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INTRODUCTION

On the basis of their association with ants, neotropical species of the genus *Acacia* may be grouped into two broad categories. 'Ant-acacias', comprising less than 10% of the species in Central America depend in varying degree on a mutualistic association with ants of the genus *Pseudomyrmex*. The plants provide their ants with shelter in swollen stipular spines and with nourishment from foliar nectaries and nutritive structures (Beltian bodies) at the leaf tips. The ants in turn provide for the plants' protection against mammalian and insect herbivores and against neighbouring plant competitors (Belt 1874; Brown 1960; Janzen 1966, 1967). Janzen (1967) has demonstrated that *Acacia cornigera* (L.) Willd. plants cannot survive after experimental removal of their associated ant colonies.

The remaining species of *Acacia* in Central America comprise the second group, the 'non-ant-acacias'. These plants do not harbour mutualistic ant colonies, nor do they possess the various morphological features of acacias with such colonies. Survival of non-ant-acacias is presumably dependent, therefore, on other means of defence against herbivores.

After noting that the foliage of non-ant-acacias was markedly bitter to human taste, whereas that of ant-acacias was mild-tasting, Janzen (1966) proposed the following hypotheses: (i) non-ant-acacias are protected from herbivores by the presence in their foliage of toxic or repellant chemicals; (ii) symbiosis with ants has been evolved by ant-acacias as an alternative means of protection; and (iii) chemical defence has subsequently been lost in the ant-acacias, possibly because maintenance of both ant and chemical defence places an unnecessary metabolic burden on the plant. In the present paper we present evidence in favour of these hypotheses.

For experiments in the laboratory we selected *A. cornigera*, an ant-acacia, *A. farnesiana* (L.) Willd., a non-ant acacia, and *A. chiapensis* Saff., a species showing intermediate characteristics. *A. cornigera* and *A. farnesiana* are widely distributed through Central America, but *A. chiapensis* is very limited in distribution. Plants of *A. chiapensis* are normally found in nature in association with ant colonies, yet they can survive in the absence of ants; therefore, this species will be regarded as a non-ant-acacia for the purposes of this study.

Since insects known to attack unoccupied ant-acacias in Central America were unavailable for our work, the southern armyworm, *Prodenia eridania* (Cramer) (Noctuidae), was selected for bioassay of the acacias. The caterpillar of this species is highly polyphagous with an outstanding ability to detoxify insecticides (Krieger 1970; Krieger & Wilkinson 1969) and, presumably, toxic compounds encountered in its wide array of food plants

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(Gordon 1961; Krieger, Feeny & Wilkinson 1971). Any demonstrated toxicity of acacia leaves to the southern armyworm should thus be of more general interest than would be similar results obtained with a more specialized phytophagous insect.

METHODS AND MATERIALS

Experimental plants and insects

Acacia seeds were collected in the field by D.H.J. at the following locations: *Acacia cornigera*, Playa Coco, Guanacaste Province, Costa Rica, 4 April 1965; *A. chiapensis*, La Granja, Veracruz, Mexico, June 1964; *A. farnesiana*, Cotaxtla Experiment Station, Veracruz, Mexico, June 1964. Seeds were notched with a file and planted in a glasshouse. Laboratory experiments were conducted on plants at least 18 months old.

Larvae of the southern armyworm were obtained from a culture which had been maintained for several generations on first leaves of kidney bean, *Phaseolus vulgaris* L., and which had been supplied originally by the Niagara Chemical Division, FMC Corporation Middleport, New York.

Specimens of the insects and plants used in this work are preserved in the Cornell University Insect Collection (lot number 1023, sublot 9) and the Bailey Hortorium, Cornell University, respectively.

Table 1. *Artificial diet for* Prodenia eridania *larvae*

Cellulose (Alphacel)*	3·00 g	Methyl-*p*-hydroxybenzoate	0·30 g
Cholesterol	0·30 g	Ascorbic acid	0·45 g
Linolenic acid	0·15 g	Casein, vitamin-free	3·00 g
Sucrose	3·75 g	Agar, granulated*	3·75 g
Wesson's salt mixture (Wesson 1932)	0·30 g	Leaf powder†	15·00 g
Wheat germ	0·75 g	Vitamin solution (McMorran 1965)	1·5 ml
Choline chloride	0·15 g	Water	267·0 ml

* Purchased from Nutritional Biochemicals, Cleveland, Ohio.

