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What Protects *Lonchocarpus* (Leguminosae) Seeds in a Costa Rican Dry Forest?

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ABSTRACT

The seeds of the six native species of *Lonchocarpus* (Leguminosae) trees in the tropical dry forest of Santa Rosa National Park in northwestern Costa Rica are rejected by all species of vertebrate seed predators. *Liomys salvini* (Heteromyidae) mice, the primary vertebrate seed predators in this habitat, were found to die of starvation rather than eat *Lonchocarpus* seeds in captivity; in contrast, some of the species of *Lonchocarpus* seeds are preyed upon by the larvae of host-specific bruchid beetles (*Ctenocolum* spp.). *Lonchocarpus costaricensis* seeds were found to be rich in at least four nonprotein amino acids and a polyhydroxypyrrolidine alkaloid. However, when this potentially toxic alkaloid, and thus potential deterrent to the mice, was added to a laboratory chow diet at concentrations approximating that in the seeds, the mice consumed the adulterated chow with no effect or only slight effect. The *L. costaricensis* seeds also contained seven kinds of flavonoids. When these potentially toxic molecules were added to lab chow as single compounds or in pairs, at concentrations approximating those found in the seeds, the mice readily consumed the adulterated laboratory chow. However, when a mix of flavonoids that represented the entire flavonoid profile of an intact seed in concentration and kind was added to laboratory chow, this diet was absolutely rejected by the mice until death by starvation. The flavonoids, rather than the peculiar small nitrogenous molecules in the *L. costaricensis* seeds, are at least one basis for seed rejection by (and thus, protection from) the mice. However, there is still the opportunity for other as yet unrecorded secondary compounds in the seed to also be a basis for seed rejection by the mice.

SEED CHEMISTRY is the evolutionary and ecological consequence of multiple challenges to a seed and its seedling. However, the relationship of a seed's chemistry to these multiple challenges is complex. The chemicals that defend a seed are also under selection for other functions such as nutrient storage, prefabricated defenses for the growing seedling, internal biochemical and physiological regulatory processes, hardness, density, and volume. Different portions of the chemical defenses of a seed are the result of both past and present selection by different species of seed predators, many of which no longer pose a threat. Ecological fitting (Janzen 1986a) plays a significant role in determining the chemical composition of a seed that is found by a potential seed predator and in determining which potential seed predators will confront a particular seed. That is to say, the ecology of most seed-seed predator rela-

tionships is in great part a function of the colonization history of a habitat and subsequent ecological adjustment by both members of the relationship, rather than the result of *in situ* evolution or coevolution. Finally, except for a few seeds protected only by a hard and/or poisonous container, each species of seed contains one to many kinds of chemicals that have the potential for deterring or poisoning a given seed predator (Janzen 1978). Simultaneously, many species of seed predators threaten a given species of seed. These seed predators range from monophagous insects (e.g., Janzen 1980) to extremely generalist vertebrates (e.g., Hallwachs 1986). Each of these animals will have different responses to a given suite of seed chemicals; one species' poison may quite literally be another species' food when it comes to seed predation (e.g., Janzen 1981, Birch *et al.* 1985, Bleiler *et al.* 1988).

While the evolutionary history of the chemical interface between seeds and seed predators is extraordinarily complex, the contemporary reality is

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that there are patterns of seed rejection and acceptance that correlate well with seed chemistry (e.g., Janzen 1977; Janzen *et al.* 1976, 1977). Here we examine the chemical basis for one such pattern: the rejection of *Lonchocarpus* seeds by vertebrate seed predators.

All six of the native species of *Lonchocarpus* trees in the deciduous and semievergreen dry forests of Santa Rosa National Park in northwestern Costa Rica mature their indehiscent, dry and wind dispersed fruits during the first half of the dry season. Adult trees normally produce hundreds to tens of thousands of fruits. The litter near a parent tree is commonly overlain by tens of the conspicuous flat fruits per m². The seeds in these fruits are often eaten by the larvae of several species of *Ctenocolum* bruchids (Janzen 1980). *Lonchocarpus* seeds are therefore unambiguously edible to at least one group of animals.

However, the Santa Rosa terrestrial habitat is also rich in vertebrate seed predators—mice, deer, curassows, collared peccaries—and these animals ignore the fallen mature *Lonchocarpus* fruits. The developing seeds of five of the six species are even ignored by the parrots that inflict severe seed predation on other legume trees in the same habitat (20 years of unpublished field notes, DHJ). *Lonchocarpus* seeds are large and soft, and enclosed in an innocuous and conspicuous papery to fibrous dry fruit that is easily torn or penetrated. It therefore appears to be the seeds' chemical traits that cause them to be rejected by the vertebrate seed predators that encounter them. However, which chemical traits produce this effect? To examine this question, we focus here on Santa Rosa's most omnipresent potential seed predator, the spiny pocket mouse (*Liomys salvini*), a small heteromyid rodent, and ask what stops it from eating *Lonchocarpus* seeds.

THE HABITAT AND ITS ORGANISMS.—The field component of this study occurred in Santa Rosa National Park (Boza & Mendoza 1981, Janzen 1986c, Fleming 1988) in the lowland tropical dry forest in northwestern Costa Rica (300 m elevation; 35 km N of Liberia, Guanacaste Province; 1978–1986). This park is contained within the larger Guanacaste National Park Project (Janzen 1986c). Most observations were made on organisms from the areas locally known as the Bosque Húmedo and Bosque San Emilio (e.g., Janzen 1986b), which lie along the paved road from the Interamerican Highway to the Administration Area of Santa Rosa. The forest in this sector of Santa Rosa is a complex fine-scale mosaic of many ages of secondary succession (1–

400 years old) in abandoned pastures and fields. Santa Rosa receives 900–2400 mm of rain per year (Janzen 1987), with a six month rain-free period from about December to mid-May. The general area has been the subject of numerous other studies of animals, plants and their interactions (e.g., Janzen 1983, Hallwachs 1986, Fleming 1988).

