the chances of pollination. Tank species of *Heliconia* have inflorescences characterized by enlarged bracts that hold many flowers partially submerged in a large volume of liquid. Experiments in which we removed bract liquid and bagged flowers from *H. wagneriana* demonstrated that these plants actively secreted bract liquid. Rates of bract liquid secretion differed between individuals, and did not correlate with three environmental factors: rainfall, number of leaves, or number of bracts. By making drain holes in inflorescences of *H. wagneriana*, we tested whether bract liquid served as a herbivore defense. Compared to experimentally drained inflorescences and inflorescences of a second species which does not have bract liquid (*H. pogonantha*), liquid in control inflorescences substantially depressed the rates of herbivory on ovaries and other reproductive structures, and reduced the incidence of nectar robbing by chewing insects. The results suggest that bract liquid and its associated maintenance structures in the inflorescences of tank *Heliconia* defend the reproductive structures from herbivory, and that herbivory may generally play a role in shaping the evolution of floral morphology.

RESUMEN

La evolución de la morfología de la inflorescencia habitualmente se considera que ha sido formada por selección natural para mejorar las oportunidades de polinización o para el control del flujo de los genes. Sin embargo, los herbívoros pueden afectar la buena salud de la planta al dañar las semillas y partes reproductivas o al robar néctar, lo que reduce las oportunidades de polinización. Las especies de tanque de *Heliconia* tienen inflorescencias caracterizadas por tener brácteas agrandadas que sostienen muchas flores parcialmente sumergidas en un gran volumen de líquido. Los experimentos en los que quitamos líquido bracteal y flores abolsadas de *H. wagneriana* demostraron que estas plantas segregan activamente líquido bracteal. Las cantidades de líquido bracteal fueron diferentes entre los individuos y no correlacionaron con tres factores del ambiente: precipitación pluvial, número de hojas, o número de brácteas. Haciendo agujeros de drenaje en las inflorescencias de *H. wagneriana*, probamos si el líquido bracteal sirvió como defensa contra los herbívoros. Al hacer la comparación de inflorescencias drenadas experimentalmente e inflorescencias de una segunda especie que no tiene líquido bracteal (*H. pogonantha*), el líquido en inflorescencias controladas disminuyó sustancialmente las cantidades de herbívoros en los ovarios y otras estructuras reproductivas, así como las ocurrencias de robo de néctar llevadas a cabo por insectos masticadores. Los resultados sugieren que el líquido bracteal y sus estructuras asociadas de mantenimiento en las inflorescencias de tanque de *Heliconia* defienden las estructuras reproductivas contra los herbívoros, y que los herbívoros quizá jueguen en general un papel en la formación de la evolución de la morfología floral.

ALTHOUGH THE EVOLUTION OF FLOWER MORPHOLOGY is generally interpreted in terms of adaptations which increase the chances of pollination or affect plant mating systems, plant fitness can also be enhanced

by reducing floral herbivory. Herbivores can reduce plant fitness by consuming or damaging the ovary, developing seeds, and other reproductive parts; and, by stealing the nectar supplies; thereby reducing the chances of pollination of the plant. However, the effects of herbivory on the evolution of inflorescence morphology have been poorly investigated.

Heliconia (Heliconiaceae) species are noted for their unusual inflorescences, which in many species

¹ Received 30 July 1988, revision accepted 23 May 1989.

² Current address: Department of Integrative Biology, University of California, Berkeley, California 94720, U.S.A.

consist of several large and colorful cincinnial bracts holding a sizable volume of liquid and many flowers at various developmental stages. Although these inflorescences have generated a great deal of interest because of the microcommunities of animals they contain in their "tanks" (reviewed in Seifert 1982), the significance of this floral structure to the plant has been relatively unstudied. By draining and covering inflorescences, Bronstein (1986) demonstrated that the tank species *Heliconia imbricata* actively pumps liquid into its cincinnial bracts (see also Seifert & Seifert 1976, Stiles 1979). Additionally, Bronstein (1986) found that *Heliconia imbricata* maintains its liquid at a basic pH, raising the possibility of active secretion of a defensive compound. The production and maintenance of this liquid probably entails some cost to the plant in terms of water balance, active transport, and chemical production, suggesting a strong selective advantage to the maintenance of liquid in the inflorescences. Several investigators have proposed that the liquid secreted and held in these inflorescences functions as a moat to prevent herbivore access to the reproductive floral parts (Skutch 1933, Stiles 1979, Seifert 1982). However, this hypothesis has not been rigorously tested. Here we present results from experimental manipulations of *Heliconia wagneriana* Petersen inflorescences, demonstrating for the first time an antiherbivore function of their morphology through the production of a liquid-filled moat around the flowers. We also show that bract liquid secretion occurs in this species and that the rates of secretion vary among individuals, rather than with several environmental factors, consistent with a hypothesis of genetic control.

