Journal of Chemical Ecology, Vol. 17, No. 12, 1991

RELATIONSHIP BETWEEN DETERRENCE AND TOXICITY OF PLANT SECONDARY COMPOUNDS FOR THE GRASSHOPPER *Schistocerca americana*

E.A. BERNAYS

Department of Entomology and Center for Insect Science
University of Arizona
Tucson, Arizona 85721

(Received July 29, 1991; accepted August 19, 1991)

Abstract—A variety of plant secondary compounds, several of which are quite widespread in nature were tested for their deterrence to the generalist grasshopper *Schistocerca americana* in short-term behavioral assays. The compounds were coumarin, salicin, tannic acid, gramine, nicotine, quinine, carvone, geraniol, abietic acid, umbelliferone, and ursolic acid. These were then tested for their post-ingestional effects over the whole of the last larval instar. Different methods were employed to mask the taste of compounds that were deterrent in order to ensure that any effects were not due to reduced feeding. In no case was there any indication of a detrimental effect or any trend suggesting one. In two cases, there was a significant increase in growth rate with the addition of the secondary compound to the diet. The evolutionary implications of these findings are discussed.

Key Words—*Schistocerca americana*, Orthoptera, Acrididae, deterrence, toxicity, plant defense, diet breadth.

INTRODUCTION

The possible evolutionary reasons for deterrent effects of plant secondary compounds on herbivores are numerous, but usually it is assumed that avoidance of chemicals or plants is an evolutionary response to some noxious quality of these materials. Yet many deterrent responses appear to be unrelated to post-ingestional toxicity (Cottee et al., 1988; Bernays and Chapman, 1987; Bernays and Graham, 1988). The question needs answering on a series of different insect species and plant compounds because, if behavioral deterrence is not associated with post-ingestional toxicity, the evolutionary reasons for the behavioral sen-

0098-0331/91/1200-2519$06.50/0

sitivity need reevaluation with respect to whether they are primarily ecological or physiological. Among grasshoppers, deterrence of chemicals in non-host plants appears to be the primary basis for rejection of plants as food (Bernays and Chapman, 1977, 1978; Chapman and Bernays, 1977; Chapman et al., 1988), and there is interest in whether rejection responses are indicators of unsuitability. The aim of this paper is to examine the behavioral effects of a range of plant secondary compounds and then to examine whether measurable noxious effects follow ingestion of them, using the acridid *Schistocerca americana* Drury. This species is polyphagous but many native American plants tested are quite unacceptable, or become so after the initial meal (Chapman and Sward, unpublished).

METHODS AND MATERIALS

Stock colonies of *Schistocerca americana* Drury (Orthoptera: Acrididae) were reared in rectangular metal cages of 64 L capacity according to the procedures used at the Centre for Overseas Pest Research (Hunter-Jones, 1961). Each day, individuals that had newly molted to the sixth instar were removed and kept in 8 L cylindrical plexiglass cages in an environment chamber kept at L:D, 12:12 and temperature, 32:28°C. They were fed on seedling wheat.

Behavioral Choice Tests. Chemicals were tested for their deterrence on 3-day-old sixth instar nymphs by presentation in choice tests on sucrose-impregnated glass fiber disks, with sucrose-only control disks for 3–4 hr at 30°C (Navon and Bernays, 1978). Experiments were also carried out in which the chemicals were presented on wheat blades, but the measurements on acceptability were done by relative amounts of leaf area removed in a 5-hr period.

Chemicals chosen for experiments were expected to be deterrent at some concentration. The ten compounds and their commercial sources are listed in Table 1. For application to wheat blades, concentrations were made up either in water with detergent, in acetone, or in alcohol, such that an appropriate dry weight concentration of the desired level was achieved. This was found to vary by about 20% around the mean value, which was considered a realistic level of variation that could occur naturally. Concentrations tested were at about the maximum recorded levels found naturally, and at one fifth of that concentration.

Performance Tests. To test the potential effects of ingestion of the plant secondary compounds, various techniques were employed. For those that had no deterrent effect on feeding behavior (tannic acid, coumarin, abeitic acid, and carvone), the material was simply added to leaves as described above (Table 1) while the controls had solvent-only treated leaves. In each experiment, each individual was fed daily on either wheat leaves with an added chemical, or control wheat leaves without the chemical. Insects were weighed and selected

TABLE 1. COMPOUNDS USED FOR THE TESTS ON BEHAVIOR AND PHYSIOLOGY OF *Schistocerca americana*, AND THEIR SOURCES

