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TOLERANCE OF ACRIDIDS TO INGESTED CONDENSED TANNIN

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Abstract—Four species of Acridoidea were fed on wheat leaves with and without the condensed tannin, quebracho. In no case was it deleterious to survival and growth at levels below about 10% dry weight on the food. Similarly, consumption, digestibility, and utilization of food were unaffected at up to 10% dry weight. At higher concentrations, however, the consumption and the efficiency of conversion of digestion were reduced, although digestibility was little affected. The possible mechanisms for such tolerance are discussed, and contrast made with insects which are very sensitive to ingested condensed tannin.

Key Words—Condensed tannin, quebracho, Orthoptera, Acrididae, digestibility, *Schistocerca*, *Locusta*, *Zonocerus*, *Chortoicetes*, peritrophic membrane.

INTRODUCTION

The condensed tannins are a heterogeneous group, but they share the characteristic of being potent protein precipitants (Haslem, 1966). They are flavonoid derivatives with an average molecular weight of 1200–1500 daltons (Swain, 1977). Their presence in plants is considered to be a primitive character, being distributed in the primitive plant groups which are vascular and in angiosperms which are woody, but being infrequent among the higher herbaceous angiosperms (Bate Smith and Metcalfe, 1957). When present at all, concentrations in leaves vary with the growth stage, but commonly reach levels of 1–5% (e.g., Bate Smith, 1973; Feeny and Bostock, 1968), although sometimes levels are as high as 20%, particularly in old leaves (Bate Smith, 1977).

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Ingestion of such compounds is believed generally to impair digestion by formation of complexes with food protein and with digestive enzymes (e.g., Feeny, 1970; Swain, 1977). Further, it has been proposed that such compounds may be expected in plants which are long-lived, widespread, conspicuous, or otherwise "apparent" to potential herbivores, which are thereby deterred from attacking them (Levin, 1971; Feeny, 1975; Rhoades and Cates, 1976). The present work is a study of the effects of an ingested condensed tannin on growth and development of four different acridids.

METHODS AND MATERIALS

Insects. Species used were *Schistocerca gregaria* (Forskål), *Locusta migratoria migratorioides* (R. & F.), *Chortoicetes terminifera* (Walker) and *Zonocerus variegatus* (L.). They were tested in groups for survival and growth, with and without condensed tannin. For the first two species 50 late first or early second instar nymphs from stock cultures were placed in a 64-liter cage and reared in a standard manner (Hunter-Jones, 1966). For each experiment there were three replicates of both the tannin treatment and the control treatment. For the latter two species, 20 late first instar individuals were reared in 11 liter cylindrical Perspex cages (for details of conditions see Bernays, 1978). Three pairs of experiments were run for each species. Counts of numbers present in each instar were made at regular intervals and adults were weighed within 24 hr of ecdysis.

In a separate experiment, consumption (C), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) were measured on individual insects over the whole of the last nymphal instar, in constant light and at a constant temperature of 30° C. Each insect was kept in a separate jar (350 ml) and each day feces and uneaten food were removed, the insect weighed, and weighed amounts of food given to each individual. Calculations were made on a dry weight basis (Waldbauer, 1968). In some instances, fecal samples were analyzed for condensed tannin (Swain and Hillis, 1959).

Food. In all cases the food was young wheat leaves taken approximately 14 days after germination, freshly cut and weighed. Each day, samples were dried so that the dry weight of the food given on that day could be calculated. The condensed tannin used was quebracho. In the survival experiments with *S. gregaria* and *L. migratoria* the material was purified and kindly supplied by T. Swain (ARC, Kew). For other experiments, the quebracho (Harshaw Chem. Co., Glasgow) was purified by washing thoroughly on a Sephadex column (Sigma, LH-20-100) with 50% methanol and then extracting with acetone. This extract was evaporated and the condensed tannin crystallized out. This was redissolved in 70% ethanol for application to wheat leaves. The leaves were dipped in the solution and the surface liquid allowed to evaporate in a cool air stream. The amount of tannin actually applied to the surface of leaves was regularly estimated by testing the dried, treated wheat leaves by the method of Swain and Hillis (1959). Concentrations varied from day to day by up to ±30% of the mean value, but weekly means were more consistent. A mean value of approximately 10% dry weight was used in experiments with *S. gregaria* and *L. migratoria*, of nearly 11% for *C. terminifera*, and just over 11% for *Z. variegatus*. This gave tannin–protein ratios of approximately 0.2.