THE TREES.—There are six species of *Lonchocarpus* with native breeding populations in Santa Rosa National Park (Janzen & Liesner 1980): *L. costaricensis* Pittier (scattered through all ages of forest succession, throughout the park), *L. acuminatus* (Schlectendal) Sousa (common in early to mid-age secondary succession in the central uplands of the park), *L. minimiflorus* Donnell-Smith (common in young to mid-age secondary succession in the central uplands of the park), *L. orotinus* Pittier (common in natural disturbance sites in the lowland western end of the park), *L. rugosus* Benthham (common in oak forest at the eastern end of the park), and *L. eriocarinalis* Micheli (common on rocky lowlands in the western end of the park). These names are in agreement with upcoming revisions of *Lonchocarpus* (M. Sousa, pers. comm.), are unambiguously applied to each of the six different species in the park, and are based on vouchers deposited in the Herbarium of the Missouri Botanical Garden, St. Louis, Missouri, and the Instituto de Biología, México City. In the host list for Costa Rican dry forest bruchids that prey on *Lonchocarpus* seeds (Janzen 1980), *L. minimiflorus* was incorrectly identified as *L. nitidus* and *L. orotinus* was incorrectly identified as *L. parviflorus*; if *L. nitidus* and *L. parviflorus* are valid species names, they occur nowhere near Santa Rosa (M. Sousa, pers. comm.). *L. costaricensis* appears to be restricted to the dry northwestern corner of Costa Rica (Nicoya Peninsula and the Guanacaste Province mainland) but the other five species are widespread in the dry Neotropics (M. Sousa, pers. comm.).

The Santa Rosa *Lonchocarpus* are all medium-sized trees. *Lonchocarpus costaricensis*, *L. rugosus* and *L. eriocarinalis* grow to 20 m in height and individuals may live at least 100 years. *Lonchocarpus acuminatus*, *L. orotinus*, and *L. minimiflorus* grow to 5–15 m height and are abundant members of woody secondary succession (e.g., Janzen 1986c); they generally live to 20–40 years of age. All six of these species of *Lonchocarpus* are bee-pollinated and deciduous in the dry season.

THE SEEDS AND FRUITS.—Santa Rosa *Lonchocarpus* seeds range from about 0.05 g (*L. minimiflorus* and

L. rugosus) to 0.2 g (*L. costaricensis* and *L. eriocarinalis*) (Janzen 1977) but are highly variable in weight within and between conspecific seed crops. *Lonchocarpus* seeds are so soft that they can easily be broken or split with the fingernail. The seed coat is paper thin, red to brown in color, and contains some tannins (PGW, pers. obs.). The contents of the yellow to beige cotyledons feel oily when crushed, seemingly owing to the liquid in small pockets scattered through the tissue; the oil content of *Lonchocarpus costaricensis* seeds may be as high as 30 percent dry weight (PGW, pers. obs.).

The 1–6-seeded fruits range from 3 to 12 cm in length. They are flat, dry, somewhat fibrous, and show no morphological indication of ever having been part of a "bait" for a frugivorous animal in their evolutionary past. They disperse by being blown off the tree (e.g., Janzen 1982b, 1986b; Augspurger 1984; Augspurger & Hogan 1983), and the fruit is released from the tree by breaking at its base. The fibrous fruit wall is easily torn by a human and could be easily opened by any vertebrate seed predator.

THE POTENTIAL VERTEBRATE SEED PREDATORS.—Santa Rosa's dry forest is rich in species of vertebrates (Wilson 1983) that prey on seeds and are inclined to open or crush fruits in search of seeds to eat. All of them are potential *Lonchocarpus* seed predators. Among these are six species of parrots (Psittacidae), great curassows (*Crax rubra*), capirs (*Tapirus bairdi*), collared peccaries (*Tayassu tajacu*), agoutis (*Dasyprocta punctata*), tepiscuintles (*Agouti paca*), white-tailed deer (*Odocoileus virginianus*), variegated squirrels (*Sciurus variegatoides*), and spiny pocket mice (*Liomys salvini*). The latter is the most omnipresent seed predator in the habitat, with densities ranging from 8 to 80 per ha among years (e.g., Janzen 1986b). Despite the fact that *Lonchocarpus* seeds are of a seed size regularly eaten by these vertebrates, there is no indication that any of them feed on mature *Lonchocarpus* seeds in Santa Rosa, except that small parrots in flocks (*Aratinga canicularis*) were observed (DHJ, pers. obs.) eating enormous numbers of immature *L. minimiflorus* seeds (well before they were ready for dispersal) from a stand in the Santa Rosa Administration Area. However, the seed crops of other species of *Lonchocarpus* are not generally fed on by parrots. Wild collared peccaries and agoutis at feeding stations ignore *Lonchocarpus* seeds when they are mixed in with other species of seeds that they do eat (W. Hallwachs, pers. comm.; DHJ, pers. obs.). A captive tepiscuintle rejected all six species of *Loncho-*

carpus seeds both in their fruits and cleaned of their fruits (Hallwachs, pers. comm.).

THE POTENTIAL INVERTEBRATE SEED PREDATORS.—With the exception of an undescribed weevil (Curculionidae) that is on rare occasions found feeding on *L. acuminatus* seeds, five species of *Ctenocolum* bruchids are the only seed predators that have been reared from hundreds of samples of wild-collected *Lonchocarpus* seeds and fruits in Santa Rosa (Kingsolver & Whitehead 1974, Janzen 1980). This habitat contains over a hundred other species of bruchids and weevils that prey on seeds (these are usually legume seeds; Janzen 1980), and hundreds of species of bugs (Hemiptera) and moth caterpillars (Lepidoptera) that feed on other species of seeds. *Ctenocolum* are restricted in Santa Rosa and elsewhere to preying on the seeds of *Lonchocarpus* and its sister genera *Piscidia* and *Dalbergia* (Kingsolver & Whitehead 1974, Janzen 1980).

