Journal of Chemical Ecology, Vol. 14, No. 2, 1988

CHEMICAL ASPECTS OF HOST-PLANT SPECIFICITY IN THREE *Larrea*-FEEDING GRASSHOPPERS

R.F. CHAPMAN, E.A. BERNAYS, and T. WYATT[1]

Department of Entomology and Division of Biological Control
University of California, Berkeley, California 94720

(Received October 15, 1986; accepted March 6, 1987)

Abstract—The host-selection behavior of three species of grasshopper feeding on creosote bush, *Larrea tridentata*, in southern California was investigated. The species were *Bootettix argentatus*, which is monophagous; *Ligurotettix coquilletti*, oligophagous; and *Cibolacris parviceps*, polyphagous. The monophagous species is stimulated to bite by nordihydroguaiaretic acid (NDGA), a compound that is characteristic of the host plant and that may comprise up to 10% of the dry weight of the leaf. Host specificity of *B. argentatus* is enhanced by deterrent responses to compounds present in the surface waxes of all non-host-plant species. Both the oligophagous and polyphagous species are deterred by NDGA at naturally occurring concentrations. Their association with *Larrea* is probably based on tolerance of the plant chemicals rather than on dependence on specific chemicals. Factors other than the chemistry of the plant probably also contribute to the specificity of *B. argentatus* and *L. coquilletti*.

Key Words—Creosote bush, *Larrea*, nordihydroguaiaretic acid, grasshoppers, monophagy, *Bootettix*, *Ligurotettix*, *Cibolacris*, Orthoptera, Acrididae, host selection, feeding deterrence.

INTRODUCTION

Creosote bush, *Larrea tridentata*, is widespread in the Mojave and Sonoran deserts in North America. Associated with it is a guild of herbivorous insects exhibiting varying degrees of specificity for the plant (Schultz et al., 1977). Rhoades (1977a, b) demonstrated that the resin which coats the outer surface

[1] Present address: Cleppa Park Field Research Station, University College, Cardiff, Newport NP1 9YT, U.K.

0098-0331/88/0200-0561$06.00/0

of *Larrea* leaves has an antidigestibility effect in vitro and obtained correlative evidence for an in vivo effect in the proscopiid grasshopper, *Astroma quadrilobatum* Mello-Leitao. While an antidigestibility effect may be the adaptive (ultimate) reason for the failure of many species to eat *Larrea* and for the specificity of some insects, it does not account for the present-day responses of insects to the plant, since some species reject it without feeding, while others are adapted to feeding on it.

In this paper we describe some of the chemical factors affecting the selectivity of three species of grasshoppers that exhibit different degrees of specificity on *Larrea*: *Bootettix argentatus* Bruner which is monophagous, *Ligurotettix coquilletti* McNeill which is oligophagous, and *Cibolacris parviceps* Walker which is polyphagous. We are concerned with establishing whether the monophagy of *B. argentatus* is determined largely by host-plant chemistry, as opposed to other ecological factors, and what sense organs are involved in selection.

METHODS AND MATERIALS

Nearly all the studies were made in 1984, 1985, and 1986 at the Boyd Deep Canyon Desert Research Center which is 10 km south of Palm Desert, Riverside County, California. The climate and flora of Deep Canyon are typical of the Colorado Desert, and the tetraploid race of *Larrea tridentata* is dominant over the alluvial plain and lower slopes of the canyon. The three species of grasshoppers feeding on *Larrea* are all relatively common.

Behavior on Plants. Observations on the feeding behavior of *Bootettix argentatus* and *Ligurotettix coquilletti* were made in open-fronted clear-plastic boxes 30 cm tall, 16 cm wide, and 8 cm deep. The open front allowed for ease of access and observation. A sprig freshly cut from the test plant was placed in a vial of water centrally on the floor of the box. Insects were transferred to the plant from vials and allowed a few seconds to settle down before others were added. The behavior of each individual was recorded continuously. In fact, most of the time the insects were stationary, and it was possible to observe and record the behavior of five insects simultaneously. Tests continued for 1 hr or until the insects left the plant, if this was earlier. Approximately equal numbers of observations were made by each observer on each plant to counteract the effects of possible observer bias. Insects were only used once in any one experiment, and they were allowed at least 24 hr in the stock cage with access to *Larrea* before being used in any other experiment. Only adults or last-instar nymphs were used.

Since *B. argentatus* feeds during the daytime (Otte and Joern, 1977), it

was tested during the day in subdued light usually at 30.0–32.5°C (range 27.5–35°C). Insects, previously collected in the field, were taken directly from stock cages containing fresh *Larrea* foliage for one series of tests simulating natural conditions (0 hr deprivation). Since selectivity of grasshoppers declines with increased time without food (Chapman and Bernays, 1977), a second series of experiments was carried out with insects deprived of food for approximately 20 h. This extreme condition provided a more rigorous test of specificity.

L. coquilletti feeds at night (Otte and Joern, 1977) and so was tested at night using red light. Under these conditions, the insects were not readily disturbed, although they could see the light and occasionally responded to it. A weak response to red light by *Locusta migratoria* L. has been recorded by Chapman (1954) and Cassier (1960). These insects were deprived of food for about 10 hr during the day to simulate normal feeding conditions; they were tested between 2000 and 2300 hr, usually at 30.0–32.5°C (range 27.5–35°C).

