Journal of Chemical Ecology, Vol. 28, No. 11, November 2002 (© 2002)

FOOD EXPERIENCE ON THE PREDATORY BEHAVIOR OF THE ANT *Myrmica rubra* TOWARDS A SPECIALIST MOTH, *Acrolepiopsis assectella*

A. M. LE ROUX, G. LE ROUX, and E. THIBOUT

Institut de Recherche sur la Biologie de l'Insecte
UMR CNRS 6035
Faculté des Science
Parc Grandmont, 37200 Tours, France

(Received January 7, 2002, accepted July 9, 2002)

Abstract—Entomophagous insects are often repelled by the secondary compounds of the plants eaten by their prey. These compounds, therefore, take on a defensive role for the phytophagous species that sequester them. Given that numerous entomophagous species are capable of learning, the effects on the foraging behavior of a repeated experience were investigated in the predatory ant *Myrmica rubra*. The sulfur amino acids methyl-cysteine sulfoxide (MCSO) and propyl-cysteine sulfoxide (PCSO) produced by *Allium* plants were identified in caterpillars of the leek moth *Acrolepiopsis assectella.* Three behavioral studies were carried out, with or without prior familiarization with caterpillars reared either on leek or on an artificial diet containing no *Allium* compounds. In choice tests with the two types of caterpillars, unfamiliarized ants displayed a preference for caterpillars reared on the artificial diet, but this preference disappeared or was reversed in both young and old ants after familiarization.

Key Words—Foraging experience, predation, ants, leek moth, prey selection, learning, *Allium*, sulfur compounds, habituation.

INTRODUCTION

Plant secondary compounds found in varying quantities in prey often lead to rejection by generalist predators. The secondary compounds, thus, have a protective role for specialist phytophagous insects (Bernays, 1989; Bernays and Cornelius, 1989; Dyer, 1995). Among the factors that can modulate this defense phenomenon are the secondary compound concentration in the plant (Bjorkman and Larsson,

* To whom correspondence should be addressed. E-mail: thibout@univ-tours.fr

0098-0331/02/1100-2307/0

1991; Hare and Eisner, 1993), the morphology of the prey (Dyer, 1995) and its defensive behavior (Bernays and Cornelius, 1989; Bjorkman and Larsson, 1991), the abundance of predators and their ability to locate the prey (Camara, 1997), or experience and learning.

The involvement of experience acquired by the predator during foraging, and observed in the bug *Podisus maculiventris* (Traugott and Stamp, 1996), has frequently been studied in ants (Dejean, 1988; Dejean et al., 1990; Fourcassié and Traniello, 1993; Hare and Eisner, 1993; Schumann and Buschinger, 1994; Dyer and Bowers, 1996). Similarly, learning seems to play a role in the predatory behavior of ants that are offered caterpillars of the leek moth, *Acrolepiopsis assectella* (Lepidoptera: Yponomeutoidea), a specialist phytophagous species of *Allium* plants (Nowbahari and Thibout, 1992). Caterpillars of the leek moth feed on the leek, *Allium porrum*, a plant that produces nonprotein sulfur amino acids, S-alk(en)yl-cysteine sulfoxides (Boscher et al., 1995) that are precursors of sulfur volatiles (Férary et al., 1998). These caterpillars are attacked markedly less often by *Formica selysi* and *Formica fusca* than those fed on an artificial diet with either little or none of these compounds (Nowbahari and Thibout, 1992).

This work addresses the following questions: Do leek moth caterpillars sequester sulfur compounds? Is it possible to generalize the dietary preference to other ant species? Can the consumption of novel or repellent prey occur as a result of familiarization during the life cycle of the predator?

METHODS AND MATERIALS

The Phytophagous Insect. The caterpillars came from two rearings. The first was carried out on the host plant, the leek (CL: caterpillars on leek), under the following conditions: 16L:8D, synchronous temperature 25°C:18°C, and 70% ± 10% relative humidity. This strain was renewed each year by caterpillars collected in leek fields around Tours, France. The second rearing was maintained for several years in the laboratory on an artificial diet containing 10% of freeze-dried leek leaf powder (Arnault, 1979), on 12L:12D, constant temperature 25°C and 70% ± 10% relative humidity. Eggs were taken from this rearing, and neonatal caterpillars were reared on an artificial diet containing neither leek powder nor leek in order to obtain caterpillars that had had no contact with leek compounds (CD: caterpillars on diet). The CL and CD used at the fifth instar, after sexual differentiation, were killed by freezing at −18°C in order to avoid escape and defensive behavior, before being introduced into the experimental device.

