

## Interactions Between Chemical and Mechanical Defenses in the Plant Genus *Bursera* and Their Implications for Herbivores<sup>1</sup>

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Traditionally, plant defenses have been divided into two main categories: chemical and mechanical defenses. The first category includes a variety of substances that are toxic, repellent, or that render plant tissues indigestible to animals. In the second category are a series of physical barriers to avoid being eaten. These include structures such as spines, trichomes, and hard, very sticky, or smooth surfaces. Studies dealing with either one of these two kinds of defenses typically assume they are independent of each other and that their actions on herbivores are additive (Dussourd and Denno, 1991; Farrell *et al.*, 1991; Becerra, 1994*b*). Yet, in some plants, structures such as glandular trichomes and secretory canals are combinations of mechanical and chemical deterrence that may interact to entrap insects in sticky and toxic secretions (Southwood, 1986; Howe and Westley, 1988; Becerra, 1994*a*).

In this article we report an investigation of plant secretory canals and their relationship with plant chemistry in the genus *Bursera*. We present data suggesting an interaction between the mechanical and the chemical components of this defense. Also we present data which suggest that the interaction between these components has profound repercussions on how *Bursera*'s herbivores defend themselves against their predators.

### SECRETORY CANALS IN PLANTS

Many species of plants produce secretions such as resins, latices, gums, and mucilages stored under pressure in networks of

canals throughout the cortex of the stems and in the leaves, where they follow the vascular bundles (Fahn, 1979; Metcalfe and Chalk, 1983). Latex and resin canals occur in more than 35,000 species (Farrell *et al.*, 1991). When such plants are damaged, there is an immediate release of fluids from injured tissues, often in copious quantities. In many species, canals transport antiherbivore repellents and toxins. For example, canals in some Apiaceae store coumarins (Berenbaum, 1991). The resin canals of Pinaceae and *Bursera* species contain terpenes (Raffa, 1991; Evans *et al.*, 2000), while those of Anacardiaceae have catechols and flavonoids (Joel, 1980; Furth and Young, 1988; Vencel and Morton, 1998). The latex of Asclepiadaceae contains cardenolides and cardiac glucosides while the Euphorbiaceae stores diterpenes (Seigler, 1979; Brower *et al.*, 1988), and the Apocynaceae and Papaveraceae contain alkaloids (Sharma and Gupta, 1994; Sacchetti *et al.*, 1999). Besides containing toxic chemicals, these exudates pose a mechanical threat to insects because they solidify upon exposure to air and may impede the movement of herbivorous insects. In large quantities secretions may also kill small insects by asphyxiation or complete entrapment (Dussourd and Denno, 1991; Becerra, 1994*a*).

### EFFECTIVENESS OF CANALS IN PLANT DEFENSE

The effectiveness of canals and their secretions for defense has been documented for many plant species. They are strong barriers against insects that are unable to deal with large quantities of latex, resin or other fluids (Farrell *et al.*, 1991; Dussourd and Eisner, 1987). Yet, canal-bearing plants can be vulnerable to phloem-sucking insects that can direct their feeding organs to avoid

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secretory canals (Johnson, 1992). These plants are also susceptible to specialized insects that can deactivate canals by cutting veins or trenches (Becerra, 1994b). In conifers, bark beetles attack the trees in mass, cutting across resin ducts and blocking the transportation of monoterpenes to the wound (Raffa, 1991). These beetles often carry spores of pathogenic fungi that infect the tree, weaken it, and accelerate its death. The milkweed beetles, *Tetraopes melanurus*, *T. tetropthalmus* and *Labidomera clivicollis*, reduce or eliminate the flow of latex in Asclepiadaceae by cutting the leaf veins. Insects consume the leaves only after the flow of latex has stopped (Dussourd and Denno, 1991). The dogbane beetle (*Chrysochus auratus*) that feeds on *Apocynum cannabinum* (Apocynaceae) chews a channel that transects major veins adjacent to the leaf margin. The rupture of the laticifers stops the flow of latex to the margin of the leaf that is subsequently consumed by these beetles (Williams, 1991).

Trenching and vein-cutting behaviors have been reported for lepidopterans, orthopterans and coleopterans, and there is a correlation between resin canal organization and method of deactivation by insects (Dussourd and Denno, 1991). Insects cut veins in plants with arborescent resin canals or in plants with laticiferous ducts that do not reticulate. In these plants, the rupture of an individual canal stops the flow of secretions beyond the cut. Insects that make trenches feed on plants in which canals reticulate and the flow of secretions at a given point is maintained through several pathways. In plants in which canals run only along major veins so that injury between veins does not generate outflow (some Convolvulaceae), insects feed between the major veins without previously trenching or cutting veins.