Protein Digestion. Apart from the study of approximate digestibility, simple experiments were carried out to investigate the effects of the tannin on protein utilization (and hence digestion). The amounts of protein present in the food and feces were estimated by measuring total nitrogen (N) in the samples by micro-Kjeldahl analysis. To correct the fecal N values for waste N, uric acid concentrations in the feces were estimated. Ground dry feces was first extracted in methanol to remove excess tannin, which was found to interfere with the reaction. It was then extracted with 0.6% lithium carbonate and treated with uricase to oxidize the uric acid to allantoin. The decrease in absorbance at 292 nm is proportional to the uric acid concentration, so that from this the uric acid concentration in the feces could be calculated.

Tannin Distribution. The distribution of tannin in the insect after ingestion for three days was examined in the following ways. First, to see if the tannin passed through the peritrophic membrane, 20 *S. gregaria* nymphs were dissected and the midgut carefully opened so that the peritrophic membrane and its contents could be removed without rupture. Then the unwashed midgut epithelium was extracted and tested for the presence of condensed

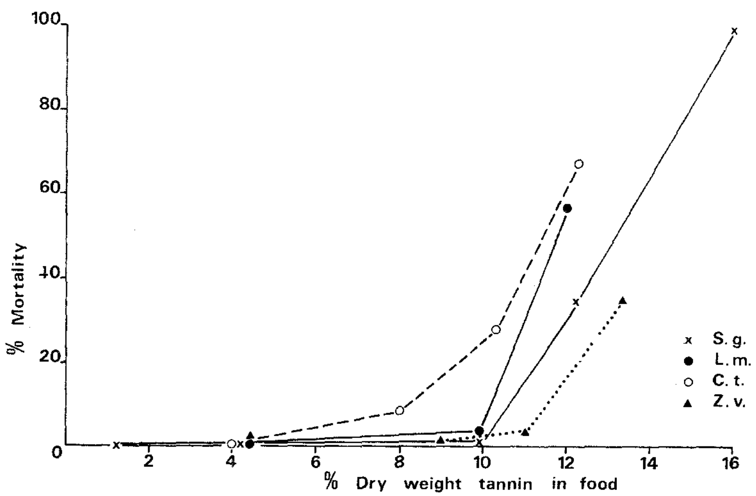

FIG. 1. Survival of insects with increasing concentrations of condensed tannin in the food. X = *Schistocerca gregaria*, ● = *Locusta migratoria*, ○ = *Chortoicetes terminifera*, ▲ = *Zonocerus variegatus*.

tannin (Swain and Hillis, 1959). Finally, fecal samples from 10 separate insects were dissected to separate the peritrophic membrane which normally surrounds the fecal pellets. The peritrophic membranes and their contents were then separately extracted and the amounts of condensed tannin associated with each were determined.

RESULTS

Survival and Growth. Survival and growth of nymphs of the four species of acridid are not affected at all by concentrations of condensed tannin below 10% dry weight. At higher concentrations, survival and the weights of the surviving adults were reduced (Figures 1 and 2). *C. terminifera* is apparently more sensitive, and *Z. variegatus* less so, than *S. gregaria* or *L. migratoria*, although the differences are not very great. Actual values and the variation at approximately 10% dry weight of tannin are shown in Table 1. This is the level at which deleterious effects begin to emerge.

Food Consumption and Utilization. At approximately 4% and 10% dry weight of condensed tannin, food consumption, digestibility, and utilization of digested food are unaffected (Table 2). At higher concentrations, however, the consumption is markedly reduced in the two species tested, approximate digestibility is slightly affected in *L. migratoria* only, while the efficiency of utilization of digested food is also greatly reduced in both species.