The Predator. The red ant *Myrmica rubra* (Hymenoptera: Formicidae), which has the ability to learn (Le Roux et al., 1997), is common in the Touraine region of France and is an omnivorous polygynous species that builds colonies of several thousand workers (Elmes and Keller, 1993). It lives in open environments such as prairies, gardens, and fairly damp wastelands and can be a predator of the leek moth. Every year, colonies were taken from the Loire riverbanks near Montlouis at the beginning of September. They were split into several groups (15–30 workers) and maintained at 12L:12D and 24°C. Each group was isolated in a nest consisting of a 15-cm-long × 1.5-cm-diam glass tube in a sleeve of black paper with a water holder at one end. The other end of the nest opened into a hunting area formed by a 5-cm-high × 8-cm-diam. cylindrical box. Twice a week, the ants received half a larva of *Tenebrio molitor* and a drop of honey in the hunting area. During the experiments, *T. molitor* and honey were replaced by two leek moth caterpillars 2 cm away from the junction between the tube and the collecting area, and 1 cm apart.

Analysis of Sulfur Amino Acid Content of Caterpillars. The two major sulfur amino acids produced by *A. porrum* are methyl-cysteine sulfoxide (MCSO) and propyl-cysteine sulfoxide (PCSO) (Freeman and Whenham, 1975). PCSO has been shown to be a repellent for formicines (Nowbahari and Thibout, 1992). The amounts of these compounds were measured in the preimaginal stages of the leek moth. Two CD taken from the diet, 2 CL taken from the leek, 2 CL starved for 5 hr, 2 chrysalids from CL, 40 exuviae from CL, and finally a 10-mg piece of green leek leaf (weight of a caterpillar is approx. 10 mg, of a chrysalis approx. 7 mg, and of an exuvia approx. 0.05 mg) were crushed in methanol. They were centrifuged for 30 min at −4°C at 6000 rpm, and the supernatant was retained. The residue obtained after evaporation of the methanol was diluted in trichloracetic acid at 6% in order to precipitate the proteins and to eliminate them by centrifugation. Amino acids were determined using derivatization coupled with reverse-phase HPLC. The Waters Pico-Tag method (Bidlingmeyer et al., 1984) used phenylisothiocyanate as a derivatization reagent (Auger et al., 1993). The HPLC system used was a Waters chromatograph consisting of a two-pump delivery system and a UV detector with a wavelength fixed at 254 nm. The column was a Waters Novapack C18 (3.9 × 300 mm). The solvent system consisted of two Waters eluents. The addition of controlled quantities of alkyl-cysteine sulfoxides synthesized in the laboratory (Auger and Thibout, 1981) allowed a quantitative study to be carried out by comparing retention time and peak areas. These operations were repeated at least 4 times (Table 1).

Experiments. All experiments were carried out under choice conditions in the ant-rearing room. An experiment began when 1 CL and 1 CD, or 2 CL, were simultaneously placed in the hunting area. A full choice test comprised five consecutive presentations of two caterpillars at 2-d intervals (D1, D3, D5, D7, and D9). Twenty-four hours after introduction, the number of caterpillars of each category taken into the nest was recorded, and their state of consumption was noted as follows: not consumed, partially consumed, or totally consumed.