#### BURSERA'S RESIN CANALS

The plant genus *Bursera* (Burseraceae) includes about 100 species distributed from southern United States to Peru. The genus diversified in the tropical dry forests of Mexico where about 80 species occur and about 70 are endemic (Rzedowski and Kruse, 1979; Becerra and Venable, 1999a, b).

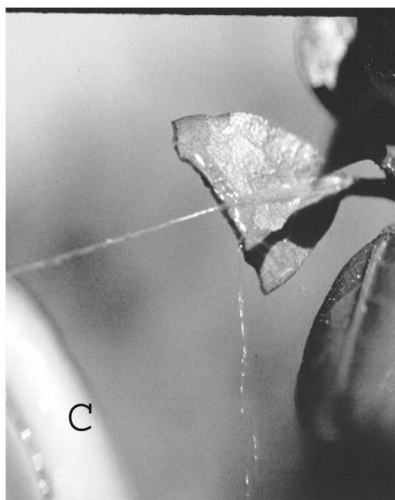
The genus is notable for its terpenoid secretions and exudates that arise from a system of arborescent resin canals (Becerra and Venable, 1990; Becerra, 1994a). As with other canal-bearing plant species, damage causes an immediate release of fluids from the site of injury. However, in some *Bursera* species resins are under considerable pressure and, when a leaf is damaged, may be released in a spectacular syringe-like squirt. In some species this squirt may travel up to two meters and lasts a few seconds (Fig. 1). About one third of the Mexican *Bursera* species are able to squirt resins when injured, while another third releases little or no fluids. The remaining third releases intermediate amounts of fluids that may partially bathe the leaf blade without producing a squirt.

There is variation in resin response among individual leaves and among plants of squirting *Bursera*. Also, water-stressed plants tend to release significantly lower amounts of resin, indicating that environmental factors can affect this response (Becerra, 1994a). We have measured the squirt response of a sample of 15 to 20 leaves in each of 100 individual plants of *Bursera schlechtendalii* over 5 yr. The variation in squirt response tends to be less variable within than among individual plants, suggesting a genetic component to the squirt response (Becerra, unpublished ms).

#### BURSERA'S SPECIALIZED HERBIVORES

The new world members of the genus *Blepharida* (Chrysomelidae: Alticinae) include about 45 species (Becerra, 1997; Furth, 1998; Evans *et al.*, 2000), many of them monophagous and feed mainly on *Bursera* or other members of Burseraceae and Anacardiaceae. *Blepharida* feeding on resin-releasing *Bursera* species cut the leaf resin canals before feeding on the leaves (Becerra, 1994a). Larvae of *Blepharida* species that feed on the *Bursera* species which release little or no fluids after damage, do not sever the leaf veins.

The squirt defense has detrimental effects even on these specialized vein-cutting herbivores. Larvae of the chrysomelid, *Blepharida schlechtendalii*, bite the midrib of *Bursera schlechtendalii* to stop the flow of



resins going to the leaf. However, larvae living on plants with a higher percentage of squirting leaves have higher mortality. Early-instar larvae are apparently unable to sever the leaf veins. They feed by mining the leaves, but they sometimes rupture the canals and die because they become covered by resins. Also, on highly squirting individuals, *B. schlechtendalii* larvae spend more time cutting veins and less time eating. This slows their growth, delays pupation, and increases their risk of predation (Becerra, 1994a).

#### *BURSERA*'S CHEMISTRY

The chemistry of *Bursera* is dominated by terpenoids, mostly monoterpenes and sesquiterpenes (Evans *et al.*, 2000), while diterpenes and triterpenes occur at a lesser extent (Peraza-Sanchez *et al.*, 1995). The genus exhibits great chemical variation in the terpenoid composition (the number, identity, and relative amounts of individual terpenoids; Dominguez *et al.*, 1973; Rzedowski and Ortiz, 1988; Syamasundar *et al.*, 1991). *Bursera* species often produce complex mixtures containing many individual compounds, often belonging to several terpenoid types. *Bursera simaruba*, for example, produces a number of mono-, sesqui- and triterpenes (Peraza-Sanchez *et al.*, 1995). The occurrence of complex mixtures of compounds, is a widespread phenomenon among plants that produce terpenoids and it has been associated with several factors (Gershenzon and Croteau, 1991). Blends of different terpenes may keep the mixture from solidifying, which may make them more effective as herbivore repellents. If the different compounds of a mixture act synergistically, greater toxicity or deterrence may result. Complex mixtures can also slow the rate at which herbivores can evolve resistance to plant defenses (Pimentel and Belloti, 1976). Finally, mixtures may also occur simply because certain bio-

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FIG. 1. Squirt-gun defense of (A) *Bursera trimera*, (B) *Bursera rzedowski*, and (C) *Bursera schlechtendalii*. When a piece of a leaf is removed, a fine syringe-like spray of resins is released.

synthetic enzymes of terpenoids produce multiple products (Colby *et al.*, 1993). Some species of *Bursera* also produce very simple resins consisting primarily of one or two simple monoterpenes. This phenomenon is less common among plants and, as we will explain in more detail in the following sections, it has very important consequences for herbivore's antipredator defense.