Cibolacris parviceps was tested behaviorally only on *Larrea*. Its readiness to feed on other plants was investigated by giving individual insects in 250-ml containers a sprig of a test plant for about 15 hr, starting at sunset. The occurrence or absence of feeding was recorded the following morning at about 0900 hours. Between tests the insects were fed on *Encelia farinosa*, a commonly occurring plant in the habitat on which they fed readily.

Preparation of Plant Extracts. Extracts of the resin on the surface of *Larrea* leaves were made using methanol, chloroform, or hexane as solvents. In each case, leaves were picked from freshly cut branches of *Larrea* plants on which *B. argentatus* was known to be present. This reduced the likelihood of selecting leaves from a bush which, for some reason, was unpalatable to this species. Bushes are known to vary in their palatability to *L. coquilletti*, and the same may be true for *B. argentatus*. Extracts were made from nine bushes. Dead leaves and reproductive parts were not included. Chloroform extracts were made by immersing the fresh leaves for 1 min at room temperature; with methanol, the leaves were immersed for 1 hr at room temperature; with hexane, they were soaked overnight at 8°C. These extracts were made from separate batches of leaves; they were not sequential. The times chosen for extraction were such that as much resin as possible was removed without the extract becoming contaminated by chlorophyll, as judged by its color. It was thus assumed that the chemicals in the extracts were largely limited to those present on the surface of the leaves. Although NDGA, when pure, is relatively insoluble in chloroform, the solubility of other resin components resulted in the bulk removal of the resin. As a result, the chloroform extract contained a high concentration of NDGA (up to 50% dry weight).

After the period of extraction, the extract was filtered, air-dried, and weighed. The residue of the leaves was oven-dried and weighed so that a mea-

sure of the dry-weight concentration of resin removed from the leaves was obtained. Subsequently the dried extracts were redissolved in the same solvents to give dry-weight concentrations on sucrose-impregnated glass-fiber disks that ranged from one to five times those on the plant.

For one series of experiments, the concentration of NDGA in a methanol extract was reduced by fractionation on an LH20 Sephadex column with methanol. Fifteen fractions were collected, and each one was examined for the presence of NDGA by comparison with the standard using thin-layer chromatography (Macherey-Nagel, Polygram SIL G/UV254, $CHCl_3$–MeOH–H_2O, 13:7:4). Fractions in which no NDGA could be demonstrated were pooled and the solvent removed. This was the NDGA-reduced fraction.

Some of these extracts were tested against all three species. Additional extracts were prepared for assay with *L. coquilletti*. This species habitually occupies some *Larrea* bushes but not others (Otte and Joern, 1975; Greenfield and Shelly, 1985). For convenience, these are called "good" and "bad" bushes, respectively. One good and one bad bush was selected by Dr. M. Greenfield on the basis of his observations. Leaf samples from each bush were oven-dried at 75°C for 3 hr and then extracted for 1 hr in methanol followed by 5 min in chloroform. In this case the extractions were sequential. Extraction in methanol reduced the dry weight of the leaves from the good bush by 14%, compared with 20% from the bad bush. Chloroform extraction resulted in a further reduction by 1.6% and 0.9%, respectively.

The concentration of NDGA in the extracts dissolved in methanol was determined on an Eyela PLC-5 liquid chromatograph system (Tokyo Rikatikai) at room temperature using a YMCA-PACK, AM-302 ODS reverse-phase column (15 cm × 6 mm) (Yamamura Chemical) equipped with Uptight precolumn (2 cm × 2 mm) (Upchurch Scientific). The mobile phase was methanol–water (7:3) at a flow rate of 1.25ml/min. The eluent was monitored by a built-in UV detector at 254 nm, and the amount of NDGA was quantified by comparison with a standard curve.

Surface extracts of nonhost plants from the habitat were made by dipping leaves into chloroform for 30 sec. Extracts were air-dried and leaves oven-dried to obtain a dry weight concentration. The extracts were tested at five times the concentration on the leaf surface because they are absorbed through the disk so that the concentration on the surface of the disk is less than the concentration applied (Woodhead, 1982).

Behavioral Observations on Responses to Plant Extracts. The responses of *B. argentatus* to contact with plant extracts or to their odor was observed in transparent plastic domes 8 cm in diameter and 3 cm high. These were made from inverted plastic champagne glasses with the stem cut off. The hole which remained served as an entry point for the insect; it was sealed with parafilm

during tests. The floor of the chamber was made of a double layer of wire gauze (1 mm mesh) on which were two semicircles of filter paper (Whatman No. 1). These were pleated so that the free edges were readily accessible to the insect for biting; *Bootettix argentatus* typically starts to feed at the edge of a leaf and had difficulty biting the edge of a disk flat on the floor of the chamber. The filter paper was untreated in control or odor experiments or treated with a chloroform surface extract of one of the test plants. In the odor experiments, a filter paper with the test extract or 1.5 g of fresh *Larrea* leaves was beneath the floor so the insect was not able to touch the extract or leaves, although the odor entered the experimental dome.