The eggs of *Ctenocolum* bruchids are glued singly to the fibrous wall of the mature or maturing fruit before the fruits disperse, and the larvae mine through the fruit wall into the seed. They require 1–2 months to emerge as adults, and then most individuals of most species spend the remainder of the year hiding in the forest and waiting for the next year's fruit crop (DHJ, pers. obs.). In the laboratory, some of the newly emerging adults will mate and oviposit on fruits or seeds, and therefore maintain continuous generations. However, there is no sign of such behavior in the field during the second half of the dry season (though in some species of *Lonchocarpus* the first beetles to emerge from eggs laid at the beginning of maturation of the fruit crop in December may start a second generation in February or March). Even if the adult beetles were capable of oviposition, further generations cannot occur during the rainy season because *Lonchocarpus* seeds rot or germinate when soaked by the first rains. Therefore, no mature fruits or seeds are available to the adult bruchids in this season.

MATERIALS AND METHODS

To explore the causes of vertebrate rejection of *Lonchocarpus* seeds in the field, seeds were offered to wild mice in captivity, and then potentially deterrent chemicals were extracted from the seeds and mixed into laboratory chow that was offered to the mice as food.

MICE.—The spiny pocket mice (*L. salvini*) used in this study were live-trapped in the secondary succes-

sional forests in the immediate vicinity of Bosque Húmedo and Bosque San Emilio. These forests are rich in adult *L. costaricensis*, *L. minimiflorus* and *L. acuminatus* and during normal foraging the mice would have encountered their seeds. The mice search for other species of seeds on the forest floor, eat some as they are found, and cache some in their underground tunnels for later consumption; they are therefore likely to harvest many more of a desirable species of seeds than they can eat in a meal (Fleming 1983; Janzen 1982a, 1986b). Before placing the seed in a pouch, the mouse usually removes it from a fruit husk (or animal dung) and leaves the husk behind. If a mouse were to harvest a *Lonchocarpus* seed, the dry fruit is large enough that the seed would be removed and the conspicuous dry fruit husk left behind. In 8 years of live-trapping more than 2000 individual mice on numerous occasions, only twice has a *Lonchocarpus* seed (*L. minimiflorus*) been found in a mouse's pouch (DHJ, pers. obs.); such seeds probably represent exploratory harvest (Janzen 1986b) rather than willingness to consume *Lonchocarpus* seeds.

FEEDING TRIALS.—Each mouse was housed in its own cubical screen cage (approximately 30 cm on a side). Cages were kept in an open air laboratory in the Administration Area of Santa Rosa, at air temperatures approximating those in the forest (18–33°C during the 24 hour cycle), but 0–6°C warmer than in their underground burrows in the daytime and during some nights. Each mouse made its own nest out of shredded toilet tissue and had excess drinking water and food. Mice were randomly chosen for treatments from among those available, or were chosen for their body weight and/or sex in those cases where these traits were matched. They were weighed each morning.

The mice normally fed at night and slept all day, unless they had rejected the diet and were therefore starving; a starving mouse would eat acceptable food at any time. "Rejection," as used here, means absolute refusal to consume the seed or seed fragments. Diet rejection can be initially difficult to detect because the mice often chip the rejected seeds into many small pieces; the mouse is apparently searching for edible subparts. Careful weighing of the chipped up seed material was not possible for each mouse because it was mixed with bedding, litter, and feces. However, when care was taken to accumulate all the chips, they added up to the initial seed weight. Furthermore, the mouse quickly stops chipping up rejected seeds and simply ignores the seed parts.

Pregnant or lactating mice were not used in feeding trials. Some of the laboratory feeding trials began on the day of a mouse's capture. Other mice began their *Lonchocarpus* experimental diets after they had been maintained in the laboratory on seeds of wild plants and laboratory rat chow, as participants in other feeding experiments (e.g., Janzen 1981). When the latter mice were used, they were used only after they had fully recovered any body weight that had been lost in a previous experiment. Previous history is not analyzed in detail here because throughout the study there was no indication that the previous diet type affected a healthy mouse's responses to *Lonchocarpus* seeds or to *Lonchocarpus* chemical derivatives. However, spiny pocket mice can physiologically "adapt" to some other species of "toxic" seeds, such as acorns (*Quercus oleoides*) (DHJ, pers. comm.). When not involved in an experiment, the mice were fed highly desired wild seeds as a staple diet (seeds of *Enterolobium cyclocarpum* and *Acrocomia vinifera*, cf. Janzen 1981, 1982c, 1986b).

In numerous cases the mice were taken off a rejected experimental diet before they could die of starvation, in order to minimize the number of mice that had to be removed from Santa Rosa's wild mouse population. Such mice are indicated in the data tables as "dying" and there was no reason to starve them to death. Mice were considered to be dying when they were losing weight at the same rate as if they had no food and they had reached a body state whereby it was evident that they would be dead within 12 hours if not given acceptable food. The latter condition is characterized by piloerection, drunken staggers, slow steps, half-closed eyes, and an emaciated appearance. These relatively objective traits are easily recognized by DHJ who initially starved many individual mice to death to "experimentally demonstrate" that a mouse who was "apparently" rejecting a diet was in fact doing so, and would not suddenly "change its mind" and start feeding after a certain number of days of rejecting a food type. Throughout the feeding trials with *L. salvini*, it was clear that once a mouse reaches the condition whereby it will die if not fed, its body weight does not decline further before death (DHJ, pers. obs.). The weight of a dying mouse at the time of being taken off a diet is therefore treated as equal to the weight of the mouse had it died. The detailed responses of these mice to starvation, and their ability to recover from starvation, will be described elsewhere.