Compound	Source	Application method	
		To leaves	To insects
Phenolics			
Coumarin	Eastman	Water	On leaves
Umbelliferone	Sigma	Alcohol	s.m. capsule
Salicin	Sigma	Water	Wax coat
Tannic acid	Sigma	Water	On leaves
Alkaloids			
Gramine	Sigma	Alcohol	s.m. capsules
Nicotine (hydrogen tartrate)	Sigma	Water	s.m. capsules
Terpenoids			
Carvone	Kodak	Alcohol	On leaves
Geraniol	Kodak	Alcohol	Cyclodextrin
Abeitic acid	Sigma	Alcohol	On leaves
Ursolic acid	Sigma	Acetone	Gelatin microcaps

for each treatment in such a way that there was considerable overlap in the weight range and no significant difference between the test and control groups. They were kept individually in 5 L tubs with gauze lids. Conditions were the same as for the sorted and aged insects described above. The amounts of leaf material given to each insect each day were approximately of the same weight as the insect. In this way, most or all of the food was eaten each 24 hr except toward the end of the instar (Bernays, 1990). There were usually at least ten test and ten control insects in an experiment. The insects were weighed daily and the dry weight measured after the final ecdysis. The growth rate and efficiency of conversion of ingested food to body mass (ECI) were calculated in the usual way (e.g. Scriber, 1978).

In some cases (umbelliferone, gramine, and nicotine) insects were dosed daily using semimicro gelatin capsules prepared as described by Szentesi and Bernays (1984). The feeding regime was as described above, but twice daily a capsule containing the chemical under test, or an empty capsule, was inserted under the labrum and between the bases of the mandibles, from which it was swallowed. The amounts of the chemical in the capsules were calculated to give the approximate percentage dry weight of food assuming all food was eaten (Table 2).

With geraniol, we attempted to mask the taste by inclusion in betacyclodextrin, as described by Usher et al. (1989). This appeared to be successful

TABLE 2. EFFECTS OF COMPOUNDS ON FEEDING BEHAVIOR OF *Schistocerca americana*, NEAR OR ABOVE THEIR LIKELY MAXIMUM NATURAL CONCENTRATIONS, AND AT ONE-FIFTH OF THAT CONCENTRATION[a]

Compound	Higher concentration used (%dw)	Effect on feeding		
		Disk high	Disk low	Wheat high
Phenolics				
Coumarin	1	− (0.06)	0	0
Umbelliferone	1	− (0.03)[b]	0	− (0.05)[b]
Salicin	1	− (0.05)[b]	0	− (0.08)
Tannic acid	5	+ (0.06)	+ (0.01)[b]	0
Alkaloids				
Gramine	1	− (0.07)	+ (0.05)[b]	0
Nicotine	1	− (0.05)[b]	0	− (0.05)[b]
Terpenoids				
Carvone	0.1	+ (0.05)[b]	+ (0.025)[b]	0
Geraniol	0.01	− (0.07)	+ (0.08)	0
Abeitic acid	0.1	− (0.08)	0	0
Ursolic acid	0.1	− (0.07)	0	0

[a] + = stimulatory, 0 = no effects, − = deterrent. *P* values (less than the value in parenthesis) based on Wilcoxon's Signed Ranks test.
[b] Significant effect.

since no more food remained in the tubs at the end of each day relative to controls.

Crystals of salicin and methylene blue dye (mixed together) were wax-coated in a simplified version of the fluid-bed Wurster chamber process (see Usher et al., 1989 for details of this chamber). Crystals were placed in a hand-pulled funnel made from glass tubing 2 cm in diameter with a plug of glass wool at the bottom. Warm air was fed into the upright funnel from below, and this airstream suspended the particles at a level somewhat above the region where the diameter expanded to that of the tubing which extended about 10 cm above. From the top of the tubing, a solution of beeswax in chloroform was sprayed onto the moving particles using a chromatographic spray. The air stream was continued for about 5 min and then turned off allowing the newly-coated particles to fall onto the plug. Blue color was not observed at this stage. When the resulting wax-coated particles were extracted with chloroform, it was found that the salicin made up about 10% of the total weight. A behavioral assay was used to test deterrency by giving individuals a choice test between wax-coated cellulose and methylene blue crystals, and wax-coated salicin and methylene blue crystals, spread on sucrose-impregnated filter paper disks. The concentration was determined to be approximately 2% dw. No deterrency was detected

and subsequent dissection showed that the dye had been released in the gut. Wax-coated particles were tested for post-ingestional effects over the instar by spreading them onto leaves using a thin layer of office glue stick material. Coated cellulose (a carbohydrate that is not digested) was used as the control. Concentrations of salicin were approximately 1–3% dw of the leaf.

Finally, with ursolic acid, gelatin walled microcapsules were prepared by complex coacervation (Thies, 1986). Afterward, these were spread as an aqueous slurry onto the wheat leaves and allowed to dry. The difficulty of controlling the concentration obtained, and of variable levels of agglomeration, meant that the amounts ingested probably varied between about 0.1 and 0.5% of the dry weight of the leaf.