Evolutionary chemical trends in the genus are not well known, although recent studies suggest that there is considerable convergence in the production of different volatile compounds by *Bursera* species (Becerra, 1997). A dendrogram of chemical similarity of *Bursera* species distinguished four main groups within the genus (Fig. 2). Many lineages in the phylogeny of *Bursera* contain plants from several of these chemical clusters (Becerra, 1997).

#### THE INTERACTION BETWEEN CHEMICAL AND MECHANICAL DEFENSES IN *BURSERA*

The dendrogram of chemical similarity of *Bursera* species showed a remarkable correspondence to groupings of species based on their squirt response. Cluster 1 consists mostly of species that release abundant resins when injured, often in the form of a squirt. Cluster 2 contains many of the species that typically do not release any fluids, and clusters 3 and 4 consists mostly of species that release smaller amounts than the species in cluster 1.

The significant positive correlation between the strength of squirt response and the first principal component of chemical composition provides some insight into the nature of the relationship between the two defenses (Fig. 3). Low scores on the first principal component signify an absence of compounds with low retention times. Therefore, highly squirting *Bursera* species tend to have highly volatile mixtures, while the mixtures of non-squirting species are less volatile.

To explore this relationship in more detail we identified compounds for four high squirting and four non-squirting species using gas-chromatography-mass spectrometry. Individual compounds were identified by matching the obtained spectra with stan-

dard mass spectral libraries (NBS 7.5K), by comparing the mass spectra and retention times of authentic standards, and by interpreting the mass spectrum (for conditions see Evans *et al.*, 2000). *Bursera schlechtendalii* and *Bursera biflora* are representative of the squirting and non-squirting syndromes respectively. The chemical mixture of terpene squirting *Bursera schlechtendalii* is highly volatile and simple. Its resin contains a mixture of monoterpenes (95%), mostly  $\beta$ -phelandrene (Fig. 4). A Simpson dominance index that summarizes the relative abundance of compounds (*i.e.*, the opposite of diversity) was 0.42 for *Bursera schlechtendalii*. The index ranges from zero (equal abundance of an infinite number of compounds) to one (100% one compound; Whittaker, 1975).

The chemical mixture of the non-squirting *Bursera biflora* is less volatile, more diverse, and more complex than the one of *Bursera schlechtendalii*. Less than 36% of its volatile mixture is composed of monoterpenes. We also found four sesquiterpenes, a diterpene, and several unidentified compounds. The dominance index of its chemical mixture was 0.17, much lower than the one of *B. schlechtendalii*.

The remaining three squirting and three non-squirting *Bursera* species that were chemically analyzed follow the same trends. The value of the chemical dominance index was much higher for the squirting species than for the non-squirting ones (Fig. 5), reflecting the dominance of their chemical mixture by only a few simple monoterpenes (Fig. 6). The resin of some squirting burseras consists almost entirely of one compound. For example, 93% of the mixture of *B. lancifolia* is  $\beta$ -phelandrene, while 75% of the mixture of *B. rzedowski* is  $\beta$ -myrcene.

The compounds of the non-releasing species are heavier and the mixture is more complex, as with *B. biflora*. The monoterpene fraction is small, compared to the squirting species, and the mixture includes more sesquiterpenes and diterpenes. Also, the relative amounts of the different compounds of the mixture tend to be more even, which is reflected in lower dominance values. This evidence suggests that there is a