The data from individual experimental animals are recorded here. Such information on this species

TABLE 1. Water, alkaloid and nonprotein amino acid content (percent dry weight) for the seeds of the six species of *Lonchocarpus* native to Santa Rosa National Park, Costa Rica (and see Fellows et al. 1979, Evans et al. 1985a, b).

Seed species	Percent water	Percent DMDP	Percent tetrahydro-lathyrine	Percent PPN ^a	Percent enduracididine ^b	Erythro-gamma-hydroxyhomo arginine
<i>Lonchocarpus costaricensis</i>	9.1	6.8	1.3	0	0	0.9
<i>Lonchocarpus acuminatus</i>	12.9	0.8	0	0.8	1.2	0
<i>Lonchocarpus eriocarinalis</i>		not available				
<i>Lonchocarpus orotinus</i>	30.1	0.9	0	1.2	1.3	0
<i>Lonchocarpus minimiflorus</i>	31.5	1.0	0	0.8	0.9	0
<i>Lonchocarpus rugosus</i>	20.8	4.0	0	0.8	0.8	0

^a 2-[2-amino-2-imidazolin-4(5)-yl] acetic acid.^b 3-[2-amino-2-imidazolin-4(5)-yl] alanine.

of wild animal was nonexistent, and though we cannot anticipate the analyses that may use these data in the future, gathering this data again would entail great effort and expense (see also Janzen 1986d).

SEEDS.—All seeds used in feeding trials were mature living seeds that had been hand-shelled out of fruits that were less than one year of age and had been stored as dry and dormant seeds. The fruits were usually collected from the ground beneath the parent after dispersal. In the cases where the fruits were collected directly from the tree crown, the fruits were always mature, dry, and ready to be dispersed by the first strong wind.

Only clean seed that was free of bruchids and bruchid damage was used in feeding trials. Spiny pocket mice also eat insects and could easily obtain nourishment from bruchid larvae inside bruchid-infested seeds. Bruchid-free seed was obtained by dropping the shelled seed into water; seeds containing bruchid larvae floated and were removed. The seeds were then dried on toweling in the sun. The amount of seed maintained in a mouse's cage was 5–10 g, which is more than twice the amount of a highly edible species of seed that was needed for a spiny pocket mouse to maintain its body weight (e.g., Janzen 1982c).

SEED CHEMICALS.—The chemicals were extracted from large samples of living seeds of *L. costaricensis* that were collected from multiple parent trees within Santa Rosa and from a forest patch 10 km north of Santa Rosa in 1983–1985. These same seeds were the basis for determinations of the chemical content of the seeds (Table 1).

The alkaloids (and uncommon amino acids) were extracted from *L. costaricensis* and the other Santa Rosa *Lonchocarpus* species by methods described in Evans et al. (1985a, b) and Fellows et al. (1979), and the flavonoids as described by Waterman and Mahmoud (1985, 1987) and Mahmoud and Waterman (1985). These chemicals were all fed to mice in Santa Rosa within a year of extraction, and are stable compounds at room temperature. Survey of seeds for nonprotein amino acids, alkaloids, and cyanogenic compounds was conducted as described in Evans et al. (1985).

The alkaloid referred to here as DMDP is 2R,5R-dihydroxymethyl-3R,4R-dihydropyrrolidine, and may more narrowly be termed a polyhydroxypyrrolidine alkaloid. It bears a close structural resemblance to the sugar fructose (in the beta-D-fructofuranose form) with the oxygen in the ring replaced by nitrogen (Fellows 1986, 1987; Fellows et al. 1986). DMDP was isolated in bulk by ion exchange chromatography (as described in Welter et al. 1976).

The flavonoids used in the feeding trials were: Flavonoid A (dimethylpraecansone B with traces of ovalichalcone), Flavonoid B (mainly 7-(3,3-dimethylallyloxy)-8-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-flavanone with some obovatol methyl ether and traces of 7-(dimethylallyloxy)-8-(3,3-dimethylallyl)-5-methoxyflavanone), Flavonoid C (mainly obovatol methyl ether with some traces of 7-(3,3-dimethylallyloxy)-8-(3-hydroxy-3-methyl-*trans*-but-1-enyl)-flavanone and 7-(dimethylallyloxy)-8-(3,3-dimethylallyl)-5-methoxyflavanone), and Flavonoid D (mainly 8-(3,3-dimethylallyl)-5,7-dimethoxyflavanone with traces of 7-(3,3-dimethylallyloxy)-8-(3,3-dimethylallyl)-5-methoxyflavanone).

TABLE 2. *Fates of Liomys salvini (spiny pocket mice), each confined to a pure diet of living mature seeds of one of the six species of Lonchocarpus native to the habitat of the mouse in Santa Rosa National Park, Costa Rica.*

Seed species	Mouse sex	Initial wt (g)	Percent weight change	Number of days	Mouse fate
<i>Lonchocarpus costaricensis</i> (April 1983)	male	41	-19	2	died
	male	62	-17	3	died
	male	53	-18	3	died
	female	32	-19	4	died
	female	52	-14	4	died
	female	55	-21	3	died
	female	47	-17	3	died
Mean			-17.9	3.1	
Standard deviation			2.2	0.7	
<i>Lonchocarpus acuminatus</i> (July 1980, April 1983)	male	63	-15	3	dying
	male	47	-19	3	dying
	male	59	-17	2	dying
	female	46	-22	6	died
	female	38	-26	7	died
	female	38	-24	4	died
	female	32	-19	2	died
Mean			-20.3	3.9	
Standard deviation			3.9	2.0	
<i>Lonchocarpus eriocarinalis</i> (December 1980, February 1983)	male	61	-23	3	died
	male	62	-16	4	dying
	male	41	-19	3	died
	female	52	-19	4	died
	female	55	-18	4	died
	female	42	-20	4	dying
	female	31	-17	3	dying
Mean			-18.9	3.6	
Standard deviation			2.3	0.5	
<i>Lonchocarpus orotinus</i> (April 1983)	male	48	-17	3	dying
	male	48	-17	3	dying
	male	46	-21	4	dying
	male	40	-20	4	dying
	female	53	-17	2	dying
	female	46	-21	5	dying
	female	45	-23	6	dying
	female	37	-16	3	dying
Mean			-19.0	3.8	
Standard deviation			2.6	1.3	
<i>Lonchocarpus minimiflorus</i> (December 1980, February 1983)	male	48	-19	3	died
	male	64	-14	3	died
	male	60	-13	3	dying
	female	30	-13	2	died
	female	23	-8	1	died
	female	44	-16	2	died
	female	50	-16	4	dying
Mean			-14.1	2.6	
Standard deviation			3.4	1.0	
<i>Lonchocarpus rugosus</i> (December 1980, February 1983)	male	63	-14	3	died
	male	51	-24	4	died
	male	62	-14	2	died
	male	47	-22	4	dying
	female	21	-14	2	died
	female	46	-17	3	died
	female	52	-19	4	died
	female	51	-22	5	died