The observations were made in red light to minimize the effects of disturbance and the behavior of each insect was recorded at 30-sec intervals for 15 minutes at 28–31°C. Insects were previously deprived of food for about 21 hr (range 17–25 hr) generally at 19–23°C. They were allowed at least 15 min to acclimate at 30°C before being tested. During each experimental session two sets of insects were watched by different observers. A fresh insect was used for each treatment, and the sequence of treatments was randomized. Equal numbers of each treatment were observed by each observer in each session to minimize bias. Experiment 1 was carried out in Deep Canyon, experiment 2 at Berkeley using insects recently collected in the Mojave Desert.

Feeding Responses to Plant Extracts. The feeding responses of insects to plant extracts were determined in experiments in which the extract was added to a glass-fiber disk (Whatman GF/A, 2.1 cm diameter) already containing 5% dry weight of sucrose. Sucrose and extracts were each added to the disks in 100 μl of the appropriate solvents. This amount was just sufficient to impregnate the whole disk. Different dry weight concentrations, approximating those on leaves, were obtained by adjusting the concentrations of the solutions. The disks were air dried after addition of sucrose and after addition of the extract.

Insects were tested individually in a choice experiment in which the test disk was paired with a second sucrose-impregnated disk additionally treated with solvent only. The amounts of the two disks eaten after 24 or 48 hr were determined by weighing. The disks were presented in plastic boxes 20 × 10 × 3 cm high with gauze insets at either end. The disks were mounted on inverted thumb tacks 5 cm from each end of the box and 1.2 cm above the floor so that the insects had easy access to the edges. A pad of wet cotton was placed centrally in the box, equidistant between the disks, to provide drinking water.

In assays with extracts of good and bad bushes against *L. coquilletti*, test disks, one from each plant, were paired. Experiments were conducted in an incubator at 30°C and in darkness since some of the extracts were colored.

Commercially available NDGA (Sigma Chemical Company, St. Louis, Missouri) was tested in the same way as the extracts, being applied to the disks

in methanol. In one experiment NDGA was added to glass-fiber disks (Whatman GF/C) without sucrose; control disks in this case were treated with solvent only (no sucrose).

RESULTS

Feeding on Larrea. All three species fed readily on *Larrea* after a period of deprivation or, in the case of *B. argentatus*, even when taken directly from the host plant (Table 1). The duration of a meal was about 5 min for all species for periods of food deprivation of 10–20 hr, and in the case of *B. argentatus* and *L. coquilletti*, nearly all the insects remained on the plant in the observation box for the hour of the experiment. More than half the *C. parviceps* left the plant.

Effects of Extracts of Larrea. Leaves of *Simmondsia*, a woody shrub similar in size to *Larrea* and present in the habitat, were completely rejected by *B. argentatus* without any attempt at biting (Table 2, and see Table 6), but all the insects tested, bit, or nibbled at the leaves when these were painted with a chloroform extract of the surface resin of *Larrea* leaves. This extract did not, however, increase the palatability of *Simmondsia* leaves for *L. coquilletti*, which nibbled, but rarely fed on the plant.

Chloroform extracts added as 29–37% dry weight to sucrose-impregnated glass-fiber disks were phagostimulatory to *B. argentatus* in two of three tests (Table 3). These extracts contained 20–50% dry weight of NDGA, giving 5–16% dry weight of NDGA on the disks.

The methanol extract of *Larrea* leaf surface did not influence the selection of *B. argentatus* for sucrose impregnated glass-fiber disks in four experiments, but was a feeding deterrent for both *L. coquilletti* and *C. parviceps*. However,

TABLE 1. FEEDING ON *Larrea*: BEHAVIORAL OBSERVATIONS ON CAGED INSECTS

Species	Time deprived (hr)	Insect stage	Number of insects: Tested	Feeding	Nibbling	Biting	Palpating	Leaving	Meal length[a]: Minute ($\bar{X} \pm$ SD)	No. of insects
B. argentatus	0	adult	30	24	2	—	—	2	3.8 ± 1.7	27
	20	IV instar	12	11	—[b]	—	—	—	5.6 ± 1.6	11
L. coquilletti	10	adult	37	28	2	—	—	2	5.6 ± 3.5	14
C. parviceps	12	adult	11	7	3	1	—	7	5.6 ± 2.9	8

[a] Derived from a different data set, hence different number of insects.
[b] Minus indicates none in this category.

TABLE 2. FEEDING ON *Simmondsia*: EFFECT OF $CHCl_3$ EXTRACT OF *Larrea* OR NDGA APPLIED TO LEAVES OF *Simmondsia* ON FEEDING RESPONSES OF *B. argentatus*, 20-HR DEPRIVED, AND *L. coquilletti*, 10-HR DEPRIVED

		Number of insects						
Species	Applied to leaf	Tested	Feeding	Nibbling	Biting	Palpating	Not responding	
B. argentatus	Solvent	10	—[a]	1[b]	—	—	9	$P = 0.00006$,[c]
	Larrea extract	10	—	7	3	—	—	Fisher's exact test
	Solvent	24	—	1[b]	1	—	22	$P < 0.005$,
	NDGA 1–1.5mg/leaf	25	1	—	5	6	13	$G = 9.852$
L. coquilletti	Solvent	10	—	4	2	—	4	$P > 0.1$,
	Larrea extract	11	2	3	2	1	3	$G = 0.353$

[a] Minus indicates none in this category.
[b] Feeding on damp tissue at base of twig.
[c] Tests on 2 × 2 table, responding–not responding; solvent–extract or solvent–NDGA.