† Leaves were freeze-dried and pulverized in a Waring blender. For control diets first leaves of *Phaseolus vulgaris* were used.

Feeding experiments with artificial diets

To minimize variation of texture, water content, and nutritional value among bioassay treatments, freeze-dried acacia leaves were incorporated into an artificial diet (5% leaf powder). The diet was a modified version of the one developed by Feeny (1968). Quantities listed in Table 1 are for preparation of 300 g of diet. The cellulose, cholesterol, and linolenic acid were mixed with a little ethyl ether, stirred thoroughly, and allowed to dry. The cellulose mixture, sucrose, Wesson's salts, and wheat germ were combined with half the water in a blender jar at room temperature. The agar was dissolved in the remaining water at the boiling point. The hot agar was poured into the blender jar, bringing the temperature of the mixture to about 65° C, after which the remaining heat-labile ingredients were added. After thorough blending the diet was poured into Petri dishes to set under refrigeration (4° C), at which temperature it was stored until used.

For each treatment twenty early fourth-instar larvae were reared on the leaf diet under constant conditions (16/8 hour light/dark photoperiod, 21° C day/19° C night, 30–40% relative humidity) in individual transparent plastic pots. An excess of diet was present at all times. The pots were half-filled with moistened vermiculite immediately prior to pupation of the larvae. Pupae were weighed and their sex determined (Butt & Cantu

1962) as soon as the pupal case hardened. Adults were weighed on emergence and paired off for mating in clear plastic boxes ($12\frac{1}{2} \times 9\frac{1}{2} \times 4\frac{1}{2}$ in.). Sections of cotton wadding saturated with dilute sucrose solution were provided for nourishment. Eggs from each female were counted and removed daily until the insect died.

Alkaloid extraction

Extraction of alkaloids from leaves of non-ant-acacias was first attempted by the method of Santizo-Mendez (1965). After failure to detect alkaloids by this method, extraction by column chromatography was attempted using a simplified version of the method of Mattocks (1961). Acacia leaves were extracted in absolute ethanol and the extract applied to a column of cation exchange resin (Dowex 50W-X, 200–400 mesh, hydrogen form; Biorad Laboratories, New York, N.Y.). The column was washed successively with ethanol and water, and any alkaloids were eluted with dilute aqueous ammonia. Chloroform extracts of the eluate were tested for alkaloids with iodoplatinate reagent (Smith 1960), cobalt thiocyanate, bromocresol green, and modified Dragendorff reagents (Punyarajun 1965).

Cyanide content of acacia leaves

The picrate paper method (Dawson 1941) was used as a qualitative test for evolved HCN. Quantitative analysis of HCN was carried out by a modification of the aeration method (Gillingham, Shirer & Page 1969). Leaves were ground immediately after picking in a Sorvall Omni-mixer micro-attachment with 100–120 mesh glass beads. It was found necessary to aspirate samples for 30 min and to run a series of standards simultaneously with plant samples. Absorbance at 500 nm was determined in a Beckman DB spectrophotometer and plotted directly against concentration to obtain a calibration plot.

β-Glucosidase activity of acacia leaves

Estimation of β-glucosidase activity was based on the method of Teas (1967). Plant material was ground as above and incubated for 20 min at 37° C in Sorensen's phosphate buffer, pH 5·3. The reaction was terminated by addition of 0·1 N NaOH.

RESULTS

Artificial diets incorporating non-ant-acacia (*Acacia farnesiana* and *A. chiapensis*) leaves proved toxic to armyworms (Table 2). The larval stage was prolonged to more than 20 days, and no larvae survived to pupate. In contrast, larvae reared on a diet containing ant-acacia (*A. cornigera*) leaves showed greater weight increases, pupated normally after 15 days and emerged as fertile adults.