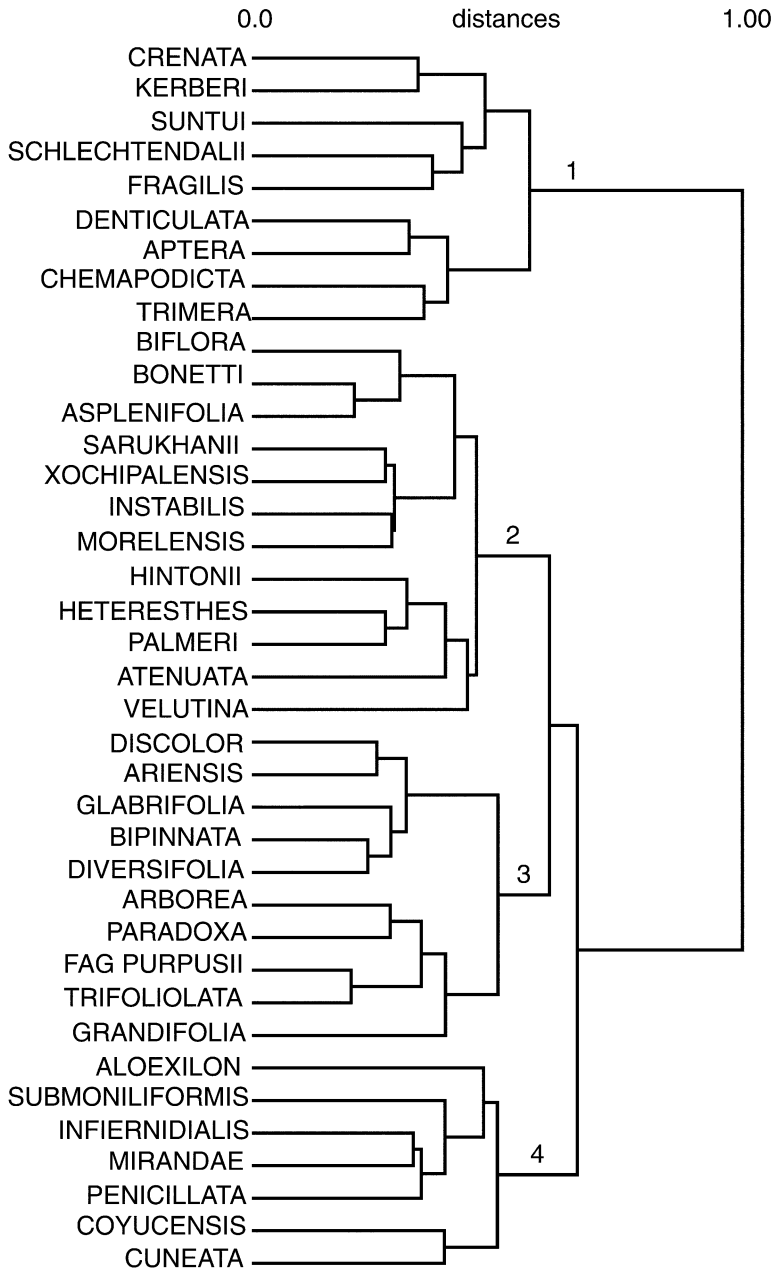


FIG. 2. Dendrogram of *Bursera* species based on chemical similarity. Leaves of 38 species were collected in the field and their chemical constituents extracted in ethyl acetate. Extracts were analyzed by gas chromatography. A matrix of Euclidean distances between these species was constructed on the basis of the presence or absence of each compound. Numbers indicate the four main chemical groups according to Ward's clustering method. For more details on methodology see Becerra, 1997.