TABLE 3. RESULTS OF CHOICE EXPERIMENTS WITH SURFACE EXTRACTS OF *Larrea* ON GLASS-FIBER DISKS[a]

	dry wt (%) on disk		*B. argentatus*			*L. coquilletti*			*C. parviceps*		
Extract	Extract	NDGA	Number feeding	Test> control[b]	*P*[c]	Number feeding	Test> control[b]	*P*	Number feeding	Test> control[b]	*P*
Chloroform	29	6	20	10	>0.1	n.t.[d]			n.t.		
	33	16	23	22	<0.01	n.t.			n.t.		
	37	8	13	11	<0.05	n.t.			n.t.		
Methanol	24	8	20	14	>0.1	14	4	<0.02	12	2	<0.01
	31	7	18	10	>0.1	n.t.			n.t.		
	33	10	11	6	>0.1	n.t.			n.t.		
	33	7	12	4	>0.05	n.t.			n.t.		
Methanol, reduced NDGA	14	1.5	19	13	>0.1	15	5	>0.1	17	4	<0.01
Hexane	1	<0.005	18	12	>0.1	n.t.			n.t.		
	5	<0.025	19	14	<0.02	19	16	<0.02	17	8	>0.1

[a]Summary: + = phagostimulatory; 0 = no effect; − = deterrent.

	B. argentatus	*L. coquilletti*	*C. parviceps*
chloroform extract	+, 0	n.t.	n.t.
methanol extract	0	—	—
methanol extract, reduced NDGA	0	0	—
hexane extract, 5%	+	+	0

[b]Number eating more of test disk than of control disk.
[c]Wilcoxon signed-ranks test, two-tailed.
[d]n.t. = not tested.

when most of the NDGA and related compounds in the extract were removed by column chromatography, reducing the NDGA concentration on the disks to 1.5%, it lost its deterrence to *L. coquilletti*.

A methanol extract of a *Larrea* bush that was habitually occupied by *L. coquilletti* (good bush) was compared in a choice experiment with one from a bush that was not commonly occupied (bad bush) (see Otte and Joern, 1975; Greenfield and Shelly, 1985), both extracts being presented at about 22% dry weight on sucrose-impregnated glass-fiber disks. The extract from the good bush was eaten by *L. coquilletti* in significantly greater quantities than that from the bad bush (Table 4), although the total amount consumed (mean of 1.2 mg/insect) was much less than in any experiment that included a sucrose-impregnated disk as a control (range of means 6.5–10.5 mg/insect, five experiments). We conclude that the extract from the good bush was deterrent to *L. coquilletti*, but less so than that from the bad bush. The dry weight concentrations of NDGA on the disk were 4% and 6%, respectively. Chloroform surface extracts of the same two bushes made after the methanol extracts did not differ in palatability and were not deterrent (10.8 mg eaten per insect, Table 4). The disks contained less than 0.002% NDGA.

A hexane extract, containing less than 0.5% NDGA, of *Larrea* leaf surface was phagostimulatory for *B. argentatus* and *L. coquilletti* and had no effect on *C. parviceps* (Table 3).

Effects of NDGA. NDGA increased the acceptability of *Simmondsia* leaves to *B. argentatus* (Table 2) and was stimulating at all concentrations from 0.005%

TABLE 4. *L. coquilletti*: RESULTS OF CHOICE EXPERIMENTS WITH SURFACE EXTRACTS OF GOOD AND BAD *Larrea* BUSHES ON GLASS-FIBER DISKS

Extract	Dry weight (%) on disc	NDGA concentration (%) on disc		Number feeding	Amount eaten (mg, $\bar{X} \pm$ SD)		Good > bad[a]	P[b]
		Good	Bad		Good bush	Bad bush		
Methanol	22–23	4	6	20	0.8 ± 0.8	0.4 ± 0.7	14	<0.05
Chloroform	6–10	0.001	0.002	17	5.4 ± 5.7	5.4 ± 5.1	7	>0.1

[a] Number eating more of extract of good bush than of bad bush.
[b] Wilcoxon signed-ranks test, two-tailed.

to 20% dry weight on sucrose-impregnated glass-fiber disks (Figure 1). The degree of phagostimulation increased from 0.005% to 5% dry weight of NDGA ($t = 3.81$, $P < 0.001$), but there was no significant difference between 5% and 10% dry weight. In experiments using 6% or 13% NDGA on glass fiber disks (GF/C) without sucrose, the insects almost invariably nibbled at the test disks, but not the controls (13 of 14 and nine of 10, respectively), but the amounts of material consumed in 24 hr were negligible. The average amounts eaten were less than 0.2 mg in both experiments, and the biggest feed was 0.4 mg, compared with 3.5 ± 1.1 mg ($\bar{X} \pm$ SE, $N = 11$) in a parallel experiment with a choice between 5% sucrose and blank disks.