TABLE 2. *Continued.*

Seed species	Mouse sex	Initial wt (g)	Percent weight change	Number of days	Mouse fate
Mean			-18.3	3.4	
Standard deviation			4.1	1.1	
Pooled controls eating mix of <i>Enterolobium</i> and <i>Acrocomia</i> seeds (1980-1983)					
Mean	20 males	51.6	-1.4	8	healthy
Standard deviation		8.6	0.3		
Mean	20 females	42.3	-0.2	8	healthy
Standard deviation		8.4	0.1		
Pooling these 40 mice					
Mean		46.2	-1.2	8	healthy
Standard deviation		9.3	0.2		
Given no food					
Mean	20 males	50.2	-17.1	3.1	dying
Standard deviation		8.8	3.0	1.0	
Mean	20 females	40.1	-18.3	3.4	dying
Standard deviation		8.8	3.0	1.2	
Pooling these 40 mice					
Mean		48.7	-17.2	3.2	dying
Standard deviation		9.3	2.8	1.2	

CHEMICALS IN DIETS.—Diets were prepared by mixing the dry trial compounds into dry lab chow finely ground in a coffee mill. The lab chow was 13 percent water, and diets were constituted as a percent of the dry weight of the lab chow (Ralston Purina Rodent Laboratory Chow 5001). The finely ground mix was then spread in a large pan, sprayed with a fine mist of absolute alcohol, and stirred until the entire mass was tacky. This soft material was then hand-pressed into small blocks. These blocks were air-dried and fed to the mice within 48 hours of preparation. More blocks were given to a mouse than it could possibly have consumed during the experiment.

All control diets of pure lab chow were prepared in the same manner. The alcohol treatment insures that the chemicals contaminate the interior of even the largest fragments of lab chow, since both the alkaloid and the flavonoids are alcohol soluble. The alcohol-treated control lab chow was very readily eaten by the mice (e.g., controls in Table 2).

RESULTS

FEEDING TRIALS WITH *Lonchocarpus* SEEDS.—Each of 44 adult spiny pocket mice were placed on pure diets of 1 of 6 species of *Lonchocarpus* seeds and all died or were dying within 1-7 days and after losing 8-26 percent of their body weight (Table

2). By inspection it is clear that these weight loss values are not significantly different from the values for 20 mice that were given no food. Likewise there was no difference among the species of *Lonchocarpus* as food. By contrast, 40 control mice on a mixed diet of *E. cyclocarpum* and *A. vinifera* seeds lost 1 percent of their body weight in the first 8 days and all survived (there are 6 less controls and 2 less experimental animals than initiated the experiment because these mice were eaten by snakes or wild black rats (*Rattus rattus*) that invaded the laboratory during the trials). Again, by inspection, these results are significantly different from either those when the mice were given no food or when the mice were fed *Lonchocarpus* seeds.

These results conform to hundreds of other feeding trials with other species of seeds (eaten and noneaten) in the same laboratory with the same species of mice. Spiny pocket mice cannot maintain their body weight or survive on a pure diet of *Lonchocarpus* seeds any better than they can on no diet at all. In contrast, these mice can maintain their body weight indefinitely on a pure (or mixed) diet of seeds that they readily eat (*E. cyclocarpum*, *A. vinifera*, *Cochlospermum vitifolium*, *Guazuma ulmifolia*, *Crescentia alata*, *Luehea speciosa*, and many other species of Santa Rosa trees; Janzen 1981, 1982c; DHJ, pers. comm.).

The reactions of the mice to *Lonchocarpus* seeds

were uncomplicated, and the same as those usually recorded with other species of seeds not eaten. The mice chipped up all the seeds given to them, as though searching for edible parts, and then continued about their daily routine of sleeping and searching for food until dead or dying. They repeatedly returned to the seeds and continued chipping them up in the first one to two days, but eventually ignored them. There was no evidence that they ate any of the seed material.

In preliminary experiments not detailed here, *Liomys* also ignored *L. minimiflorus* and *L. costaricensis* seeds mixed in with acceptable species of seeds in the laboratory. Likewise, when dishes of seeds of many species were set out in the Santa Rosa forest, spiny pocket mice consistently left behind the seeds of *L. costaricensis*, *L. rugosus*, and *L. minimiflorus* after what appeared to be some exploratory harvest on the first night of encounter (Janzen 1986b). Additionally, free-ranging wild *Liomys* quickly learned to leave *L. costaricensis* seeds behind when collecting mixed species of seeds from dung (Janzen 1986b).