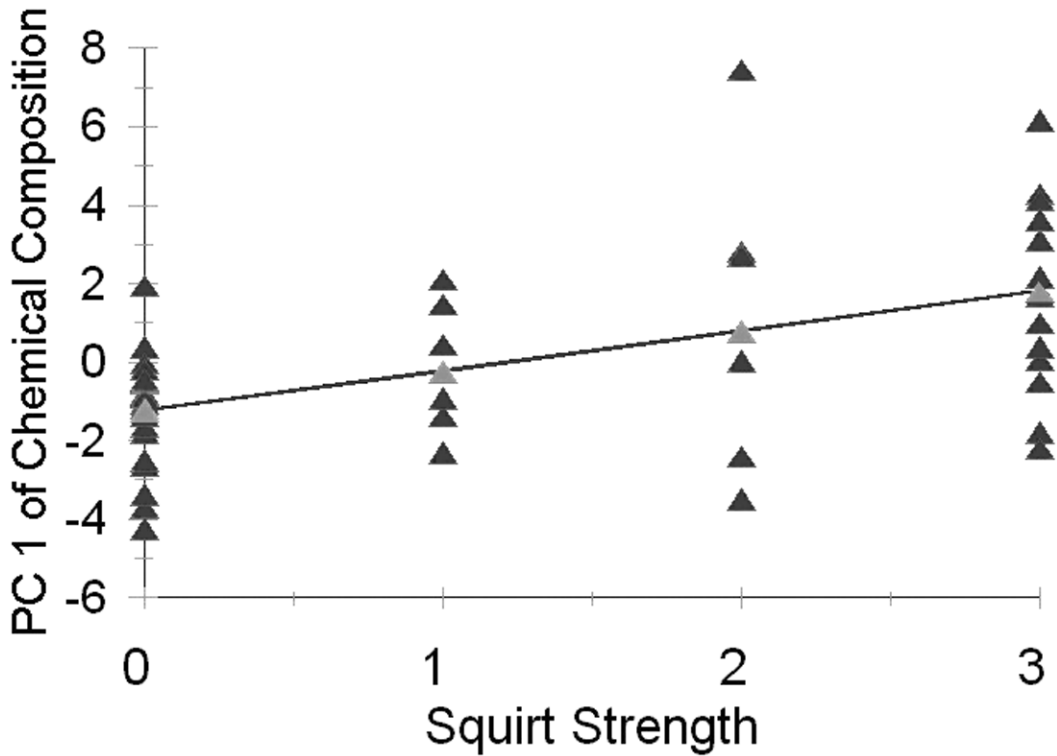


FIG. 3. Linear regression of the volatile chemical composition of *Bursera* species (first principal component) as a function of squirt strength ( $R^2 = .27$ ,  $P < 0.0002$ ). Chemical constituents were extracted and chemically analyzed as described in the text. Relative amounts of the main 10 to 15 compounds were used for the Principal Component Analysis. Low scores on the first principal component signify a lack of compounds of low retention time. Squirt strength was documented by cutting 5 to 20 leaves on multiple individuals per species with nail clippers. Squirt strength was ranked from 0 (no release) to 3 (squirting into the air) corresponding to an increasing amount of liquids released. Measurements were averaged and rounded.

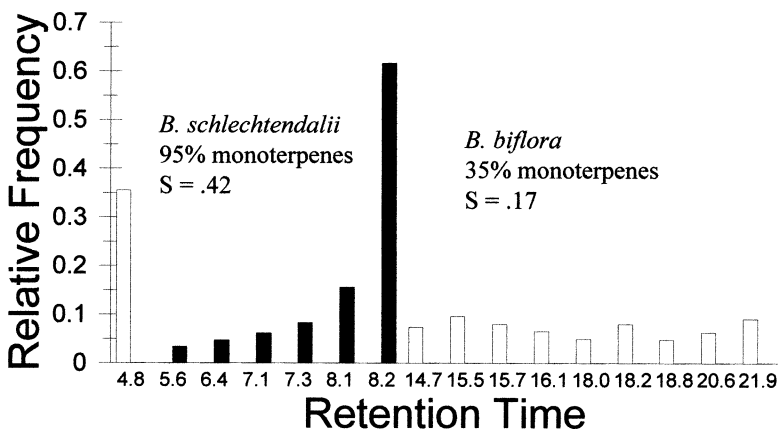


FIG. 4. Chemical analyses of the squirting *Bursera schlechtendalii* (black bars) and the non-resin releasing *Bursera biflora* (white bars). Bars indicate the relative amounts of the different compounds found, according to their retention times in the gas-chromatograph. S is the Simpson index of chemical dominance (lower values of S correspond to increased chemical diversity; see text for details).

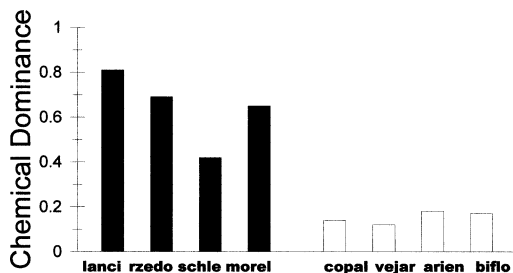


FIG. 5. Dominance Index values for the mixture of four squirting *Bursera* species (black bars) and for four non-squirting ones (white bars). Lower values correspond to increased chemical diversity. Lanci = *B. lancifolia*, rzedo = *B. rzedowski*, schle = *B. schlechtendalii*, more = *B. morelensis*, copa = *B. copalifera*, vejar = *B. vejar-vasquezii*, arien = *B. ariensis*, biflo = *B. biflora*.

correlation between mechanical and chemical defenses in *Bursera*. Squirting species tend to have highly volatile and simple mixtures composed mostly of one or two simple monoterpenes, while non-squirting species have heavier and more complex mixtures. While it is easy to speculate on the defensive significance of complex chemical combinations, it is more difficult to envision the adaptive value of a simpler chemistry. Perhaps the link to terpene squirting provides a clue.

From a physical point of view, squirting requires the resin to be in a liquid, low-viscosity state. In other plant-herbivore systems, the physical properties of the resins are determined by their terpenoid composition. In conifers, for example, the diterpene content of resin has a direct influence on the rate of resin crystallization (Schuck, 1982; Cruteau, 1987; Gershenson and Croteau, 1991). Thus, the required liquid state of resins may determine the high monoterpene fraction in squirting species of *Bursera*. Yet, this does not explain why only one or two monoterpenes dominate the mixture.