Preliminary Behavioral Experiment. The ability to distinguish easily between males and females (visible testicles in the male caterpillar) was used as

TABLE 1. CONCENTRATIONS OF SULFUR AMINO ACIDS OF DIVERSE LEEK MOTH INSTARS FROM TWO REARINGS[a]

Instar	*N*	MCSO	PCSO
CD	4	0	0
CL	6	0.9 (0.3–2.7)	3.7 (0.7–5.6)
CL St.	5	t	2.1 (0.8–6.8)
NL	8	0.1 (0–1)	0.9 (0–2.0)
EL	5	7.2 (0-35.8)	62.9 (10.3–137.5)
LL	4	1.5 (0-5.9)	39.1 (13.3–70.7)

[a] Leek leaves are used as control. Values are mean (range). CD: fifth instar caterpillars reared on diet without leek; CL: fifth instar caterpillars reared on leek; CL St.: CL starved for five hour; NL: nymphs (chrysalid) from leek rearing; EL: exuviae from leek rearing; LL: Leek leaves; t: traces; *N*: number of repetitions.

a method of avoiding confusion between CL and CD, i.e., by alternately placing the CL of one sex with the CD of the other sex. In addition, the right–left placing of the caterpillars was also alternated in order to eliminate a potential effect of environmental dissymmetry. Ten groups of 30 workers were tested with some CL in order to check that *M. rubra* behaved in the same way towards males and females.

Behavior of Ants. A first experiment allowed 10 groups of 30 workers that had never been placed previously with leek moth caterpillars (unfamiliarized) to choose between CL and CD. Moreover, ten groups of 15 young workers were fed for 30 days on 2 CL every 2 d (young familiarized). A choice test was then carried out between CL and CD. Subsequent to the behavioral modifications observed after familiarization in young workers, a comparable experiment was done using old workers collected during the previous year and kept in the laboratory for 6 months. Thirty days before the choice test, the 10 groups of 20 old workers (old familiarized) were fed every 2 d with 2 CL in the same way as the young workers. The number of caterpillars taken back to the nest and their state of consumption were noted. The data were compared using a χ^2 contingency test.

RESULTS

Amount of Sulfur Amino Acids in Caterpillars. The sulfur amino acids MCSO and PCSO synthesized by the leek were not detected in the CD (Table 1). The two compounds were generally found in the preimaginal stages of CL. The CL only just taken from the leek were richer in sulfur amino acids than those starved for several hours. The chrysalids arising from rearing on leek were low in sulfur amino acids, whereas the corresponding exuviae were rich. The concentrations of sulfur amino acids measured in the leek moth do not reflect exactly the concentrations

TABLE 2. LEEK MOTH CATERPILLARS FROM TWO REARINGS CONSUMED IN 24 HR BY UNFAMILIARIZED OR FAMILIARIZED (YOUNG AND OLD) *Myrmica rubra* WORKERS, ACCORDING TO DIET OF CATERPILLARS[a]

	Unfamiliarized (N)		Young familiarized (N)		Old familiarized (N)	
Consumed	CL	CD	CL	CD	CL	CD
Totally	27	49	18	24	42	32
Partially	8	0	18	10	6	7
Not	15	1	14	16	2	11
P	< 0.001*		0.19		0.02*	

[a] Data are compared by column using χ^2. *$\alpha < 0.05$. For defiinition of CL and CD, see Table 1.

in the hostplant (Table 1). MCSO and PCSO were found in lower quantities in the phytophagous insect than in the green leek leaves, with the exception of the exuviae.

Preliminary Behavioral Experiment. The preliminary experiment concerning choice between male and female caterpillars by unfamiliarized workers showed no significant difference between the two sexes. The numbers of males and females consumed in 24 hr were not statistically different (28% vs. 26% not consumed, 22% vs. 34% partially consumed, and 50% vs. 40% totally consumed; $\chi^2 = 2.229$, $P = 0.32$). It was, thus, possible to use sexual dimorphism as a marker of the origin of *A. assectella* caterpillars.

Behavior of Workers. When the unfamiliarized ants had the choice between a CL and a CD, the number of CD totally or partially consumed was greater than that of the CL (Table 2). After early dietary familiarization with CL, the young (1-month-old) workers showed no preference for either caterpillar category (Table 2). However, in 6- to 8-month-old workers, dietary familiarization with CL led to a tendency to prefer the CL (Table 2).