FEEDING TRIALS WITH CHEMICALS

Alkaloids: Screening of the seeds of five of the six species of Santa Rosa *Lonchocarpus* for potentially toxic low molecular weight nitrogenous compounds disclosed no cyanogens but did locate four guanidino nonprotein amino acids (Evans *et al.* 1985a) and DMDP at high concentrations (Table 1); at least four of these five nitrogenous compounds are known to be toxic to insects (Janzen *et al.* 1977; Fellows 1986, 1987; Fellows *et al.* 1986; Evans *et al.* 1985a). The uncommon amino acids tetrahydroalathyrine and erythro-gamma-hydroxyhomoarginine were present only in *L. costaricensis* and are potentially part of the seed's defenses. However, these two compounds were not tested in this study as they are obviously not what keeps the mice from eating *Lonchocarpus* seeds, since they occur in only one of the species of the *Lonchocarpus* surveyed. PPN and enduracididine are likewise potential defensive uncommon amino acids found in the Santa Rosa *Lonchocarpus* seeds (Table 1), but were absent from *L. costaricensis*, and these are therefore not what keep the mice from eating *L. costaricensis* seeds. Finally, in an unpublished screening of numerous uncommon amino acids in *L. salvini* diets, the mice have generally shown no aversion to consuming laboratory chow adulterated up to 10 percent with uncommon amino acids (DHJ, pers. obs.). However, the alkaloid DMDP was found to be

present in *L. costaricensis* and *L. rugosus* seeds at 4–7 percent concentration and in much smaller amounts in three other species.

Spiny pocket mice feeding on a diet of laboratory chow containing 5 percent dry weight DMDP showed a small but significant weight loss over a 7–8 day period as compared to the controls on pure laboratory chow (Table 3; $t = 2.4367$, 18 df, $P < 0.05$). The mice readily consumed all of the experimental chow and in amounts approximately equal to that of the control mice. One mouse died abruptly without weight loss, and probably died of factors unrelated to the experiment.

The experimental mice became adjusted to feeding on adulterated laboratory chow during the experimental feeding but had substantial weight loss to recover when placed on pure laboratory chow. When they were again given pure laboratory chow, 9 of the 10 averaged a daily rate of weight gain of about twice that of the control mice during the same ten days (Table 3). A 4–8-day delay in a weight-gain response to a pure laboratory chow diet (*i.e.*, the control mice, Table 3) is commonplace with spiny pocket mice at Santa Rosa. The great individual variation in response to pure laboratory chow is also commonplace with these mice.

Flavonoids: It seems likely that DMDP is not the chemical that was stopping the spiny pocket mice from eating *Lonchocarpus* seeds. One of us (PGW) then searched *L. costaricensis* seeds for flavonoids and a total of 7 were isolated (Waterman & Mahmoud 1985), at an overall concentration of 9–11 percent dry weight flavonoids (PGW, pers. obs.); there may be as many as 13 more in very low concentrations (PGW, pers. obs.) (see total list in Materials and Methods). Three of these that could be obtained in relatively large amounts were first tested as a two-compound mix and separately on the mice. A mixture of equal parts of Flavonoid B and Flavonoid C was used to adulterate the laboratory chow at 10 percent dry weight flavonoids for ten mice (5 males and 5 females), and the mice eagerly ate this food as their sole diet for two days. Laboratory chow containing 10 percent Flavonoid D was also eaten readily by the same ten mice as their sole diet for two days. On both diets, the mice sustained no significant loss of weight, and clearly demonstrated that taken separately, these flavonoids did not deter feeding. Flavonoid A from the *L. costaricensis* seeds was not available in large enough amounts to be used in these trials, but was mixed back in with all the other flavonoids in the final feeding trials.

All flavonoids that had been extracted from *L.*

TABLE 3. *Effect of DMDP on Liomys salvini when incorporated at five percent (dry weight) in laboratory chow*

	Initial weight (g)	Percent weight change	Number days experiment	Percent wt change after next 10 days on laboratory chow
Experimental diet				
Male	41	-7.3	8	13.2
Male	43	-9.3	8	12.8
Male	44	-2.3	7 ^a	died
Male	41	-4.9	7 ^a	0
Male	39	-10.3	7 ^a	10.3
Female	40	-2.5	8	7.7
Female	40	-2.5	8	2.6
Female	40	-10.0	8	19.4
Female	43	-16.3	8	16.7
Female	43	-7.0	8	10.0
Mean		-7.24 ^b		10.30
Standard deviation		4.45		6.23
Laboratory chow				
Male	53	1.9	8	9.2
Male	34	14.7	8	5.1
Male	59	1.7	8	0
Male	54	-7.4	8	6.0
Male	63	-15.9	8	5.7
Female	34	11.8	8	10.5
Female	39	0	8	2.6
Female	38	-5.3	8	8.3
Female	30	3.3	8	0
Female	36	-2.8	8	2.9
Mean		0.10 ^b		5.03
Standard deviation		8.91		3.66

^a These mice exhausted their food after 7 days.^b Significantly different from experimental diet by inspection.

costaricensis seeds were then mixed back together, and this material was used to adulterate laboratory chow at 13 percent of the dry weight; this concentration was used because at that time it was believed to be the overall concentration of all flavonoids taken together (as mentioned above, the true concentration lies somewhere between 9 and 11 percent). Mice

offered only this diet refused to eat the laboratory chow and reached a moribund state due to starvation (Table 4) as rapidly as did mice given no food or a diet of pure *Lonchocarpus* seeds (Table 2).

Mice given a pure diet of ground *L. costaricensis* seed from which only the flavonoids had been extracted (Table 5) lived significantly longer than did

TABLE 4. *Effect of crude L. costaricensis flavonoids on Liomys salvini when incorporated at 13 percent laboratory chow dry weight.*

Experimental diet	Initial weight (g)	Percent weight change	Number days experiment	Mouse fate
Male	61	-23	5	dying
Male	56	-29	4	dying
Male	56	-25	5	dying
Female	40	-28	4	dying
Female	47	-26	5	dying
Female	46	-20	3	dying
Female	40	-25	3	dying
Female	41	-22	5	dying
Mean		-24.8		
Standard deviation		3.01		

TABLE 5. Response of *Liomys salvini* to a pure diet of ground *L. costaricensis* seeds with only the flavonoids extracted.