Alkaloids and amines have been discovered in the foliage of many species of *Acacia* (Table 3). Since the presence of such compounds in leaves of *A. farnesiana* and *A. chiapensis* could have accounted for the toxicity of these species to *Prodenia eridania* larvae, exhaustive analyses were carried out for alkaloids and amines in the leaves of both *Acacia* species (see Methods section). No trace of such compounds was found, even though Santizo-Mendez (1965) has reported an unidentified alkaloid from *A. farnesiana* in Guatemala (Table 3). Webb (1952) failed to find alkaloids in *A. farnesiana* leaves in Australia, so that the report of Santizo-Mendez may have resulted from extraction of stems (Tunmann & Rosenthaler 1931) with the leaves.

The other class of secondary chemicals, widely represented in the genus *Acacia*, bitter in

Table 2. *Comparison of growth of* Prodenia eridania *larvae on 5% freeze-dried leaf diets of three species of acacia (initial number of larvae was twenty on each diet;* $\bar{X} \pm S.E.$)

Species	Acacia type		Mean initial larval weight (mg)	Mean peak larval weight (mg)	Number of pupae	Number of adults	Mean fecundity† (eggs/female)
Acacia farnesiana	Non-ant	I*	10·7 ± 0·3	201·3 ± 8·3	1	0	0
		II	10·3 ± 0·2	106·5 ± 3·9	0	0	0
A. chiapensis	Non-ant	I	10·3 ± 0·5	126·0 ± 5·8	0	0	0
		II	10·4 ± 0·2	112·4 ± 5·3	0	0	0
A. cornigera	Ant	I	10·3 ± 0·3	256·1 ± 12·9	18	17	73 ± 11
		II	10·3 ± 0·2	356·0 ± 36·7	18	17	405 ± 135

* Constant conditions for trials I were 24° C, 70% relative humidity, 16/8 hour light/dark photoperiod. Trials II were conducted under conditions described in the Methods section.

† Females which laid no eggs were not averaged.

taste and potentially toxic to insects, is that of the cyanogenic glycosides (Table 4). These compounds may be hydrolysed by enzymes or by dilute acids to yield a sugar and an α-hydroxynitrile, which dissociates to release HCN (Fig. 1). Qualitative tests, using the method of Dawson (1941), revealed the presence of cyanide in the leaves of both *A. farnesiana* and *A. chiapensis*, but not in the leaves of *A. cornigera*. Quantitative analyses (see Methods section) revealed that the leaves of *A. chiapensis* released approximately ten times as much HCN as did those of *A. farnesiana* and that cyanide content in both species was higher in the young than in the mature leaves (Table 5). We have extracted and purified the cyanoglycoside from *A. chiapensis*, and its characterization is now in progress. It appears to be a previously unknown compound. J. Secor and E. E. Conn (personal communication) have found both linamarin and lotaustralin in the leaves of *A. farnesiana* from Australia.

Table 3. *Reported occurrence of alkaloids and amines in the foliage of the genus* Acacia

Australia and New Zealand		
A. kettlewelliae *A. adunca* *A. harpophylla*	β-Phenylethylamine and/or derivatives	Fitzgerald, J. S. (1964). *Aust. J. Chem.* **17,** 160–2.
A. prominens section	β-Phenylethylamine and/or derivatives	White, E. P. (1944). *N.Z.Jl Sci. Tech.* (B) 25, 139–42; (1951). *ibid.* **33,** 54–60; (1954). *ibid.* **35,** 451–5.
A. acacinea	β-Phenylethylamine	
A. argentea *A. polystacha*	*N-Cinnamoyl-N-methylhistamine*	Fitzgerald, J. S. (1964). *Aust. J. Chem.* **17,** 375–8.
A. complanata	*N*-Methyltetrahydroharman, tetrahydroharman	Johns, S. R. *et al.* (1966). *Aust. J. Chem.* **19,** 1539–40.
A. phlebophylla	*N,N*-Dimethyltyramine	Rovelli, B. & Vaughn, G.N. (1967). *ibid.* **20,** 1299–1300.
A. podalyriaefolia *A. cultriformis*	Tryptamine	White (1951).
A. retinodes (?)	Nicotine	Fikenscher, L. H. (1960). *Pharm. Weekblad,* **95,** 233–5.
Twelve species	Unidentified alkaloids	White, E. P. (1944), (1951).
Americas		
A. berlandieri	*N*-methyl-β-phenylethylamine, tyramine, *N*-methyltyramine, hordenine	Camp, B. J. & Lyman, C. M. (1956). *J. Am. Pharm. Assoc.* (sci. edn), 719–21. Adams, H. R. & Camp, B. J. (1966). *Toxicon,* **4,** 85–90.
A. farnesiana	Unidentified alkaloid(s)	Santizo-Mendez, J. I. (1965). *Esc. Farm. Guatemala,* **26,** 1–5.