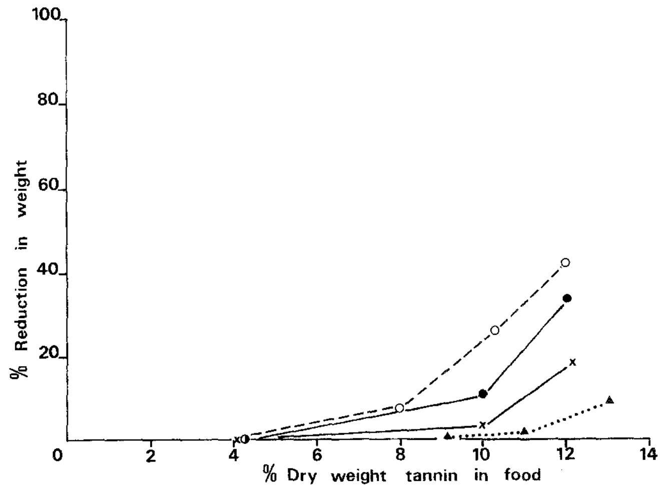

FIG. 2. The percent reduction in adult weights of insects reared on food with different concentrations of condensed tannin compared with control insects having no condensed tannin. For key to symbols see Figure 1.

TABLE 1. OVERALL PERFORMANCE OF FOUR SPECIES WHEN TANNIN IS ADDED TO FOOD

	Mean % mortality (%)			Development time (days)[b]		Adult weights[c]			
						Treated		Control	
Species	Tannin[a]	Treated	Control	Treated	Control	♂	♀	♂	♀
S. gregaria	10	35	34	33	32	1198 ± 111	1435 ± 162	1193 ± 106	1507 ± 105
L. migratoria	10.2	20	23	30	29	806 ± 113	1047 ± 138	908 ± 141	1163 ± 149
C. terminifera	10.8	52	40	35	29	187 ± 21	236 ± 32	240 ± 19	285 ± 26
Z. variegatus	11.2	35	35	90	81	510 ± 56	590 ± 47	566 ± 40	649 ± 44

[a] Percent on dry weight basis.
[b] Time for 50% of survivors to reach the adult stage.
[c] Mean values (mg) ± SE.

TABLE 2. CONSUMPTION (C), APPROXIMATE DIGESTIBILITY (AD), EFFICIENCY OF CONVERSION OF DIGESTED FOOD (ECD), AND WEIGHT INCREASE DURING LAST NYMPHAL INSTAR WITH DIFFERENT CONCENTRATIONS OF CONDENSED TANNIN IN DIET[a]

Species	Treatment	C[b]	AD	ECD	% weight increase	Instar length (mean days)
S. gregaria	controls	654 ± 24	34 ± 4	33 ± 5	76 ± 18	12.4
	4% tannin	650 ± 27	38 ± 4	37 ± 6	78 ± 16	12.2
	10% tannin	645 ± 19	35 ± 4	31 ± 5	71 ± 12	12.6
	[c] controls	623 ± 31	36 ± 5	68 ± 8	98 ± 9	
	[c] 16% tannin	343 ± 40[d]	32 ± 6	31 ± 7[d]	50 ± 9[d]	
L. migratoria	controls	591 ± 41	44 ± 2	28 ± 4	116 ± 13	11.4
	4% tannin	596 ± 30	45 ± 2	26 ± 3	110 ± 10	11.5
	10% tannin	637 ± 17	42 ± 2	22 ± 5	92 ± 10	12.8
	[c] controls	517 ± 35	41 ± 3	71 ± 13	114 ± 7	
	[c] 15% tannin	204 ± 30[d]	30 ± 2	17 ± 3[d]	31 ± 10[d]	
C. terminifera	controls	702 ± 41	38 ± 4	36 ± 4	100 ± 7	7.9
	8% tannin	643 ± 32	31 ± 5	30 ± 6	90 ± 7	8.5
Z. variegatus	controls	741 ± 16	46 ± 3	18 ± 2	102 ± 6	19.4
	4% tannin	751 ± 21	53 ± 4	16 ± 4	99 ± 11	21.0
	10% tannin	736 ± 15	50 ± 6	18 ± 4	102 ± 9	19.6

[a] 10–20 in each treatment. Mean values are given ±SE.
[b] C in amount eaten/100 mg initial wt.
[c] Experiment run for 10 days only and terminated before ecdysis; hence high ECD levels in controls.
[d] Control and treated values significantly different (*t* test, $P < 0.001$).