Experimental diet	Initial weight (g)	Percent weight change	Number days experiment	Mouse fate
Male	52	-23	9	died
Male	61	-13	12	thin but healthy
Female	39	-15	12	thin but healthy
Female	40	-23	9	died
Female	44	-7	13	thin but healthy
Mean		-16.2		
Standard deviation		6.87		

those given no food (Table 1) or given laboratory chow with 13 percent flavonoids (Table 4) (obvious by inspection). The three surviving mice appeared to lose at least as much weight on a diet of flavonoid-free seed meal as did mice on a diet of laboratory chow with 5 percent DMDP (Table 5 *vs.* Table 3). However, when mice were given a pure diet of ground *L. costaricensis* seed from which the flavonoids, the uncommon amino acids, and DMDP had been removed (Table 6), they still lost weight as fast as when given seed with only the flavonoids removed (Table 5).

DISCUSSION

DMDP is clearly not responsible for the fact that *Liomys* mice would rather starve to death than eat *Lonchocarpus* seeds, since they readily eat laboratory chow adulterated with it to a level equal to that occurring in *Lonchocarpus costaricensis* seeds. However, there was significant weight loss by the mice when they were restricted to a diet of laboratory chow adulterated with DMDP at the concentration (about 5%) at which it occurs in the seeds of two species of *Lonchocarpus*. Numerous other unpublished studies with *Liomys* feeding on other seeds

and artificial diets (DHJ, pers. obs.) do not aid in determining whether the DMDP-induced weight loss is biologically significant. A seed diet that initially causes a weight loss may later have no apparent negative effect, with the mouse later regaining its body weight and maintaining it on this diet. Alternatively there may be a gradual decline in body weight and eventual death. When DMDP was fed to laboratory rats in their drinking water at a rate of about 25 mg per day, it had no effect on either body weight or water intake (P. Dorling, pers. comm.). However, the *Liomys* ate approximately 2.5 mg per g body weight of DMDP per day while the rats ate 0.08 mg per g body weight per day. While DMDP is relatively ineffective against mouse gut digestive disaccharidases (compared with other glucosidase-inhibiting alkaloids of plant origin, Scofield *et al.* 1986), it has been shown to inhibit a range of other mammalian glycosidases, including enzymes of glycoprotein processing (Cenci di Bello *et al.* 1985) and this may have contributed to the weight loss we observed.

DMDP likewise does not deter *Ctenocolum tuberculatum* from eating *L. costaricensis* seeds; the larvae of this beetle have a gut alpha-glucosidase that is about 100 times as resistant to DMDP as

TABLE 6. Response of *Liomys salvini* to a pure diet of *L. costaricensis* seeds with the flavonoids, uncommon amino acids, and DMDP extracted.

Experimental diet	Initial weight (g)	Percent weight change	Number days experiment	Mouse fate
Male	57	-16	13	thin but healthy
Male	56	-9	12	thin but healthy
Female	48	-22	13	thin but healthy
Female	42	-14	12	thin but healthy
Female	53	-28	9	died
Female	51	-24	12	thin but healthy
Mean		-18.8		
Standard deviation		7.05		

is the same enzyme in the gut of the larvae of the bruchid *Callosobruchus maculatus* (Nash *et al.* 1986, Fellows *et al.* 1989). *C. maculatus* feeds on the seeds of the relatively undefended cow pea (*Vigna sinensis*) (Janzen *et al.* 1977). DMDP is highly toxic to *C. maculatus* and other insects when incorporated in their diets (Evans *et al.* 1985a, Fellows 1986). However, it is striking that the larva of the brilliantly colored neotropical moth *Urania fulgens* (Uranidae) eats the foliage of *Omphalea diandra* (Euphorbiaceae), which is rich in DMDP (LEF, pers. comm.; N. Smith, pers. comm.); this moth is restricted to *Omphalea* for its larval diet (Smith 1983).

On the other hand, there still remains the possibility that DMDP may negatively influence *Liomys* in its relative ranking of the desirability or suitability of seeds in the diet. It may also make a *Lonchocarpus* diet more energetically expensive to *Ctenocolum* or *Liomys* than if it were absent. It is reasonable to hypothesize that DMDP is one of the reasons why some of the other 100-plus species of bruchid and weevil seed predators in Santa Rosa (Janzen 1980) have not ecologically or evolutionarily shifted to preying on *Lonchocarpus* seeds.

Laboratory chow adulterated with just some of the major flavonoids was not overwhelmingly repellent to *Liomys*. However, when all the seed's flavonoids were mixed into chow at the 13 percent concentration, which is just slightly more than the 11 percent concentration at which they normally occur, they totally deterred the mice. *Lonchocarpus* seeds are not totally repellent to spiny pocket mice as shown by the fact that they will chip them up thoroughly when starving. This has, however, little or nothing to do with direct feeding because a rodent can chip up potential food with its incisors without ever taking it into its mouth.

The total set of flavonoids in a *Lonchocarpus* seed clearly does not stop *Ctenocolum* bruchid larvae from developing, even though flavonoids can be toxic to other insects (Hedin & Waage 1986). As with DMDP, the bruchids are somehow either detoxifying, avoiding, or using the flavonoids.

It is tempting to accept the flavonoids as the answer to the question of why *Liomys* do not prey on *Lonchocarpus* seeds. However, while *Lonchocarpus* seed meal with both the DMDP (and the non-protein amino acids) and the flavonoids removed was eaten, this diet still led to severe weight loss (Table 6). There are at least three possibilities. The rejection of extracted seed meal could be due to incomplete extraction of the DMDP, uncommon amino acids and/or flavonoids from the ground

seeds. However, these potentially toxic compounds would be present only in trace amounts and nothing about the feeding trials suggests that such trace amounts would cause severe weight loss. The extracted *Lonchocarpus* seed meal may still contain some toxic compounds of a quite different chemical class than that for which we assayed. The seed meal itself may be of relatively low nutrient value. All three caveats also could apply. Since the seed itself is primarily stored food reserves for the seedling, a low seed nutrient value seems unlikely unless the flavonoids, DMDP, and nonprotein amino acids (about 15% of the seed's dry weight) are themselves also seedling nutrients. A complication is added by the fact that the extraction process also removes the oils from the seed. However, the mouse should be able to compensate by simply eating more seed meal; there was no sign of such "overconsumption" by the mice whose feeding pedigrees are presented in Table 6.