Qualitative tests for cyanide were carried out also on the foliage of several other species of *Acacia*, either on glasshouse-reared plants or on plants growing wild in Central America (using Dawson's (1941) test modified for the field). In all cases the non-ant-acacias were cyanide-positive, while the ant-acacias were cyanide-negative (Table 6).

Some species of acacia in Africa (Steyn 1935) and Australia (Finnemore & Gledhill 1928) have been reported to contain cyanogenic glycosides but no hydrolytic enzymes to liberate HCN. However, we tested leaves of *A. cornigera*, *A. farnesiana* and *A. chiapensis* for β-glucosidase activity (see Methods section) and found a similar level for β-glucosidase activity in all three species (Table 7).

To examine the toxicity of HCN to armyworms, artificial diets were prepared contain-

Table 4. *Reported occurrence of cyanogenic glycosides in the foliage of the genus* Acacia

Australia		
A. glaucesens	*l*-Mandelonitile-*β*-glucoside (sambunigrin)	Finnemore, H. & Cox, C. B. (1928). *J. Proc. R. Soc. N.S.W.* **62,** 369–78.
A. cunninghamii *A. doratoxylon* *A. cheelii* *A. longifolia*	Unidentified cyanoglycoside	Finnemore, H. & Gledhill, W. C. (1927). *J. Coun. scient. ind. Res. Aust.* **1,** 254. Hurst, E. (1942). *The Poison Plants of New South Wales.* N.S.W. Poison Plants Committee, Sydney.
A. paramattensis *A. pulchella* *A. paucijuga* *A. cunninghamii*	Mandelonitrile glucoside	Secor, J. & Conn, E. E. (1971), personal communication.
A. farnesiana	Unidentified cyanoglycoside	Herbert, D. A. (1922). *Philippine Agric.* **11,** 11–16.
A. farnesiana	Linamarin and lotaustralin	Secor, J. & Conn, E. E. (1971), personal communication.
Americas		
A. greggii (?)	Unidentified cyanoglycoside	*Rep. Ariz. agric. exp. Stn* (1934), No. 45, 44.
Africa		
A. lasiopetala *A. stolonifera*	Dimethylketenecyanhydrin-*β*-glucoside (acaciapetalin)	Rimington, C. (1935). *S. Afr. J. Sci.* **32,** 154–71.
A. giraffae *A. robusta*	Unidentified cyanoglycoside	Steyn, D. G. (1950). *Veldtrust*, **11,** No. 11, 15–16, 31.
A. tortilis heteracantha = *A. litakuensis*	Unidentified cyanoglycoside	Watt, J. M. & Breyer-Brandwijk, G. (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd. edn. Livingstone, Edinburgh.

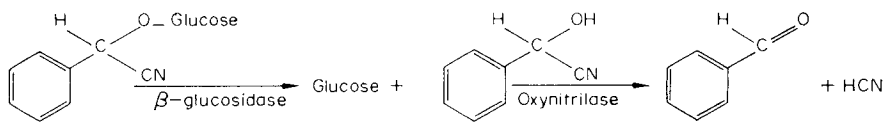

FIG. 1. Hydrolysis of a representative cyanogenic glycoside.

Table 5. *Content of cyanide in non-ant-acacia leaves* ($\bar{X} \pm S.E.$)

Species	*μ*g HCN/100 mg leaf (fresh weight)* Young leaves†	Mature leaves†	Freeze-dried leaves
Acacia chiapensis	36·3 ± 3·4	22·3 ± 0·3	11·6 ± 2·4
A. farnesiana	3·7 ± 0·5	2·9 ± 0·3	1·9 ± 0·7

* Average of three trials.