TABLE 3. NITROGEN (N) CONCENTRATIONS IN FOOD AND FECES OF INSECTS FED WITH AND WITHOUT TANNIN, TOGETHER WITH FECAL VALUES CORRECTED FOR URIC ACID CONTENT, AND PERCENTAGE UTILIZATION OF DIETARY N AFTER CORRECTION[a]

	Treated			Control		
	Total N (g/100 g)[b]	Total N minus uric acid N (g/100 g)	N utilization (%)	Total N (g/100 g)[b]	Total N minus uric acid N (g/100 g)	N utilization (%)
Leaves	7.6 ± 0.1			7.8 ± 0.2		
S. gregaria feces	5.0 ± 0.4	3.2	58	5.2 ± 0.3	3.3	58
L. migratoria feces	5.4 ± 0.5	3.7	51	5.8 ± 0.6	3.8	51

[a] Six determinations in each case.
[b] Mean values ±SE.

Digestion of protein was apparently unaffected by the presence of condensed tannin in the food. From the levels of N in the food and feces, the percentage uptake of dietary N was estimated at 36% and 34% in control *S. gregaria* and *L. migratoria*, respectively, while the utilization in the presence of 10% dry weight tannin was 40% and 31%, respectively. These values are increased if the N of fecal uric acid is subtracted from the total measured fecal N, but there is still no effect from the presence of tannin (Table 3).

Fate of Condensed Tannin. Quantitative measurements of condensed tannin in feces suggest that the bulk of it is passed out with the feces (Table 4). Thus when food contains 10% tannin, if the AD is 40% (the overall mean in all species), then the expected value in the feces is 14%. None of the measured values fell below 14%. No positive reaction was found for condensed tannin in the tissues of the midgut. It is thus assumed that the peritrophic membrane prevents the passage of condensed tannin through to midgut epithelium. This may be partly a simple filtration process, but the peritrophic membrane may also play another role in selectively adsorbing tannin since high proportions (always 30%) of fecal tannin were always associated with the peritrophic membrane.

DISCUSSION

Concentrations of condensed tannin below 10% had no effects on the growth or survival of the acridids but were deleterious at concentrations at and above this. The effects were similar in the four species tested, but it is interesting that the polyphagous species *S. gregaria* and *Z. variegatus* were a little less sensitive than the two graminivorous species *L. migratoria* and *C. terminifera*, which will encounter little or no condensed tannin in their natural diets.

Studies on individual insects showed that consumption, approximate

TABLE 4. CONDENSED TANNIN IN FECES AFTER FEEDING ON WHEAT WITH APPROXIMATELY 4% AND 10% CONDENSED TANNIN[a]

	4% tannin in food		10% tannin in food	
	Amount in feces (mg/100 mg)	% in peritrophic membrane	Amount in feces (mg/100 mg)	% in peritrophic membrane
S. gregaria	6 ± 1	48	15 ± 3	41
L. migratoria	6 ± 1	44	14 ± 3	33
C. terminifera	6 ± 1	43	14 ± 3	32
Z. variegatus	7 ± 1	40	15 ± 2	37

[a] Mean ±SE for 10 insects fed on test diets for 48 hrs.

digestibility, and efficiency of conversion of digested food were little affected at 10% dry weight or below. At higher concentrations, however, consumption in both *S. gregaria* and *L. migratoria* was markedly reduced as was the ECD. Approximate digestibility was little effected in *L. migratoria* and not at all in *S. gregaria*, even at these high levels. The utilization of nitrogen was also unimpaired by the addition of up to 10% dry weight of condensed tannin in the diet. These results suggest that the poor growth and survival at higher concentrations are due largely to a decrease in consumption. This in turn may lead to an increase in the level of locomotor activity (Ellis, 1951) which will thus be a major contributory factor in the reduced efficiency of conversion of digested food to body substance, since much of the absorbed material will be expended as energy. The insects are apparently reducing food intake at tannin concentrations which have little deleterious effect, at least on digestive functions, since the AD at 15% dry weight was little affected, although consumption was approximately halved.