DISCUSSION

The concentrations of MCSO and PCSO in the caterpillars of the leek moth were generally lower than in the plant and showed a high variability, as indicated by the minimal and maximal values. This variability could come from the hostplant itself, as, the *Allium* genus displays large variations in the concentrations of these compounds, depending on the season, the variety, the organ, or the amount of sulfur in the soil, (Lancaster et al., 1984). While the concentration of these amino acids in the phytophagous insect depends on the plant, it is not an exact reflection of this. The caterpillar contains more PCSO than MCSO, although both are particularly abundant in the cuticle since the exuviae are rich in them, whereas the chrysalids contain little. This agrees with the results obtained in certain Lepidoptera specialized on plants producing alkaloids in which the cuticle contains the majority of the alkaloids present in the caterpillars (Montllor et al., 1991). Moreover, a high quantity of the sulfur compounds found in the caterpillar must come via the digestive tract. The reduction in these compounds in starved caterpillars in comparison to those that have fed, probably comes from the presence of leek particles in the digestive tract. The accumulation of sulfur amino acids in the caterpillars of the leek moth makes plausible the previous hypothesis on the role of alkyl-cysteine sulfoxides of *Allium* in the protection of *A. assectella* against *Formica* ants (Nowbahari and Thibout, 1992). This role is confirmed in our choice tests, as the unfamiliarized *M. rubra* workers preferred the CD that had never been in contact with the leek.

After repeated encounters with the CL avoided prey, *M. rubra* workers chose their prey differently from unfamiliarized workers. They equally attacked the two categories of caterpillar and, in the case of the old workers, preferred the previously avoided caterpillars to those with which they had been familiarized, i.e., the CL. This adaptation phenomenon is not limited to any particularly sensitive period in young adults, as it is also found in old workers. Moreover it cannot be an imprinting process as has been observed several times in ants (Jaisson, 1975, 1980; Beckers et al., 1994; Schumann and Buschinger, 1994). It is more of a habituation process, which gradually leads to the disappearance and possibly to the inversion of the initial response of the unfamiliarized workers.

Based on the results obtained here, it would be interesting to study the behavior of the ants when they came into contact with the cuticle or the gut of the caterpillars that are rich in sulfur compounds.

Acknowledgments—The authors thank, in particular, J.M. Amé and N. Mandon, and also M. Bodin, S. Lepiffe, S. Lhuilier, M. Martaresche, and L. Servain for their help during the experiments. They are also grateful to Pr. A. Lenoir for comments on the manuscript and to Dr. A. Hefetz for English revision.

REFERENCES

ARNAULT, C. 1979. Influence de substances de la plante-hôte sur le développement larvaire d'*Acrolepiopsis assectella* (Lepidoptera; Acrolepiidae) en alimentation artificielle. *Entomol. Exp. Appl.* 25:64–74.

AUGER, J. and THIBOUT, E. 1981. Émission par le poireau, *Allium porrum*, de thiosulfinates actifs sur la teigne du poireau, *Acrolepiopsis assectella* Z. (Lepidoptera). *C.R. Acad. Sci. D Paris* 29:217–220.

AUGER, J., MELOUKI, F., VANNEREAU, A., BOSCHER, J., COSSON, L., and MANDON, N. 1993. Analysis of *Allium* sulphur amino acids by HPLC, after derivatization. *Chromatographia*-36:347–350.

BECKERS, R., LACHAUD, J. P., and FRESNEAU, D. 1994. The influence of olfactory conditioning on food preference in the ant *Lasius niger* (L.). *Ethol. Ecol. Evol.* 6:159–167.

BERNAYS, E. A. 1989. Host range in phytophagous insects: the potential role of generalist predators. *Evol. Ecol.* 3:299–311.

BERNAYS, E. A. and CORNELIUS, M. L. 1989. Generalist caterpillar prey are more palatable than specialists for the generalist predator *Iridomymex humilis*. *Oecologia* 79:427–430.

BIDLINGMEYER, B. A., COHEN, S. A., and TARVIN, T. L. 1984. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr. A* 336:93–104.

BJORKMAN, C. and LARSSON, S. 1991. Pine sawfly defence and variation in host plant resin acids: a trade-off with growth. *Ecol. Entomol.* 16:283–280.

BOSCHER, J., AUGER, J., MANDON, N., and FERARY, S. 1995. Qualitative and quantitative comparison of volatile sulphides and flavour precursors in different organs of some wild and cultivated garlic. *Biochem. Syst. Ecol.* 23:787–791.