Both *L. coquilletti* and *C. parviceps* were deterred by 5% and 10% NDGA on glass-fiber disks (Figure 1), and in both cases significantly fewer insects fed on the test disks with the higher (10%) concentration of NDGA (*Ligurotettix*, $P = 0.009$; *Cibolacris*, $P = 0.005$; Fisher's exact test). At concentrations of 0.5% dry weight and below, *C. parviceps* did not distinguish between test and

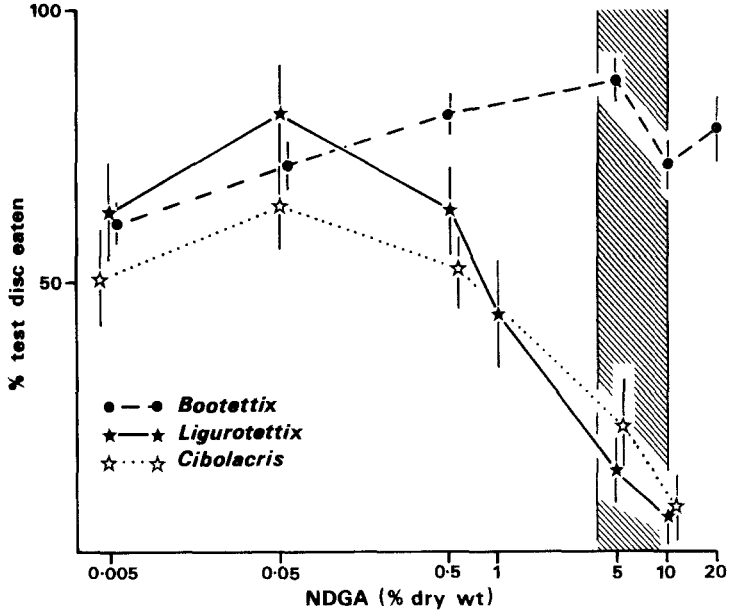

FIG. 1. Responses of each species to different concentrations of NDGA on sucrose-impregnated glass-fiber disks. Ordinate shows the amount of test disk consumed as a percentage of the total consumption by weight (test + control disks): 50% indicates no preference; >50%, phagostimulation; <50%, deterrence. Each point is based on a minimum of 16 insects. Results are of tests lasting 48 hr except for 20% which was only 24 hr. Vertical lines indicate standard errors. Hatched area shows range of normally occurring concentrations on *Larrea* leaves.

control disks. In the case of *L. coquilletti*, however, the pooled data for 0.005–0.5% dry weight indicate a significant phagostimulatory effect (Wilcoxon signed-ranks test, $P < 0.01$).

Behavioral Responses. Observations on the behavior of *B. argentatus* were carried out to determine which sensilla were involved in acceptance of the host plant. Normally, before starting to feed, a grasshopper tests the chemical quality of the potential food by touching it with the palps, "palpating," and biting. Rejection of the plant may follow either of these activities, or the insect may go on to feed. A chloroform extract of the leaf surface on filter paper induced more palpation and biting than was observed on untreated filter papers in two separate experiments (Table 5). The higher level of response with the extract may have resulted from information received via contact chemoreceptors on the tarsi or from olfactory receptors, probably on the antennae. When presented only with the odor of the extract or of *Larrea* leaves, significantly more palpation and biting occurred with the odor than without it in one experiment, but not in two others.

Responses to Other Plants. B. argentatus did not feed on any of 19 other plant species, most of which were common in the habitat at Deep Canyon. Undeprived insects rarely palpated and even after a 20-hr deprivation only a few insects palpated, bit, or attempted to nibble (Table 6). Other species of

TABLE 5. *B. argentatus*: BEHAVIORAL RESPONSES TO CONTACT WITH $CHCl_3$ SURFACE EXTRACTS OF *Larrea* AND NONHOST PLANTS (COMPARED WITH CONTROL IN SAME EXPERIMENT)

			Number of insects			
Plant	Experiment	Stimulus	Tested	Palpating and/or biting	*G* statistic[a]	*P*
Larrea	1	extract	14	12	21.007	<0.01
	2	extract	17	15	8.382	<0.05
	2	whole leaf odor	16	11	2.452	>0.05
	1	extract odor	12	6	6.831	<0.05
	2	extract odor	16	11	2.452	>0.05
Hyptis	2	extract	16	5	0.336	>0.05
	2	extract odor	16	5	0.336	>0.05
Encelia	2	extract	16	8	0.248	>0.05
	2	extract odor	16	5	0.336	>0.05
Control	1	—	16	1	—	—
	2	—	17	7	—	—

[a] *G* test, probabilities adjusted to take account of multiple comparisons.