The absence of any marked effect on digestion is unexpected. This may result partly from the very high level of protein in the diet, amounting to approximately 50% dry weight of the wheat leaves. A separate element of tannin tolerance may relate to the peritrophic membrane. The fact that this contains such a high proportion of the fecal tannin suggests that it may be of benefit by selectively adsorbing tannin so that the effective gut concentration is reduced. This can only take place behind the foregut, but even during digestion in the foregut fluid movement to and from the midgut (Baines, 1979) will ensure that tannin will also be present in the midgut soon after the start of feeding. It is also possible that some tannin is adsorbed onto the cellulose in the wheat so that the effective concentration in the food is reduced (Swain, personal communication).

Larval Lepidoptera examined so far are much more sensitive to the presence of condensed tannin. Relatively small quantities of oak leaf condensed tannin (1%) in an artificial diet severely restricted growth of the larvae of *Operophtera brumata* (Feeny, 1968), and extremely low levels of cotton condensed tannin (0.1%) in artificial diet restricted growth of *Heliothis armigera* (Chan et al., 1978). Both artificial diets were rich in protein so that these caterpillars, at least, are very sensitive. The distribution of the tannin throughout the diet in these experiments may have resulted in more thorough complexing of dietary tannin compared with the present work, but the deleterious effect is very great. In both species, condensed tannin at concentrations of less than 3% dry weight is considered to account for the relative resistance of particular ages and varieties of host plant (Feeny, 1970; Chan et al., 1978). In respect of the artificial diets used for the caterpillars above, it is difficult to invoke the protein-binding theory (i.e., reduction in available protein for growth, as suggested by Feeny, 1968) since the tannin–

protein ratio is so low that only a small fraction of the protein in the diet will be unavailable. Such a theory could be relevant in a natural situation, however, if protein levels are relatively low. Thus, the oak tree in September in Britain has protein levels of only about 12%, and the added presence of over 5% tannin could certainly make protein availability a limiting factor for growth (Feeny, 1970). For caterpillars at least, it is likely that there is some sensitivity to condensed tannin which is not explained by the protein-binding mechanism, unless the tannin selectively binds with the digestive enzymes. It is also possible that it is a potent antifeedant, since the above studies with artificial diet did not separate reduced food intake from other deleterious effects. Whatever may be the cause of the excessive sensitivity, it appears to be absent in acridids.

Levels of 10% dry weight of tannin had very little effect on acridids, and it follows that, providing protein levels are relatively high, condensed tannin in the food plant is unimportant, particularly as levels commonly do not exceed 5% dry weight in green leaves (Long, 1971), although in some species they certainly reach much higher levels, and have possibly been greatly underestimated in the past (Bate Smith, 1977). The combination of high tannin levels together with very low protein levels is more likely to have deleterious effects on digestion and growth, by severely restricting protein availability. Experiments with *S. gregaria*, however, using an artificial diet containing 18% protein and 18% tannic acid (i.e., hydrolyzable tannin) showed no effect on approximate digestibility and growth. Even when the protein level was reduced to 9% and the tannic acid kept at 18%, differences between control and test insects were not measurable after the first 1–2 days (Bernays and Chamberlain, 1980). There must be extraordinary resistance to the binding of tannin with food protein or enzymes in the gut of the locust since it is usual for a given weight of tannin to precipitate an approximately equal weight of protein (Goldstein and Swain, 1965; Feeny, 1969), at least in the prevailing gut pH of 6.5–7 (van Sumere et al., 1975). A similar resistance to both condensed tannin and hydrolyzable tannin occurs in the beetle *Paropsis atomeria* Ol. which feeds and grows well on *Eucalyptus* species with protein–tannin ratios of less than 1 and with protein concentrations of less than 10% (Fox and Macauley, 1977).

It is possible that primitive insects acquired some resistance to condensed tannin at an early stage in evolution, since the initial radiation occurred when plants were already very rich in such compounds (Bernays, 1978). The exact nature of the apparent difference between the Acridoidea, which appear to retain such resistance to tannins, and the more recently evolved Lepidoptera, which do not, remains to be found.

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