Protease inhibitors and lectins (phytohemagglutinins), two widespread groups of legume seed toxins, are possible additional toxic chemicals. However, *Lonchocarpus costaricensis* and other Santa Rosa *Lonchocarpus* show only very mild trypsin inhibitor activity (Janzen *et al.* 1986). Furthermore, *Liomys* eagerly consume laboratory chow diets containing up to 25 percent soybean trypsin inhibitor and maintain their body weight in the process (Janzen 1981). On the other hand, *Liomys* mice starve to death rather than eat uncooked black beans (*Phaseolus vulgaris*) but eat cooked ones eagerly (Janzen 1981). Lectins are the only known potentially defensive compounds in black beans (e.g., Janzen *et al.* 1976) but a first screening of *Lonchocarpus* seeds for three categories of lectins (Janzen *et al.* 1986) showed no activity. Lectins, however, may be very active against one substrate and show no response to others, so a negative result in this screening is not conclusive.

In conclusion, it is clear that the flavonoids are an absolute barrier to the consumption of *Lonchocarpus* seeds by *Liomys* mice. However, DMDP may have some mild negative effect in the absence of the flavonoids and may reinforce the impact of the flavonoids. Furthermore, until the entire secondary compound profile of *Lonchocarpus* seeds is known and tested against *Liomys*, there is no way to know if the flavonoids are the only absolute deterrent of a *Lonchocarpus* seed against *Liomys*. For example, while *L. minimiflorus* and *L. orotinus* seeds are as rich in total flavonoids (Waterman & Mahmoud 1987, Mahmoud & Waterman 1985) as are the seeds of *L. costaricensis* (PWG, pers. obs.), there

is one unconfirmed report that *L. rugosus* seeds lack flavonoids (Gomes *et al.* 1981); if confirmed, this lack suggests that at least *L. rugosus* seeds contain yet another kind of repellent or toxic chemical for the mice.

This investigation of the defenses of *Lonchocarpus* illustrates two contextual problems in the interpretation of the chemical defenses of seeds. First, in contemporary time the nonpredation on *Lonchocarpus* seeds by hundreds of species of potential insect seed predators in Santa Rosa habitats may be due to the lack of either ovipositional stimulants or the right nutrient compounds, as much as due to the presence of deterrent or toxic chemicals. The secondary compounds may themselves be an important food for the seed predator, as appears to be the case with the larvae of the bruchid *Caryedes brasiliensis* metabolizing the canavanine in *Dioclea megacarpa* seeds (Rosenthal 1983, Rosenthal & Janzen 1983, Bleiler *et al.* 1988). Even on an evolutionary time scale, there is no reason to expect the interactions present to be as fully developed evolutionarily as is physiologically possible. The fact that *Ctenocolum* is restricted to *Lonchocarpus* (and *Dalbergia* and *Piscidia*) seeds (Kingsolver & Whitehead 1974) suggests that some peculiar chemical traits of these seeds are essential to the physiology of the beetle larvae; it is striking in this context that these three genera are all well known for the formation of appreciable amounts of flavonoids and related compounds (PGW, pers. obs.). Additionally, it is likely that other species of seeds (including over a hundred species of legumes in Santa Rosa, Janzen & Liesner 1980) contain chemicals that *Ctenocolum* larvae cannot tolerate or have not evolved to tolerate. We suspect that the beetle larvae are in fact using the uncommon amino acids, alkaloids, flavonoids and perhaps other secondary compounds in the seeds as dietary items; they may even need them in combination.

Second, a secondary compound is eaten as a portion of a meal. That meal may well contain items that fully or partially compensate for a detrimental impact of the secondary compound on digestive or physiological processes. For example, DMDP has been shown to have relatively little effect on laboratory mouse (*Mus musculus*) gut disaccharide digestion (Schofield *et al.* 1986) but kills cells in culture at high concentrations (Cenci di Bello 1985) and

inactivates insect gut alpha-glucosidases (Nash *et al.* 1986 and LEF, pers. obs.). The fact that DMDP does not have a severe effect on *Liomys* may be due to *Liomys* glucosidases being exceptionally resistant to inhibition (as might be expected of an animal that feeds on many species of potentially toxic seeds); laboratory chow containing so much disaccharide that partial inhibition of its digestion still leaves an absolutely large amount being digested; and/or, laboratory chow contains enough other high quality energy sources to compensate for a reduced yield from disaccharides. Equally, smaller amounts of flavonoids added to an otherwise innocuous diet of lower nutrient value than laboratory chow might deter the mice as thoroughly as did 13 percent flavonoids in laboratory chow. This is to say that it might take a considerably greater amount of chemical weaponry to protect a seed with a high nutrient value than a seed with a low nutrient value, both on a contemporary and evolutionary time scale.

This esoteric exploration of the seed defenses of a tropical seed intersects with the widespread current interest of how the specific traits of these distinctive molecules interact with cell function. DMDP, for example, inhibits enzymes required for the elaboration of the side-chains of glycoproteins (Fellows 1986). It is, therefore, in demand as a laboratory tool for probing the biochemistry of such things as cancer metastasis and the immune response. An assessment of the potential of a compound such as DMDP for clinical use, necessitating whole animal responses such as those that we report here, has been hindered by scarcity and expense of the chemicals. The present study shows that at least one mammal can tolerate high levels of DMDP in its diet for several days without irreversible adverse effects.

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