TABLE 6. FEEDING RESPONSES OF THE THREE SPECIES TO A RANGE OF PLANTS OCCURRING IN THE HABITAT OR WITHIN THE RANGES OF THE INSECTS ELSEWHERE

Plant family and species	*Bootettix argentatus*										*L. coquilletti*					*C. parviceps*	
	Nondeprived					20-hr-deprived											
	Number tested	Feeding	Nibbling	Biting	Leaving plant	Number tested	Feeding	Nibbling	Biting	Leaving plant	Number tested	Feeding	Nibbling	Biting	Leaving plant	Number tested	Number feeding
Acanthaceae																	
Beloperone californica			not tested			8	—	—	—	3	9	—	1	2	5	not tested	
Asteraceae																	
Ambrosia dumosa	10	—[a]	—	—	8	9	—	—	—	8	10	—	7	1	1	not tested	
Bebbia juncea	10	—	—	—	9	11	—	—	—	10	10	—	—	2	3	15	14
Encelia farinosa	10	—	—	—	6	10	—	—	1	8	9	—	1	—	5	9	9
Hymenoclea salsola			not tested			10	—	—	—	6			not tested			not tested	
Perityle emoryi			not tested			9	—	—	—	6	9	—	1	3	6	not tested	
Peucephyllum schotti			not tested			10	—	1	—	9	9	—	1	2	5	not tested	
Buxaceae																	
Simmondsia chinensis	8	—	—	—	5	9	—	—	—	8	10	—	3	4	1	15	—
Chenopodiaceae																	
Atriplex canescens			not tested					not tested			10	8	1	—	—	not tested	

Fabaceae																		
Acacia greggi	10	—	—	—	6	10	—	—	—	9	10	—	8	1	—	not tested		
Cercidium floridum	8	—	—	—	5	9	—	—	—	8	11	—	5	3	1	10	8	
Krameriaceae																		
Krameria parvifolia	not tested					10	—	1	—	7	9	1	6	2	9	not tested		
Lamiaceae																		
Hyptis emoryi	9	—	—	—	6	8	—	—	—	7	10	—	4	—	8	not tested		
Nyctaginaceae																		
Boerhaavia coccinea	not tested					8	—	—	—	4	9	—	3	1	6	not tested		
Solanaceae																		
Lycium andersonii	not tested					not tested				10	10	—	—	5	not tested			
Zygophyllaceae																		
Fagonia laevis	10	—	—	—	8	10	—	—	—	8	10	7	—	—	3	not tested		
Larrea tridentata	30	26	2	—	—	12	11	—	—	—	37	28	2	—	—	10	9	
Tribulus terrestris	11	—	—	—	8	10	—	—	—	7	12	—	5	3	3	14	14	
Poaceae																		
Aristida sp.	11	—	—	—	9	11	1	1	1	6	10	—	2	1	5	15	9	
Cynodon dactylon	10	—	—	—	7	11	—	—	1	6	10	1	1	1	2	12	3	
Bouteloua aristidoides	not tested					9	—	—	—	6	8	—	—	1	5	not tested		
Triticum vulgare	not tested					9	—	—	1	6	9	1	—	1	5	not tested		

[a] Minus indicates none in this category.

Zygophyllaceae, the family to which *Larrea* belongs, were no more acceptable than plants from other families. Only on the grass, *Aristida*, was a sustained attempt at feeding made by one insect. This individual chewed at a leaf blade for 8 min, but during this time, although the leaf tissue was crushed, hardly any was removed. One other insect also nibbled and one bit on the *Aristida*. With nearly every plant offered, a majority of the insects left the plant within the duration of the experiment (1 hr) without apparently palpating on the surface. Only the tarsi had made contact with the leaves.

Surface extracts of 11 of these species were tested in choice tests on sucrose-impregnated glass-fiber disks. In all cases, the extracts were strongly deterrent and, apart from *Beloperone* and the grasses, virtually no feeding occurred on the test disks (Table 7). The chloroform extracts of *Encelia* and *Hyptis* on filter papers did not, however, influence behavior in observations lasting 15 min (Table 5).

In contrast with *B. argentatus*, one or more individuals of *L. coquilletti* bit or nibbled at all the different plants offered, and three species, *Atriplex*, *Lycium*, and *Fagonia*, were readily eaten by most insects tested. Individual insects also fed on the grasses (Table 6). *L. coquilletti* commonly left the plants on which it did not feed.

TABLE 7. *B argentatus*: RESULTS OF CHOICE EXPERIMENTS WITH SURFACE EXTRACTS OF NONHOSTS ON GLASS-FIBER DISKS

Plant family and species	Number feeding	Number eating test disk	Number eating more test than control	P^a
Acanthaceae				
Beloperone californica	16	8	3	<0.01
Asteraceae				
Ambrosia dumosa	17	0	0	<0.01
Bebbia juncea	16	1	0	<0.01
Encelia farinosa	14	0	0	<0.01
Hymenoclea salsola	11	0	0	<0.01
Perityle emoryi	13	3	1	<0.01
Peucephyllum schotti	15	0	0	<0.01
Fabaceae				
Cercidium floridum	20	1	1	<0.01
Lamiaceae				
Hyptis emoryi	17	0	0	<0.01
Poaceae				
Cynodon dactylon	15	8	2	<0.01
Triticum	16	13	3	<0.01

[a] Wilcoxon signed-ranks test, two-tailed.

The feeding responses of *C. parviceps* were only tested on eight plant species in five families. It ate all the plants (Table 6), although not always in large amounts.