METHODS

We studied *Heliconia wagneriana* at the La Selva biological field station in Heredia Province, Costa Rica (10°27'N, 84°0'W) from January through March 1987, and in February 1989. In this species, cincinnial bracts hold up to 60 ml of liquid and flowers contain large (\bar{x} = 105 μ l) concentrated (\bar{x} = 32% sugar) nectar supplies.

To determine whether this species secretes bract liquid, we removed liquid from one bract of nine different plants and monitored liquid replacement over the next 3 days. Using plastic bags, we covered the inflorescences containing the bracts without liquid to prevent the collection of rainwater in the bracts. We measured daily rainfall, number of leaves on each plant, and number of opened bracts on

each inflorescence to test for possible environmental correlates of liquid secretion rate.

To test the hypothesis that the bract liquid acts as a herbivore defense, we arbitrarily chose 11 individuals from different locations, and selected 1 inflorescence from each. We eliminated the liquid from each experimental bract by cutting a drainhole (1 × 1 cm) at the bract base. Bracts with a drainhole above the bract liquid line served as controls. Each inflorescence contained two experimental and two control bracts. Bracts open sequentially at weekly intervals from bottom to top. We used the two most recently-opened bracts (1 and 2 weeks of age) and the two bracts next in line to open (0 and -1 weeks of age) on each inflorescence, and spread treatments equally across each bract age. The ineffectiveness of three drain holes reduced the number of experiment/control pairs used and produced a slight bias in the age of the experimental bracts toward younger ones relative to control bracts. This bias slightly reduced the chances of detecting an herbivore effect, because herbivores had a shorter time period to find younger drained bracts. Because of low sample sizes resulting in part from the inadvertent loss of 8 experimental and 10 control bracts prior to examining them for nectar robbing, we also sampled 20 additional untreated bracts for evidence of nectar robbing.

We initiated the liquid-draining experiment on 25 January 1987, and examined bracts after 36 days for the amount of herbivory on the (cincinnial) bracts and floral bracts, the presence of damage to the ovaries, the presence of nectar robbing (*i.e.*, holes cut in the nectary walls of the flower), and the presence of ants in the bracts. Ovaries sampled ranged in age from those in undeveloped flowers through those starting to ripen as fruit. Because the flowers dry up and loose shape following their presentation, we only scored the presence or absence of nectar robbing within any bract, rather than the frequency within each bract. Our observations included flowers which had not yet opened to permit pollination, as well as open flowers. Additionally, we only considered holes chewed at the base of the floral nectary as evidence of nectar robbing. Hence, other forms of nectar robbing, notably birds removing the entire flower, were not detected. Nectar robbing is thus conservatively estimated, but the undetected nectar robbing is unlikely to be affected by bract liquid.

We also sampled, for signs of nectar robbing and herbivore damage, 50 bracts from 4 clumps of *H. pogonantha* Cufodontis, a concurrently-flowering

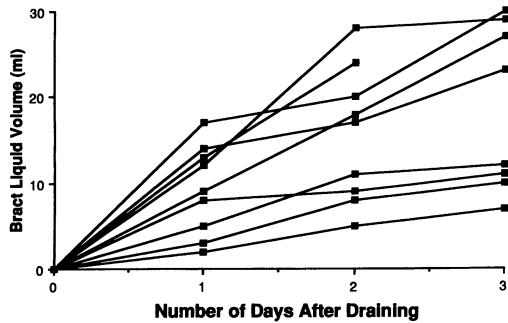


FIGURE 1. Volume of liquid held in bracts of *H. wagneriana* covered with plastic bags plotted against time following bract liquid removal. Each set of connected points represents an individual plant. After removing time effects, liquid volumes differ significantly between individuals ($P < 0.01$), with two statistically distinguishable ($P < 0.05$) groups.

species with pendant inflorescences (*i.e.*, the bracts are oriented like a canopy over the flowers and cannot hold liquid). Examining a species that naturally occurs without bract liquid further insured that differences seen in the drained bracts of *H. wagneriana* were not experimental artifacts, but reflected natural herbivory levels.

RESULTS

In bracts where we removed liquid and covered them with bags, liquid accumulated at an average rate of 9.2 ± 5.2 ml after one day ($42.5 \pm 23.1\%$ of original volume), demonstrating that this species secretes bract liquid (Fig. 1). After factoring out the increase in liquid volume over time with linear regression, we found that individual plants differed significantly in the volume of liquid secreted per day (ANOVA, $F_{8,17} = 4.2$, $P < 0.01$). Plants could be separated statistically ($P < 0.05$) into two groups: those with low (3 ml/day) and those with high (10

ml/day) liquid secretion rates (Fig. 1). Low volume plants grew interspersed with high volume plants, thus differences in bract liquid secretion did not correlate with geographic location. Mean rates of liquid secretion among plants did not correlate with number of leaves a plant possessed ($r = 0.219$, $P > 0.3$), or number of open bracts on an inflorescence ($r = 0.232$, $P > 0.25$). Daily rates of liquid secretion did not correlate with rainfall during the previous 24 hours ($r = 0.014$, $P > 0.95$).