RESULTS AND DISCUSSION

Eight of the ten compounds deterred feeding at some concentration, although in only four cases were there significant effects at concentrations in the likely natural range, while three were significantly phagostimulatory at lower concentrations (Table 2). In no case was there any significant depression of growth rate or efficiency of conversion of food to body mass when insects ingested the chemicals over a whole instar (Tables 3 and 4). On the contrary, tannic acid and salicin appeared to cause slight increases in growth rates (Table 3). Similarly, Cottee et al. (1988) found a singular lack of oral toxicity in eight

TABLE 3. EFFECTS OF INGESTED PLANT SECONDARY COMPOUNDS ON GROWTH RATE OF SIXTH INSTAR *Schistocerca americana* NYMPHS[a]

	Test	Control	*F*	*P*
Coumarin	13.5(0.2)	13.3(0.2)	1.13	0.3618
Umbelliferone	13.8(0.2)	14.0(0.3)	1.42	0.2588
Salicin	14.7(0.2)	13.6(0.3)	4.54	0.0481[b]
Tannic acid	14.5(0.2)	13.6(0.3)	4.43	0.0400[b]
Gramine	13.4(0.3)	13.4(0.2)	0.66	0.5462
Nicotine	13.7(0.2)	13.4(0.2)	0.08	0.5611
Carvone	13.6(0.2)	13.2(0.3)	0.09	0.6302
Geraniol	13.5(0.3)	13.6(0.2)	0.93	0.3259
Abietic acid	14.7(0.3)	14.0(0.3)	1.11	0.4333
Ursolic acid	14.7(0.3)	14.8(0.3)	0.12	0.5639

[a] Values are given in mg dw increase per day, with standard error in parenthesis. *F* and *p* values are from analyses of covariance where weight gain is examined in relation to initial weight as well as treatment.

[b] Significant effects.

TABLE 4. EFFECTS OF INGESTED PLANT SECONDARY COMPOUNDS ON ECI OF SIXTH INSTAR *Schistocerca americana* NYMPHS[a]

	Test	Control	*p*
Coumarin	12.5 (0.4)	12.0 (0.5)	NS
Umbelliferone	13.1 (0.5)	12.6 (0.6)	NS
Salicin	13.3 (0.5)	12.8 (0.4)	NS
Tannic acid	14.1 (0.6)	13.0 (0.4)	<0.05
Gramine	11.9 (0.6)	12.7 (0.4)	NS
Nicotine	13.3 (0.5)	13.3 (0.4)	NS
Carvone	11.8 (0.4)	12.2 (0.5)	NS
Geraniol	12.0 (0.3)	12.4 (0.4)	NS
Abietic acid	14.5 (0.6)	13.7 (0.5)	NS
Ursolic acid	13.0 (0.5)	14.1 (0.6)	NS

[a] Number in each test and control between 10 and 15. Values are given in percentages with standard error in parenthesis. *P* values are from *t*-tests.

plant secondary compounds when tested with the related species, *Schistocerca gregaria* (the desert locust), and Bernays (1990) found no detrimental post-ingestive effects of several deterrents for *Locusta migratoria*.

The lack of postingestive effects of the secondary compounds tested precluded the establishment of a meaningful ranking so that correlation analyses between deterrence and toxicity were not really possible. Cottee et al. (1988) demonstrated a weak correlation between behavioral deterrence of compounds and their toxicity following injection into the hemolymph in both *Locusta migratoria* and *Schistocerca gregaria* (when this is re-analyzed and Spearmans rank correlation done, $p < 0.05$). On the other hand, when compounds were administered by cannulation into the midgut, no correlation was found when deterrence was compared with oral toxicity.

In a comprehensive study by Harley and Thorsteinson (1967) on another polyphagous grasshopper, *Melanoplus bivittatus*, results with steroids and alkaloids also indicate a total lack of correlation between deterrence and toxicity. Interestingly, in that study, care was taken to use plant chemicals occurring in the habitat in which the insect occurs.

Although these experiments were undertaken to examine components of fitness, the tests are obviously not definitive. Feeding on such chemicals over a lifetime may be detrimental while feeding over one instar is possibly not long enough for effects to be seen. However, it might be expected that with below optimal levels of food, additional stresses involved would render insects vulnerable to any deleterious effects of plant secondary compounds. For example, protein shortage may lead to less effective detoxification processes and water

shortage to less effective excretion via the Malpighian tubules. Experiments by Boys (1981) in which *Locusta migratoria* was dosed with gramine at several concentrations covering the natural range, showed no effects on growth rate, fecundity, or longevity. Usher et al. (1989) also found that grindelic acid was deterrent to *Schistocerca americana*, but that when the taste was masked and the food eaten, no detrimental effects could be detected. Tannic acid has been tested with the related *Schistocerca gregaria* and also found to have no detrimental effects even when it is ingested at relatively high levels for the whole of nymphal life (Bernays et al., 1980).