Another explanation for the peculiar chemical simplicity of squirting burseras is a relaxation of the need for chemical defenses. If the release of a highly pressurized liquid is an effective antiherbivore defense, the chemical nature of the individual components may not be as important as in non-releasing *Bursera* species. One single toxic

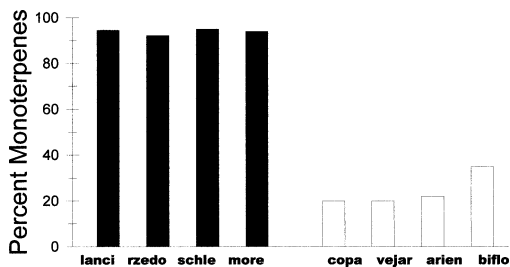


FIG. 6. Percentage of monoterpene compounds found in five squirting *Bursera* species (black bars) and four non-squirting ones (white bars). Lanci = *B. lancifolia*, rzedo = *B. rzedowski*, schle = *B. schlechtendalii*, more = *B. morelensis*, copa = *B. copalifera*, vejar = *B. vejar-vasquezii*, arien = *B. ariensis*, biflo = *B. biflora*.

or repellent compound that satisfies the required physical demands of the squirt defense may be an effective, economical option.

Accumulation of terpenoids is more expensive per gram than accumulation of most other primary and secondary metabolites (Gershenson, 1994). Their high cost is due to extensive chemical reduction mediated by ATP and NADPH and the expense of producing specific enzymes that catalyze the reactions along the biosynthetic pathway. The costs are high because many of these enzymes are highly specific for the individual reactions of terpenoid biosynthesis. Producing mixtures with more individual compounds requires production of more specific enzymes. Also, simple compounds often require fewer steps, which decreases their substrate and cofactor costs. Thus, producing a very simple mixture of one or two simple monoterpenes may be an adaptation of squirting plants to alleviate the high metabolic costs of terpenoid synthesis.

Interaction between mechanical and chemical defenses involving terpenoids has been documented in at least one other system. The volatile compounds found in the leaves of *Artemisia annua* differ depending on whether biotypes have glandular trichomes or not (Tellez *et al.*, 1999). The monoterpenes  $\alpha$ -pinene and pinocarvone are predominant in the essential oil of biotypes with leaves bearing glandular trichomes. The essential oil from glabrous leaves consists mostly of a blend of several sesquiterpenes, of which germacrene-D and

TABLE 1. Comparative biology of defense and antipredator defense in the genus *Blepharida* and its hosts.\*

Host species	<i>Blepharida</i> species	Plant releases fluids when damaged	Larvae cut veins	Larvae with fecal shield	"Boxing-like" defensive display <sup>b</sup>
<i>Bursera</i>					
<i>B. attenuata</i>	<i>B. alternata</i>	Yes	Yes	Yes	No
<i>B. grandifolia</i>	<i>B. pallida</i>	Yes	Yes	Yes	No
<i>B. instabilis</i>	<i>B. alternata</i>	Yes	Yes	Yes	No
<i>B. chaemapodicta</i>	<i>B. alternata</i>	Yes	Yes	Yes	No
<i>B. crenata</i>	<i>B. lineata</i>	Yes	Yes	Yes	No
<i>B. denticulata</i>	<i>B. lineata</i>	Yes	Yes	Yes	N/o
<i>B. fagaroides</i>	<i>B. multimaculata</i>	Yes	Yes	Yes	No
<i>B. fagaroides</i>	<i>B. gabrielae</i>	Yes	Yes	Yes	No
<i>B. fragilis</i>	<i>B. alternata</i>	Yes	Yes	Yes	No
<i>B. kerberi</i>	<i>B. lineata</i>	Yes	Yes	Yes	No
<i>B. kerberi</i>	<i>B. sparsa</i>	Yes	Yes	Yes	No
<i>B. morelensis</i>	<i>B. verdea</i>	Yes	Yes	Yes	Yes
<i>B. odorata</i>	<i>B. atripennis</i>	No	No	Yes	Yes
<i>B. paradoxa</i>	<i>B. gabrielae</i>	Yes	Yes	Yes	No
<i>B. schlechtendalii</i>	<i>B. schlechtendalii</i>	Yes	Yes	Yes	No
<i>B. trifoliolata</i>	<i>B. gabrielae</i>	Yes	Yes	Yes	No
<i>B. trimera</i>	<i>B. lineata</i>	Yes	Yes	Yes	No
<i>B. aptera</i>	<i>B. gabrielae</i>	Yes	Yes	Yes	No
<i>B. aptera</i>	<i>B. multimaculata</i>	Yes	Yes	Yes	No
<i>B. discolor</i>	<i>B. gabrielae</i>	No	No	Yes	No
<i>B. discolor</i>	<i>B. multimaculata</i>	No	No	Yes	No
<i>B. fagaroides</i> var. <i>fagaroides</i>	<i>B. multimaculata</i>	Yes	Yes	Yes	No
<i>B. fagaroides</i> var. <i>purpusii</i>	<i>B. multimaculata</i>	Yes	Yes	Yes	No
<i>B. asplenifolia</i>	<i>B. flavocostata</i>	No	No	No	Yes
<i>B. copalifera</i>	<i>B. flavocostata</i>	No	No	No	Yes
<i>B. copalifera</i>	<i>B. balyi</i>	Yes	Yes	Yes	No
<i>B. cuneata</i>	<i>B. unknown species</i>	No	No	No	N/o
<i>B. excelsa</i>	<i>B. bryanii</i>	No	No	Yes	No
<i>B. hintoni</i>	<i>B. flavocostata</i>	No	No	No	Yes
<i>B. sarukhanii</i>	<i>B. flavocostata</i>	No	No	No	Yes
<i>B. velutina</i>	<i>B. flavocostata</i>	Yes	No	No	Yes
<i>B. biflora</i>	<i>B. flavocostata</i>	No	No	No	Yes
<i>Rhus</i>					
<i>R. glabra</i>	<i>B. rhois</i> <sup>c</sup>	No	No	Yes	Yes