† Leaves designated 'young' were light-coloured leaves at the apex of growing tips; 'mature' leaves were darker and farther from the growing tips.

Table 6. *Occurrence of HCN in foliage of various ant and non-ant acacia species*

Species*	Type of acacia	Presence of HCN
Acacia farnesiana	Non-ant	+
A. chiapensis	Non-ant	+
A. macracantha Humb. & Bonpl.	Non-ant	+
A. cochliacantha Humb. & Bonpl. × *hindsii*†	Non-ant	+
A. cornigera	Ant	−
A. gentlei	Ant	−
A. hindsii Benth.	Ant	−
A. sphaerocephala Schl. & Cham.	Ant	−
A. collinsii Saff.	Ant	−

* *A. macracantha* was tested in the field in Mexico by D. Strong. Glasshouse-reared plants of the remaining species were tested; in addition *A. farnesiana*, *A. cornigera*, and *A. collinsii* were tested in the field in Costa Rica by D.H.J.

† Only those hybrids that were morphologically very similar to *A. cochliacantha* produced HCN; those that resembled *A. hindsii* produced no HCN.

ing the only commercially available cyanogenic glycoside, amygdalin (mandelonitrile-β-gentiobioside). Amygdalin (Nutritional Biochemicals, Cleveland, Ohio) was added to a control diet incorporating freeze-dried bean leaf (*Phaseolus vulgaris*) powder in such quantities that the HCN released approximated the levels calculated for the non-ant-acacia leaf diets above. At such levels HCN did not prove toxic to armyworms (Table 8).

Table 7. *Estimated β-glucosidase activity in acacia leaves*

Species	β-Glucosidase activity (μg *p*-nitrophenol/100 mg/20 min)
Acacia cornigera	8·0
A. chiapensis	16·0
A. farnesiana	8·0

It was considered possible that all Central American acacias may contain a chemical which is synergistic with cyanogenic glycosides but non-toxic by itself. To test this, diets were prepared by adding the above concentrations of amygdalin to a diet of ant-acacia (*Acacia cornigera*) leaves. Again these diets were not toxic to armyworms (Table 9). The HCN concentrations were then increased by as much as 100-fold, but such concentrations of HCN were not toxic to armyworms (Table 9). The assumption had been made that

Table 8. *A comparison of growth of* Prodenia eridania *larvae on artificial diets containing a cyanogenic glycoside (amygdalin) (the initial number of larvae on each diet was twenty;* $\bar{X} \pm S.E.$)

	Mean initial weight (mg)	Mean days to pupation	Mean pupal weight (mg)	Mean days to emergence	Mean adult weight (mg)
5% bean (*Phaseolus vulgaris*) leaf (control diet)	10·2 ± 0·5	12·9 ± 0·2	312·9 ± 7·4	22·7 ± 0·2	169·9 ± 6·0
5% bean leaf + 0·02% amygdalin	10·3 ± 0·7	12·7 ± 0·2	291·5 ± 5·6	23·0 ± 0·2	158·1 ± 7·7
5% bean leaf + 0·2% amygdalin	10·3 ± 0·6	12·9 ± 0·2	284·2 ± 9·6	23·0 ± 0·2	145·3 ± 7·3

since lethal concentrations of HCN were so low for vertebrates (Spector 1955), the importance of the carbonyl component of the aglycone as a potentially toxic compound was negligible by comparison. To eliminate the possibility that the unknown carbonyl components of the cyanoglycosides of non-ant-acacias were the toxic compounds, artificial diets were prepared by addition of a pure cyanogenic gum extracted from *A. chiapensis* leaves (Feeny & Rehr, unpublished) to a control diet of bean leaf powder. The carbonyl component of the aglycone was not toxic to armyworms at the levels fed (Table 10). It was confirmed by the picrate paper test that all the diets incorporating cyanogenic glycosides did indeed release HCN.