CAMARA, M. D. 1997. Predator responses to sequestered plant toxins buckeye caterpillars: are tritrophic interactions locally variable? *J. Chem. Ecol.* 23:2093–2106.

DEJEAN, A. 1988. Memory effect on predatory behaviour of *Odontomachus troglodytes* (Formicidae-Ponerinae). *Behaviour* 107:131–137.

DEJEAN, A., CORBARA, B., and OLIVA-RIVERA, J. 1990. Mise en évidence d'une forme d'apprentissage dans le comportement de capture des proies chez *Pachycondyla* (= *Neoponera*) *villosa* (Formicidae, Ponerinae). *Behaviour* 115:175–187.

DYER, L. A. 1995. Tasty generalists and nasty specialists? Antipredator mechanisms in tropical Lepidopteran larvae. *Ecology* 76:1483–1496.

DYER, L. E. and BOWERS, M. D. 1996. The importance of sequestered iridoid glycosides as a defense against an ant predator. *J. Chem. Ecol.* 22:1527–1539.

ELMES, G. W. and KELLER, L. 1993. Distribution and ecology of queen number in ants of the genus *Myrmica*, pp. 294–307, *in* L. Keller (ed.), Queen Number and Sociality in Insects. Oxford University Press.

FÉRARY, S., KELLER, J., BOSCHER, J., and AUGER, J. 1998. Fast narrow-bore HPLC-DAD analysis of biologically active thiosulfinates obtained without solvent from wild *Allium* species. *Biomed. Chromatogr.* 12:104–106.

FOURCASSIÉ, V. and TRANIELLO, J. F. A. 1993. Effects of experience on food-searching behavior in the *Formica schaufussi* (Hymenoptera: Formicidae). *J. Insect Behav.* 6:287–299.

FREEMAN, G. G. and WHENHAM, R. J. 1975. A survey of volatile components of some *Allium* species in terms of S-alk(en)yl-L-cysteine sulfoxides present as flavour precursors. *J. Sci. food Agric.* 26:1869–1886.

HARE, J. F. and EISNER, T. 1993. Pyrrolizidine alkaloid deters ant predators of *Utetheisa ornatix* eggs: effect of alkaloid concentration, and prior exposures of ants to alkaloid-laden prey. *Oecologia* 96:9–18.

JAISSON, P. 1975. L'imprégnation dans l'ontogenèse des comportements de soins aux cocoons chez la jeune fourmi rousse *(Formica polyctena* Forst.). *Behaviour* 52:1–37.

JAISSON, P. 1980. Environmental preference induced experimentally in ants (Hymenoptera: Formicidae). *Nature* 286:388–389.

LANCASTER, J. E., MC CALLION, B. J., and SHAW, M. L. 1984. The levels of S-alk(en)yl-L-cysteine sulfoxides during the growth of the onion (*Allium cepa* L.). *J. Sci. Food Agric.* 35:415–420.

LE ROUX, G., LE ROUX, A. M., and BOULAY, R. 1997. Apprentissage et mémorisation lors du déménagement du couvain chez *Myrmica ruginodis* Nyl. (Formicidae) : influence de l'âge, pp. 369-373, *in* Actes 29ème Coll. SFECA, Albi, Publication Université Paul Sabatier, Toulouse, France.

MONTLLOR, C. B., BERNAYS, E. A., and CORNELIUS, M. L. 1991. Responses of two Hymenopteran predators to surface chemistry of their prey: significance for an alkaloid-sequestering caterpillar. *J. Chem. Ecol.* 17:391–399.

NOWBAHARI, B. and THIBOUT, E. 1992. Defensive role of *Allium* sulphur compounds for leek moth *Acrolepiopsis assectella* Z. (Lepidoptera) against generalist predators. *J. Chem. Ecol.* 18:1991–2002.

SCHUMANN, R. D. and BUSCHINGER, A. 1994. Imprinting effects on host-selection behaviour of colony-founding *Chalepoxenus muellerianus* (Finzi) females (Hymenoptera, Formicidae). *Ethology* 97:33–46.

TRAUGOTT, M. S. and STAMP, N. E. 1996. Effects of chlorogenic acid- and tomatine-fed caterpillars on the behavior of an insect predator. *J. Insect Behav.* 9:461–476.