DISCUSSION

Analysis of gut contents has revealed that *Bootettix argentatus* is strictly monophagous on *Larrea*; *Ligurotettix coquilletti* is oligophagous, although *Larrea* commonly constitutes a major host plant; and *Cibolacris parviceps* is polyphagous, feeding on *Larrea* only to a limited extent and not, in general, being dependent on it (Otte and Joern, 1977; Joern 1979). The present results demonstrate that the different relationships of these species with *Larrea* are at least partly governed by the chemical characteristics of the plant, extending the work of Rhoades (1977a), who clearly showed the importance of the leaf-surface resin in selection by *C. parviceps* and had circumstantial evidence relating the resin content of the leaves to acceptability by *B. argentatus* and *L. coquilletti*.

The sticky resin covering the leaves of *Larrea* may comprise 26% or more of the dry weight of the young leaves, sometimes falling to around 10% on older leaves of the same sprig and also varying between bushes. Phenolic aglycones comprise over 80% of the resin and the major component is nordihydroguaiaretic acid (NDGA), so that this single compound constitutes 4–10% of the dry weight of the leaf in samples taken from Deep Canyon (Greenfield et al., 1987). The resin also contains a range of flavonoid aglycones (Mabry et al., 1977; Rhoades, 1977a). In addition, Seigler et al. (1974) recorded the presence of a range of wax esters (even-numbered, C_{46}–C_{56}), most of which were from the external surface of the stems, but some of which were probably also on the leaves.

In the present experiments, *B. argentatus*, even after 20 hr without food, fed only on *Larrea*; none of the other plants from the habitat was eaten, and they were almost invariably rejected by the insect without biting. Clearly the insect responds to the surface chemicals or to the odors of the plants. The importance of the surface resin is confirmed by the fact that a chloroform extract of the surface induced the insects to bite and nibble at leaves of *Simmondsia* to which it was applied and to feed on sucrose-impregnated glass-fiber disks. The extract used in these experiments contained 50% dry weight of NDGA, which itself was a stimulant over a range of concentrations from 0.005–20% dry weight of the disks. The experiments in which NDGA was presented without sucrose suggest that it is a biting factor since, although virtually all the test disks were bitten, very little was ingested. These experiments were carried out with GF/C disks, not GF/A as in all other experiments, so it is possible that the failure to ingest was related to the physical differences of the disk, although we consider this unlikely.

The hexane surface extract was also phagostimulatory at 5% dry weight. This extract contained less than 0.025% NDGA, which may have produced the positive effect, although the presence of other phagostimulants cannot be excluded. Although the methanol surface extract contained NDGA at relatively high concentrations, it was not phagostimulatory. Presumably the effects of the NDGA were offset by unidentified deterrents.

The behavioral observations using a chloroform extract show that palpation and biting are induced by tarsal contact and/or olfaction. The much stronger response on contact suggests that contact chemoreception of the resin is of primary importance, but a response to odor occurred in one of three experiments, so that an olfactory response cannot be ruled out, especially since the odor presented was that of the surface extract and so will, presumably, have contained only a proportion of the many volatiles known to be present in *Larrea* (Mabry et al., 1977). An electroantennogram could be recorded from an antenna stimulated by the odor of leaves, so the odor was perceived by the insect, although the antennogram did not differ in magnitude from that produced by the leaves of nonhost plants (Wyatt, unpublished).

When presented with nonhost plants, nearly all the *B. argentatus* left the plants without attempting to feed or even palpating. This may have been due to the lack of specific phagostimulants characteristic of the host or to deterrent effects of the nonhosts. The behavioral experiments with *Encelia* and *Hyptis* provided no evidence of rejection, although the longer-term disk tests show that the surface extracts of all the nonhosts, including *Encelia* and *Hyptis*, contained feeding deterrents. This was true even of the two grass species tested, but on these more than half the insects did feed on the test disk, whereas with extracts of most other plants no feeding occurred at all. Mature grasses are generally low in biochemically active plant secondary compounds (Bernays and Barbehenn, 1987), although some strains of *Cynodon* are cyanogenic (this was not tested), and it is significant that the only nonhost on which *B. argentatus* made sustained attempts to feed was the grass *Aristida*. In this case, the combination of the fibrous food and specialized mandibles of the insect (Chapman, unpublished) apparently resulted in a mechanical barrier to feeding.

We conclude that the specificity of *B. argentatus* to *Larrea* involves a positive response to NDGA. This is the first example of an acridid adapted to respond positively to a characteristic host-plant chemical, although probably other instances are to be expected in other monophagous species (Bernays and Chapman, 1978). This parallels the suggestion by Rhoades (1977b) that the *Larrea* resin provides feeding cues to the monophagous caterpillar of the moth *Semiothisa colorata*. The specific response to NDGA in *Larrea* is complemented by the deterrent responses to surface compounds of nonhost plants, a combination similar to that exhibited by many relatively specific phytophagous insects (Chapman and Bernays, 1977). This is not to suggest that the chemical

cues are solely responsible for the specificity of *B. argentatus*. The insect is cryptic in *Larrea* foliage, and it probably responds to visual features of the plant (Otte, 1981).