Table 9. *A comparison of the growth of* Prodenia eridania *larvae on artificial diets containing ant-acacia leaves* (Acacia cornigera) *and increasing concentrations of a cyanoglycoside amygdalin*) (*initial number of larvae on each diet was twenty;* $\bar{X} \pm S.E.$)

	Mean initial weight (mg)	Mean days to pupation	Mean pupal weight (mg)	Mean days to emergence	Mean adult weight (mg)
5% *A. cornigera*	10·3±0·5	18·3±0·7	180·3±5·7	26·8±0·9	96·5±5·9 (N = 17)
5% *A. cornigera*+ 0·02% amygdalin	10·8±0·4	15·7±0·2	219·1±3·6	27·7±0·2	108·7±2·4 (N = 20)
5% *A. cornigera*+ 0·2% amygdalin	10·8±0·5	16·4±0·3	242·6±6·2	28·5±0·3	129·7±3·7 (N = 17)
5% *A. cornigera*+ 1·0% amygdalin	12·2±0·5	15·9±0·3	231·2±6·7	28·5±0·4	111·5±5·5 (N = 20)
5% *A. cornigera*+ 2·0% amygdalin	12·2±0·5	17·3±0·6	228·9±4·9	30·2±0·6	111·5±5·2 (N = 19)

As a final confirmation that HCN alone could not account for the toxicity of non-ant-acacia leaves to armyworms, severed *A. chiapensis* leaves were allowed to stand at room temperature for 72 h until almost completely depleted of HCN. As a control, *A. cornigera* leaves were treated equally. Diets of non-ant-acacia leaves depleted of HCN proved only slightly less toxic to armyworms than cyanogenic leaves (Table 11), while air-dried ant-acacia leaves were even more palatable to the larvae than freeze-dried leaves had been.

DISCUSSION

Hydrocyanic acid, in theory, appears to be an ideal defensive chemical. Its site of action is at the cellular level, so it is potentially lethal to any organism with an electron transport system involving cytochromes. Unlike the alkaloids, in which toxicity has been established primarily for vertebrates, HCN is toxic to a wide spectrum of organisms, including plants. Indeed cyanophoric plants must conjugate their unstable α-hydroxynitriles with a sugar for self-protection. Intact cyanophoric plants release no HCN (Jones 1966), implying that the glycoside and glycosidase are stored separately within the cells or in different cells (Armstrong & Armstrong 1931). Damage to the leaf, by an herbivore for example, brings the substrate and enzyme together with subsequent evolution of HCN.

The rôle of HCN production by plants in defence against herbivores has often been suggested (Robinson 1930; Jones 1966; Conn 1969). The toxic effect on domestic animals

Table 10. *Effects on* Prodenia eridania *larvae of incorporating a cyanogenic gum extracted from* Acacia chiapensis *leaves into the artificial diet (initial number of larvae was twenty;* $\bar{X} \pm S.E.$)

	Mean initial weight (mg)	Mean days to pupation	Mean pupal weight (mg)	Mean days to emergence	Mean adult weight (mg)	Number of adults
0·02% cyanoglycoside + 5% bean leaf	11·4 ± 0·8	13·3 ± 0·5	309·4 ± 7·7	23·3 ± 0·3	157·6 ± 7·1	16
0·02% cyanoglycoside + 5% bean leaf + 0·002% emulsin	14·8 ± 0·8	11·9 ± 0·3	314·5 ± 8·3	22·4 ± 0·5	159·6 ± 6·2	19
5% bean leaf + 0·002% emulsin	14·6 ± 0·7	11·4 ± 0·3	328·1 ± 8·3	21·3 ± 0·2	164·8 ± 6·7	20

Table 11. *Comparison of growth of* Prodenia eridania *larvae on 5% diets of air-dried ant and non-ant-acacia leaves and on 5% diets of freeze-dried non-ant-acacia leaves (initial number of larvae on each diet was twenty;* $\bar{X} \pm S.E.$)

