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SELECTIVE PREDATION ON CHEMICALLY DEFENDED CHRYSOMELID LARVAE
A Conditioning Process

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Abstract—Laboratory experiments showed that female *Tenthredo olivacea* prefer to prey upon insects of a previously encountered species, instead of upon unknown ones. This has been observed when comparing two natural prey of the sawfly, the larvae of *Phratora vitellinae* and those of *Plagiodera versicolora.* The two species secrete copious amounts of defensive secretion, the first salicylaldehyde, and the latter a mixture of cyclopentanic monoterpenes. The predator appears less reluctant when encountering a species whose secretion has been previously experienced. A selective pressure might thus exist favoring rare secretions, which is consistant with the well-known diversity of defensive compounds among sympatric insects.

Key Words—Coleoptera, Chrysomelidae, leaf-beetle larva, *Phratora vitellinae, Plagiodera versicolora,* Hymenoptera, Tenthredinidae, sawfly, *Tenthredo olivacea,* predation, conditioning, defensive secretion.

INTRODUCTION

Many chrysomeline larvae are protected by nine pairs of eversible glands. When the insect is disturbed, droplets of secretions are produced and form a chemical barrier against enemies (Figure 1A). These secretions are chemically diverse: so far, six different methylcyclopentanoid monoterpenes, five aromatic compounds, three alkenyl acetates, one alkyl acetate, and one hydrocarbon have been identified in various species. This diversity is even greater when the proportions of the compounds are considered (Pasteels et al., 1982, 1984) and remains poorly understood. Host-plant chemistry has

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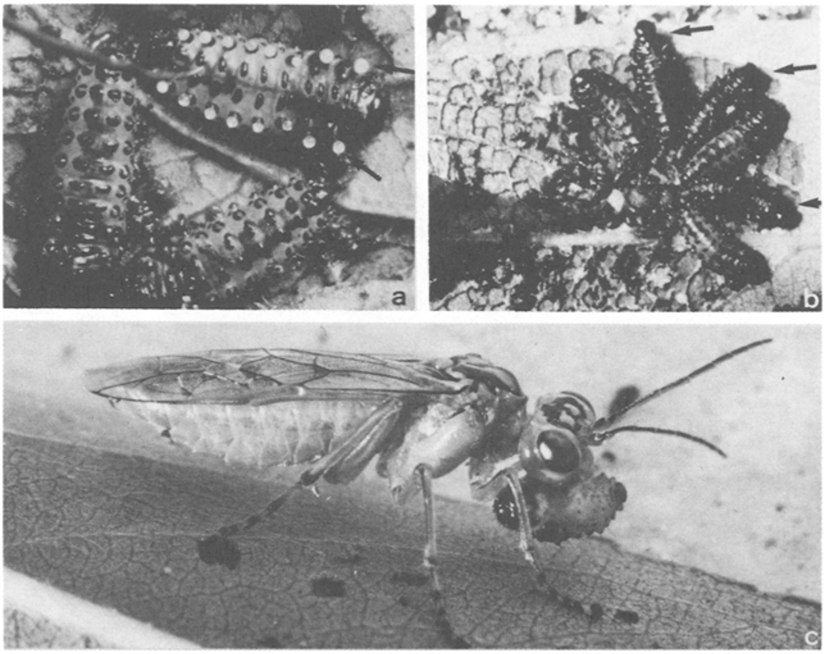

FIG. 1. (A) A third instar *Phratora vitellinae* larva has been disturbed with a seeker and has everted its abdominal glands, secreting droplets of a salicylaldehyde–water emulsion (arrows) (× 10). (B) Larvae of *Phratora vitellinae,* and *Plagiodera versicolora* (arrows) forming a common aggregate on a *Salix* leaf, in the laboratory. Larvae of both species are more or less the same size and shape and look very much alike (× 5). (C) A female *Tenthredo olivacea* holding freshly captured prey (*Phratora vitellinae*) in its mandibles (× 5).

some influence (Rowell-Rahier and Pasteels, 1982; Pasteels et al., 1983b), but the selective pressure exerted by predators remains totally ignored. Generalized predators like ants are effectively repelled, whatever the nature of the secretion (Blum et al., 1972, 1978; Pasteels et al., 1983b; Sugawara et al., 1978; Wallace and Blum, 1969). Other insect predators, however, are able to overcome the chemical defense of chrysomelid larvae and even seem to specialize on these prey (Fabre, 1891; Clausen, 1940; Jolivet, 1950; Whitehead and Duffield, 1982).

We demonstrate here that at least one of these predators, the female sawfly, *Tenthredo olivacea* Kl., shows some preference between sympatric chrysomeline larvae. This differential predation, however, is not intrinsic, but depends on previous experiences with a given prey species.

METHODS AND MATERIALS

The sawflies were observed and field-collected in a poplar nursery belonging to the Rijksstation voor Populierenteelt, at Geraardsbergen, Belgium. Female adults were kept singly in the laboratory, in Petri dishes (30 cm diameter) half filled with moist plaster. They were fed with full grown chrysomelid larvae, *Plagiodera versicolora* Laich. and *Phratora vitellinae* (L.) These larvae fed on a *Salix* leaf fastened in the dish so that the sawfly had access to its two sides.