Cincinnial bracts and floral bracts in drained treatments had a higher incidence of herbivore damage than did controls (Table 1). Herbivores lightly damaged cincinnial bracts, presumably because of their toughness, but heavily damaged the softer floral bracts on occasion. Drained bracts produced significantly fewer undamaged ovaries (Table 2, paired *t*-test, $P < 0.0005$) and contained a significantly higher percentage of damaged ovaries (Table 2, paired *t*-test, $P < 0.0005$) than did undrained bracts. Ovary damage ranged from slight chewing on the ovary wall, to tunneling through the ovary into the seed chambers, to total consumption of the ovary down to the base of the flower stalk. For those bracts in which we examined flowers, nectar was robbed only from drained bracts (Table 1).

The percentage of *H. pogonantha* ovaries damaged did not differ significantly from ovaries in drained *H. wagneriana* bracts (*t*-test, $P > 0.5$), but was significantly higher than that of control *H. wagneriana* bracts (Table 2, *t*-test, $P < 0.005$). We observed no *H. pogonantha* flowers robbed of nectar.

DISCUSSION

H. wagneriana secretes bract liquid (see also Orellana & Alarcon 1985), like *H. imbricata* (Bronstein 1986). The rate of bract liquid secretion differs among individual plants, and declines as bracts fill

TABLE 1. Percentages (N) of bracts or ovaries with evidence of herbivory in experimental and control treatments.*

Treatment	Damaged ovaries	Grazed cincinnial bracts	Grazed floral bracts	Nectar robbed bracts
Drained	39 (283)	100 (19)	100 (19)	27 (11)
Undrained	14 (311)	21 (19)	10 (19)	0 (29)**
<i>P</i>	<0.001	<0.001	<0.001	0.02

* Probabilities from χ^2 test with 1 df except for nectar robbing, where an exact probability is calculated.

** Sample includes 20 additional bracts from untreated inflorescences.

TABLE 2. Mean (standard deviation) number of healthy ovaries and percentage of damaged ovaries per bract in control and experimental *H. wagneriana* treatments and in untreated *H. pogonantha* inflorescences. N = sample size.

Treatment	# Healthy ovaries per bract (SD)	% Damaged ovaries per bract (SD)	N
Drained <i>H. wagneriana</i>	9.1 (3.6)	38.0 (5.4)	19
Undrained <i>H. wagneriana</i>	14.0 (3.0)	11.5 (4.0)	19
<i>H. pogonantha</i>	6.8 (4.2)	36.9 (14.0)	50

up. Such differences between individuals could reflect genetic differences between plants, or phenotypic responses to the environment. If environmental factors control liquid secretion rate, one might expect several relationships to be revealed. Water availability might affect secretion rate; hence, a correlation with rainfall would be expected. Plant condition, reflected in the number of leaves, might determine how much liquid a plant could deliver to the inflorescences; hence, a positive correlation with leaf number would be expected. The number of other bracts on the inflorescence might negatively correlate with liquid secretion rate, because these bracts also demand liquid from the plant. None of these relationships holds; hence, the data remains consistent with a hypothesis of genotypic differences in liquid secretion rate, providing material for evolution to act. The causes of interplant variation obviously require further work.

Bract liquid clearly reduced herbivory on *H. wagneriana* inflorescences. Reproductive structures suffered increased herbivore damage in the absence of bract liquid. Because damage to ovaries and seeds may reduce reproductive success, the more than three-fold increase in ovary damage in the absence of bract liquid probably results in lower plant fitness; hence, represents a potential selective pressure for the production and maintenance of bract liquid.

Reduced hummingbird pollination may also occur when bracts contain no liquid. We observed instances of nectar robbing only in drained bracts. Increased nectar robbing may reduce plant fitness because it reduces the reward available to pollinators and may damage flowers, affecting floral attractiveness to pollinators. We could not collect fruiting data to determine pollination efficiency, which would provide a stronger test of the effects of nectar robbing. We observed ants in association with flowers robbed of nectar, but the size and shape of the holes cut in the flower wall suggest that insects other than ants may have been involved.