\* Data are based on personal observations made by J. Becerra and D. L. Venable during multiple visits to natural populations of each *Bursera* species over three to six years depending on the species. Fluid release was documented by cutting multiple leaves on multiple individuals with nail clippers. Vein cutting was directly observed *in vivo* (from Evans *et al.*, 2000).

<sup>b</sup> See text for description. This behavior was assayed by approaching larval heads with forceps; n/o, Not observed.

<sup>c</sup> Vencil and Morton (1998).

$\beta$ -caryophyllene are the major components. Thus, biotypes that rely on both mechanical and chemical defenses have more volatile chemical mixtures, while biotypes that depend solely on chemical protection have more complex and less volatile chemical combinations.

#### IMPACT OF THE INTERACTION OF *BURSERA*'S DEFENSES ON *BLEPHARIDA*'S ANTIPREDATORY DEFENSE

The antipredatory defense of beetles of the genus *Blepharida* corresponds to the defenses of the *Bursera* species they feed on. Larvae of *Blepharida* that feed on squirting *Bursera* festoon themselves with their own feces to form fecal mounds or shields. The collection of their feces is the result of a dorsal anus and a neuromuscular propulsion system that conveys feces forward over the larvae (Vencl and Morton, 1998). These species may also release an anal secretion or regurgitate when attacked or disturbed by predators (Evans *et al.*, 2000). Larvae of species that feed on hosts releasing little or no fluids when damaged typically do not regurgitate, release an anal secretion, or carry their feces on their backs. Instead, they rear their heads up to face their insect predators in a "boxing-like" display and swing their abdomens rapidly and forcefully (Table 1).

To understand the role of host chemistry in *Blepharida*'s counterdefensive strategies, we analyzed the chemistry of a *Bursera-Blepharida* interaction typical of the squirting strategy and another interaction typical of the non-squirting strategy (Evans *et al.*, 2000). We analyzed the leaves and squirt of *Bursera schlechtendalii* and the larvae, feces, regurgitate and anal secretion of *Blepharida schlechtendalii* (Table 2). The volatile components of the resin of *Bursera schlechtendalii* contain primarily  $\beta$ -phellandrene and limonene. Extracts of the larvae and enteric discharges of *Blepharida schlechtendalii* contain all of the volatile compounds identified from the plant. Therefore, although this beetle species is able to disarm the high pressure resin defense of its host, the plant compounds are ingested and excreted in the fecal shields.

We also analyzed leaves of the non-

TABLE 2. Chemical analysis of the extracts of *Blepharida schlechtendalii* and *Bursera schlechtendalii*.<sup>a</sup>

Retention <sup>b</sup>	Chemical	CAS #	Leaf	Resin	Larvae	Regurgitate	Anal secretion	Shield
5.64	Nonane	111-84-2	3.4%	2.4%	3.1%			
6.36	Alpha-pinene	80-56-8	4.7%	2.8%	3.8%			
6.91	Benzaldehyde	100-52-7			3.9%			
7.08	Sabinene	3387-41-5	6.2%	9.5%	4.3%	7.6%	9.6%	9.7%
7.30	Beta-myrcene	123-35-3	8.3%	6.3%	3.1%			
8.12	Limonene	138-86-3	15.6%	18.0%	16.4%	18.5%	20.1%	17.0%
8.18	Beta-phellandrene	555-10-2	61.7%	61.0%	65.2%	74.0%	70.3%	73.3%

<sup>a</sup>From Evans *et al.*, 2000.