Perhaps, if levels are high enough, detrimental effects would ensue (Cottee et al., 1988), but there is an interesting possibility that grasshoppers are much more highly sensitive behaviorally to plant secondary compounds, than is required for reasonable protection from ingestion of them.

From the evolutionary point of view, a poor correlation between deterrency and oral toxicity would indicate that avoidance responses may often have evolved under selective pressures unrelated to post-ingestional toxicity. Similarly, the tendency toward restriction of host range observed in insect herbivores may be unrelated to their inability to tolerate plant secondary compounds. Host range may be restricted for quite diverse and ecological reasons rather than for mainly physiological reasons. In such a case, non-host plants may become deterrent and unacceptable even when they are chemically suitable for growth and development (Bernays and Chapman, 1987; Bernays and Graham, 1988).

Acknowledgments—This work was funded by NSF Grant BSR-8705014. Various students and other colleagues helped in different ways, but special thanks go to Barbara Usher, Roger Wrubel, Ray Barbehenn, Michelle Murgia, and Junji Hamai.

REFERENCES

BERNAYS, E.A. 1990. Plant secondary compounds deterrent but not toxic to the grass specialist acridid *Locusta migratoria*: Implications for the evolution of graminivory. *Entomologia Exp. Appl.* 54:53–56.

BERNAYS, E.A., and CHAPMAN, R.F. 1977. Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.). *Ecol. Entomol.* 2:1–18.

BERNAYS, E.A., and CHAPMAN, R.F. 1978. Plant chemistry and acridoid feeding behaviour, pp. 99–141, *in* J.B. Harborne (ed.). Biochemical Aspects of Plant and Animal Co-evolution. Phytochem. Soc. Europe Symp. No. 15.

BERNAYS, E.A., and CHAPMAN, R.F. 1987. Evolution of plant deterrence to insects, pp. 159, *in* R.F. Chapman, E.A. Bernays, J.G. Stoffolano (eds.). Perspectives in Chemoreception and Behavior. Springer-Verlag, New York.

BERNAYS, E.A., and GRAHAM, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69:886–892.

BERNAYS, E.A., CHAMBERLAIN, D.J., and MCCARTHY, P. 1980. The differential effects of ingested tannic acid on different species of Acridoidea. *Entomologia Exp. Appl.* 28:158–166.

BOYS, H. 1981. Food selection by some graminivorous Acrididae. D. Phil. thesis, University of Oxford, England.

CHAPMAN, R.F. and BERNAYS, E.A. 1977. The chemical resistance of plants to insect attack. *Pont. Acad. Sci. Scripta varia* 41:603–643.

CHAPMAN, R.F., BERNAYS, E.A., and WYATT, T. 1988. Chemical aspects of host-plant specificity in three *Larrea*-feeding grasshoppers. *J. Chem. Ecol.* 14:561–579.

COTTEE, P.K., BERNAYS, E.A., and MORDUE, A.J. 1988. Comparisons of deterrency and toxicity of selected secondary plant compounds to an oligophagous and a polyphagous acridid. *Entomologia. Exp. Appl.* 46:241–247.

HARLEY, K.L.S., and THORSTEINSON, A.J. 1967. The influence of plant chemicals on the feeding behavior, development, and survival of the two-striped grasshopper, *Melanoplus bivittatus* (Say), Acrididae, Orthoptera. *Can. J. Zool.* 45:305–319.

HUNTER-JONES, P. 1961. Rearing and Breeding Locusts in the Laboratory. Anti-Locust Research Centre, London. 12 pp.

NAVON, A., and BERNAYS, E.A. 1978. Inhibition of feeding in acridids by non-protein amino acids. *Comp. Biochem. Physiol.* 59A:161–164.

SCRIBER, J.M. 1978. The effects of larval feeding specialization and plant growth form on the consumption and utilization of plant biomass and nitrogen: An ecological consideration. *Entomologia Exp. Appl.* 24:695–710.

SZENTESI, A., and BERNAYS, E.A. 1984. A study of behavioural habituation to a feeding deterrent in nymphs of *Schistocerca gregaria. Physiol. Entomol.* 9:329–340.

THIES, C. 1986. How to Make Microcapsules. Course manual, Washington University, St. Louis, Missouri.

USHER, B.F., BERNAYS, E.A., BARBEHENN, R.V., and WRUBEL, R.P. 1989. Oral dosing of insects with feeding deterrent compounds. *Entomologia Exp. Appl.* 52:119–133.