Species	Acacia type	Treatment	HCN	Mean initial larval weight (mg)	Mean peak larval weight (mg)	Number of pupae	Mean days to emergence	Number of adults
Acacia chiapensis	Non-ant	Air-dried	–	11·9 ± 0·6	234·6 ± 15·5	2	43 ± 0·0	2
A. chiapensis	Non-ant	Freeze-dried	+	10·4 ± 0·3	112·4 ± 5·3	0	–	0
A. cornigera	Ant	Air-dried	–	12·2 ± 0·5	596·9 ± 36·4	19	26·5 ± 0·3	19

and humans of high levels of cyanogenic glycosides in food plants is well documented (Seddon & White 1928; Anon 1934; Viehover 1940; Coop & Blakely 1950; Steyn 1950; Razafirmahery 1953; Moran 1954). Evidence is accumulating which suggests that cyanoglycosides are also toxic or repellant to insects. Greshoff (1907) reported that HCN evolved by *Arum maculatum* L. killed any insects which entered the plant after pollination. The presence of high concentrations of dhurrin (*p*-hydroxymandelonitrile-β-glucoside) in some varieties of *Sorghum vulgare* Pers. has been suggested as the cause of resistance to insects in those varieties (Painter 1951). The resistance of certain varieties of bush beans and lima beans to the Mexican bean beetle, *Epilachna varivestis* Muls., has been linked to high concentrations of linamarin (methylethylketonecyanhydrin-β-glucoside) (Nayar & Fraenkel 1963). Cyanogenesis in birdsfoot trefoil, *Lotus corniculatus* L., has also been implicated as a significant factor in the resistance of trefoil to a variety of fungi (Millar & Higgins 1970), and Bunge (McIlroy 1951) reported bactericidal action by some cyanogenic glycosides.

HCN is toxic to most insects. Indeed the cyanide jar is the usual means employed by entomologists for killing insect specimens. However, certain insects are capable of feeding on cyanogenic plants (Jones, Parsons & Rothschild 1962; Lane 1962). This ability depends partly on the enzyme rhodanese, which converts cyanide to thiocyanate (Parsons & Rothschild 1964). On the basis of our experiments the southern armyworm appears to be a cyanide-tolerant insect, though the mechanism of cyanide tolerance is as yet unknown.

While cyanogenic glycosides may not ensure complete protection against phytophagous insects, they appear to limit the range of insects capable of exploiting cyanophoric plants. Janzen (1967) has shown that the removal of all ants from *Acacia cornigera* plants results in defoliation of those acacias by a broad spectrum of insect species, normally host-specific to a wide variety of mimosaceous and other legumes. In contrast, the array of insects able to feed on *A. chiapensis* plants from which ants have been removed is much smaller, and significantly the insects are those adapted to feed on the non-ant species *A. macracantha*. *A. farnesiana* is not sympatric with *A. chiapensis*, but the insects feeding on it are closely related to those feeding on *A. macracantha* (D.H.J., unpublished).

In addition to their rôle of combating insect herbivores, acacia ants clear leaves of fungal spores and dust. D. H. Janzen (unpublished) has observed that ants on *A. chiapensis* are less efficient than ants of *A. cornigera* in clearing this minute debris. HCN, with its known fungicidal activity, may again function to protect the non-ant-acacia. Vine pruning is the only rôle of the acacia ants which a secondary chemical cannot conceivably fill. It is perhaps for this reason that *A. chiapensis*, a plant of wet regions where vines thrive, maintains an ant colony in addition to cyanoglycosides.

Since armyworms appear, on the basis of our experiments, to tolerate HCN but are killed on ingestion of non-ant-acacia leaves, the presence in non-ant-acacia leaves of a second toxic chemical is suggested. The presence of cyanogenic glycosides and of the additional toxic chemical appears to confer protection on non-ant-acacias against a broad spectrum of phytophagous insects, and possibly also against fungi and bacteria. Such chemical defence is not present in the ant-acacias, in which symbiosis with ants serves as an alternative means of protection.

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SUMMARY

(1) Certain species of *Acacia* in Central America gain protection against herbivores by means of a mutualistic association with ants. We have found that the foliage of other acacia species, not protected by ants, is toxic to larvae of the southern armyworm when it is incorporated into an artificial diet; diets of ant-acacia foliage, however, support normal growth.

(2) Leaves of several species of non-ant-acacia were found to be cyanogenic, while ant-acacia leaves contain no cyanide.

(3) The southern armyworm proved to be a cyanide-tolerant insect, so that the presence in non-ant-acacia leaves of an additional toxic chemical is suggested.

(4) We found no evidence of alkaloids in the foliage of the toxic non-ant-acacias.

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