Ligurotettix coquilletti exhibits a disjunct oligophagy. Otte and Joern (1975, 1977) recorded seven plants in the gut contents including *Larrea*, *Atriplex*, *Simmondsia*, and grass, and Ball et al. (1942) recorded feeding on *Franseria* (=*Ambrosia*) *dumosa*. Diet breadth, however, was more restricted within any one locality (Otte and Joern, 1977). In the current experiments, this species occasionally nibbled at *Simmondsia*, but did not eat it even when it was coated with the surface extract of *Larrea*. It did, however, eat *Atriplex*, *Lycium*, and *Fagonia* as well as *Larrea*. Populations are known to exist in areas in which *Larrea* is absent, and *Atriplex* and *Lycium* are the dominant shrubs (Greenfield, personal communication); clearly it is not dependent on any one plant species.

Methanol extracts of *Larrea* leaf surface were deterrent to *L. coquilletti*, even that from a good bush normally inhabited by this species. This extract was, however, less deterrent than that from a normally uninhabited bush. Rhoades (1977a) observed that the resin content of *Larrea* leaves from unoccupied bushes was, on average, higher than that of occupied bushes. This was also true in our case, but in the experiment the two extracts were present in similar dry weight concentrations (22–23%) on the glass-fiber disks. Our experiment suggests that the significant difference to the insects is the composition of the resin rather than, or perhaps, as well as, its quantity on the leaf. The disks from the good bush contained 4% dry weight of NDGA, compared with 6% in the disks from the bad bush. Over this range of concentrations, the deterrent effect of NDGA changes rapidly (Figure 1), and this could account for the differences in amounts eaten. Greenfield et al. (1987), in a parallel study of *L. coquilletti*, recorded longer meals on foliage with low NDGA–resin ratios.

The hexane extract of *Larrea* leaves is phagostimulatory. The compound responsible has not been identified, although low levels of NDGA, similar to those in the extract, are phagostimulatory.

L. coquilletti is cryptic on the stems of *Larrea*, and Otte and Joern (1977) point out that it is equally cryptic on the stems of *Atriplex* and *Simmondsia*. The same is true of *Lycium*. This suggests that visual cues may be important in host-plant selection by this species, although concealment on the nonhost, *Encelia*, seems equally effective (Greenfield, personal communication). If so, the chemical aspects of selection may be permissive rather than providing key sign stimuli. That the insect is not dependent on specific chemical signals, at least in the initial stages of selection, is suggested by the frequency with which biting and nibbling occur on nonhost plants. These are all ultimately unpalatable, but the deterrence in many cases depends on the internal constituents of the plant, not primarily on its surface properties.

Cibolacris parviceps is polyphagous (Otte and Joern, 1977; Rhoades,

1977a; Joern, 1979), feeding on a wide range of plants from numerous families. In the current experiments, all the extracts of *Larrea* leaf surface, as well as NDGA, were deterrent. Rhoades (1977a) and Schultz et al. (1977) showed that this species feeds preferentially on the oldest *Larrea* leaves, presumably those with the lowest resin contents. Removal of the resin with ether greatly enhanced the palatability of both young and old leaves and eliminated the differential between them (Rhoades, 1977a). The implication is that *C. parviceps* is able to tolerate *Larrea*, rather than having a specific response to it.

Bernays and Chapman (1978) suggest that polyphagous Arididae are, in general, less sensitive than oligophagous species to deterrence by plant secondary compounds, some of which may even become phagostimulatory. Host range and amounts eaten by these insects are determined by the balance between phagostimulatory and deterrent properties of a plant for that species of grasshopper. The results with *C. parviceps* are consistent with this thesis, although the meager data do not allow any more certain conclusions to be drawn.

All three species of grasshopper studied are Gomphocerinae (Jago, 1971), a subfamily comprised almost entirely of grass-feeding taxa. Otte and Joern (1977) discuss the evolutionary pressures that may have led to the current habits of these *Larrea*-feeding species. They envisage a series of steps in which insects fed on shrubs when grasses were no longer available, through the ability to feed entirely on a range of shrubs, and finally to specialization on *Larrea* associated with its high level of spatial and temporal predictability. It seems evident that the chemical relationships between the insects and the plant are largely a consequence of the pressures of desertification. *B. argentatus* have evolved a dependence on the plant, and at least one specific chemical is stimulatory over a wide range of concentrations. In contrast, it seems that both *L. coquilletti* and *C. parviceps* tolerate *Larrea*, but the requirement of *L. coquilletti* for suitable stems for concealment has perhaps become associated with a more limited tolerance of other plants than is true for *C. parviceps*. Thus the first exhibits oligophagy, the second polyphagy.

Acknowledgments—We are greatly indebted to Michael Greenfield for sharing his knowledge of *Larrea* as a grasshopper habitat with us, and to Alan and Vic Muth for making the facilities at Deep Canyon freely available to us. We also appreciate the assistance received from Drs. G. de Boer, K. Downum, I. Kubo, D. Light, D. Stanley-Samuelson, and Mr. R. Wrubel. Thanks are due to the UK AFRC Wain Fellowships for financial support of T.W.

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