<sup>b</sup>Retention time in minutes.

<sup>c</sup>Chemical Abstracts Service Registry Number.

TABLE 3. Chemical analysis of the extracts of *Bursera biflora* and *Blepharida flavocostata*.<sup>a</sup>

Retention <sup>b</sup>	Chemical	CAS#	Identification <sup>c</sup>	Leaf	Larvae	Feces
4.79	Alpha pinene	80-56-8	1	35.5%		
5.20	Benzaldehyde	100-52-7	1		4.8%	
7.22	Monacetin	106-61-6	2		4.0%	
10.22	Benzene acetaldehyde-alpha ethylidene	4411-89-6	2		3.2%	
12.57	Caryophyllene	87-44-5	1			3.4%
13.31			4			3.8%
14.69	A sesquiterpene		3	7.4%		2.2%
15.00	Octanoic acid	124-07-2	2			5.2%
15.20			4		5.4%	
15.45	A sesquiterpene		3	9.6%		
15.65	A sesquiterpene		3	8.0%		
16.05	A sesquiterpene		3	6.5%		
16.47			4			3.0%
16.57			4			5.0%
16.60			4		6.7%	
16.88			4		5.4%	
17.56			4			3.4%
17.97			4	4.9%		
18.22			4	8.0%		
18.35	A diterpene		3		9.7%	2.7%
18.83	Palmitic acid	57-10-3	2	4.8%	4.9%	8.0%
19.22	Ethyl palmitate	628-97-7	2		12.7%	6.2%
20.15			4			19.6%
20.50			4		11.1%	22.1%
20.59	Phytol	150-86-7	1			
21.16	Ethyl linoleate	544-35-4	2	6.3%	5.0%	5.4%
21.27	Ethyl oleate	111-62-6	2		19.3%	
21.55	Stearic acid	57-11-4	2		7.7%	
21.63			2			4.1%
21.90			2	9.1%		6.0%

<sup>a</sup> From Evans *et al.*, 2000.<sup>b</sup> Retention time in minutes.<sup>c</sup> Chemical Abstracts Service Registry Number.<sup>d</sup> Method of Identification: 1. Gas chromatographic retention coincidence and mass spectral matching with an authentic sample, 2. Mass spectral matching with a library spectrum, 3. Interpretation of the mass spectrum, 4. Unidentified.

squirting *Bursera biflora* and larvae and feces of its herbivore *Blepharida flavocostata* (Table 3). The chemical analyses of the body and frass of *Blepharida flavocostata* revealed almost no match to the constituents found in *B. biflora*. Thus, this beetle species does not sequester the chemical compounds of its host in its body or feces. Perhaps the greater chemical complexity of their host makes it more difficult or more energetically expensive to sequester compounds for antipredator defense. It seems then, that the antipredator strategy of *B. flavocostata* is primarily behavioral, consisting of the previously described boxing display.

The antipredatory function of fecal shields has been experimentally demonstrated in *Blepharida* beetles. Larvae of *Blepharida rhois* with intact shields were avoided by the generalist predatory ant *Formica subserica*, while larvae without shield were readily captured (Vencl and Morton, 1998). Furthermore, larvae whose natural shields were removed and then reared on a diet of lettuce were defenseless. However, larvae reared on lettuce, regained full protection when their shields were removed and replaced with host-derived shields. Thus, host-derived shields can be effective anti-predatory barriers.

#### SUMMARY

It is apparent that an interaction exists between the chemical and the mechanical components of the squirt gun defense in *Bursera*, which has resulted in some species having powerful squirts but simple chemistry. Squirting burseras tend to have more volatile chemical mixtures dominated by one or two monoterpenes while non-squirting burseras have mixtures involving sesquiterpenes and diterpenes. This interaction has had important consequences for the tactics that *Blepharida* beetles employ for their own defense. *Blepharida* feeding on squirting plants have responded evolutionarily by cutting the resin canals, which often makes them more susceptible to predation. These beetles also compensate for their higher risk of predation by utilizing the defensive compounds present in the plant against their own predators. *Blepharida* species that feed

on non-squirting, chemically more complex plants do not obtain the indirect benefit of protection from their hosts and have developed an alternative behavioral defense.

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