Herbivory and nectar robbing appear to have

been strong selective forces favoring bracts enlargement and liquid secretion. The similarity of herbivore damage in *H. pogonantha* compared to experimentally drained *H. wagneriana* bracts indicates that the absence of the ability to hold liquid in *H. pogonantha*, and probably in other pendant species, represents a cost in terms of herbivore damage to ovaries. *H. pogonantha* appears to have developed other defensive mechanisms to combat nectar robbing. *H. pogonantha* grows tightly-packed floral bracts and flowers, and develops plant hairs within the inflorescence, making insect access to the nectary difficult. Furthermore, Orellana and Alarcon (1985) show that the flowers of *H. pogonantha* are thicker and tougher than those of *H. wagneriana*, which would make chewing holes in the nectary wall more difficult for insects. The cost of herbivory in *H. pogonantha* may be offset by its ability to exploit a wider range of habitats and flowering times than tank species (see below).

The use of bract liquid appears to effectively defend *Heliconia* flowers from herbivory, but may result in several costs to the plant. Secreting bract liquid may partially dictate the habitats and flowering times a *Heliconia* species can use. Because the production of bract liquid by the plant requires a great deal of water, species with tank inflorescences can carry out this strategy most easily during the wet season (late May through late December at La Selva), or in damp habitats near streams and rivers. Of the four *Heliconia* species with liquid-holding inflorescences at La Selva, only *H. wagneriana* blooms primarily during the dry season (Stiles 1975), but both pendant species flower at that time. Because *H. wagneriana* flowers during the dry season, its pattern of habitat occupancy appears affected by bract liquid secretion relative to *H. pogonantha*. *H. wagneriana* is more than eight times more abundant than *H. pogonantha* in moist alluvial habitats at La Selva, but less than half as abundant in dry habitats (Stiles 1975). This species may provide an unusual case where herbivores indirectly affect the habitat

and phenology of a species through the physiological costs of herbivore defense, rather than directly through differences in herbivore pressure between habitats.

Interestingly, under some conditions, the strategy of tank *Heliconia* may change as water stress and herbivory load change. Bronstein (1986) reports that under dry conditions in Corcovado National Park, Costa Rica, *H. imbricata* maintained little bract liquid. This strategy appeared in an area of low herbivory, because no evidence of elevated nectar robbing or herbivory was found, in contrast to our results. Whether this lack of bract liquid results simply from extreme physiological stress or represents an adaptive response to some trade-off between herbivory and water availability remains to be investigated. Because genetic variation in liquid secretion rate may exist among individuals (see above) a hypothesis of different evolutionary strategies remains plausible.

In conclusion, bract liquid and the associated morphological structures of the inflorescence to maintain it, function as an herbivore defense in *H.*

wagneriana. Whether these function only as defensive structures is debatable, since the enlarged bracts are generally vividly colored to attract bird pollinators. Nevertheless, our results suggest that herbivory probably played a large part in the evolution of these inflorescences, and that herbivore defense might be a worthwhile avenue of investigation in other studies of the evolution of floral morphology.

ACKNOWLEDGMENTS

We thank R. Carroll, C. Hoffman, M. Oemke, J. Stout and the members of OTS 87-1 for helpful discussion and companionship during the study, and J. Bronstein, R. Cruden, G. Orians, G. Stiles, and J. Wetterer for useful comments on the manuscript. We also thank D. A. Clark and D. B. Clark for logistical help and access to the La Selva facilities, and L. Overman for translation of the abstract. The Graduate School and Department of Zoology of the University of Washington, a Jessie Smith Noyes grant administered through OTS, NSF grant OCE-8614463 to R. T. Paine, and an NSF predoctoral fellowship provided support for Tim Wootton. Support for I-Fang Sun was provided by the Botany Department and the Program in Evolutionary Ecology and Behavior, University of Iowa.

LITERATURE CITED

- BRONSTEIN, J. L. 1986. The origin of bract liquid in a neotropical *Heliconia* species. *Biotropica* 18: 111-114.
- ORELLANA, R., AND C. A. ALARCON. 1985. El papel del agua en las inflorescencias de *Heliconia wagneriana* Petersen. *In* *Ecología de Poblaciones* 85-2, pp. 493-501. Organization of Tropical Studies, Durham, North Carolina.
- SEIFERT, R. P. 1982. Neotropical *Heliconia* insect communities. *Q. Rev. Biol.* 57: 1-27.
- , AND F. H. SEIFERT. 1976. Natural history of insects living in florescences of two species of *Heliconia*. *J.N.Y. Entomol. Soc.* 84: 233-242.
- SKUTCH, A. F. 1933. The aquatic flowers of a terrestrial plant, *Heliconia bihai*. *L. Am. J. Bot.* 20: 535-544.
- STILES, F. G. 1975. Ecology, flowering phenology, and hummingbird pollination of some Costa Rican *Heliconia* species. *Ecology* 56: 285-301.
- . 1979. Notes on the natural history of *Heliconia* (Musaceae) in Costa Rica. *Brenesia* 15: 151-180.
-