During the first three days, the sawflies were fed with only one or the other chrysomelid species. Three larvae were given the first day and the consumed larvae replaced each morning. The number of larvae consumed was counted four to five times a day, and the following morning before the replacement of missing larvae. During the 4th day, the sawflies were given the choice between three larvae of both species, and the missing larvae were replaced after each count.

RESULTS

Female adult sawflies were observed in a nursery exploring the leaves of young poplars to find prey and to oviposit. These poplars were infested with the chrysomeline larvae, *P. vitellinae,* which were preyed upon by the sawflies. While feeding (Figure 1C), the predators completely chewed the larvae and left only small pellets of cuticle. Behaving in this way, they cannot avoid being contaminated by the copious secretions of a salicylaldehyde–water emulsion (Wain, 1943).

In other circumstances, *T. olivacea* was observed exploring *Salix* spp. infested by larvae of another chrysomeline, *P. versicolora.* This latter species has not been observed in the nursery. Both chrysomelines, however, are sympatric, and they have been observed in other locations feeding on the same *Salix* tree. In the laboratory at least, they even form common aggregates (Figure 1B). They look very much the same, but *P. versicolora* differs considerably from *P. vitellinae* in the chemistry of its defensive secretion, producing a water emulsion of a mixture of two methylcyclopentanic monoterpenes, plagiodial and plagiolactone. The precise composition of the secretion depends on the geographical origin of the larvae. In larvae collected in Belgium, plagiodial amounts to 70% of the volatiles and plagiolactone for 30% (Pasteels et al., 1982).

Predation on third-instar larvae of both chrysomelines by female sawflies collected in the nursery was compared in the laboratory. The sawflies were divided into two groups of 30 and fed during three days with larvae of either *P. vitellinae* or *P. versicolora.* In all, 217 *P. vitellinae* larvae, and only 168 *P. versicolora* larvae were consumed ($P < 0.001$, χ^2). This could suggest that the latter species is better protected from *T. olivacea* predation, its defensive secretion being more efficient. A closer look at the results, however, indicates another possibility.

A comparison of the daily consumption rate in the two groups shows that, during the first and second days, predation was much higher on *P. vitellinae* than on *P. versicolora* (Figure 2). This indicates that at first the sawflies were reluctant to feed on *P. versicolora.* With nothing else to eat

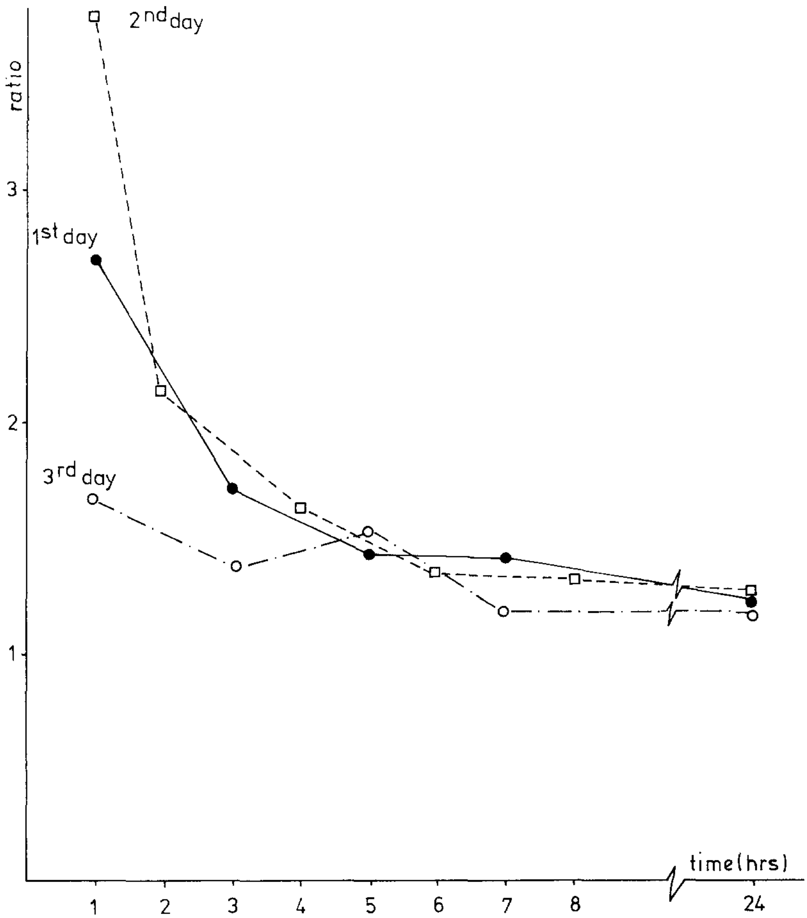

FIG. 2. Ratio of the number of *Phratora vitellinae* to the number of *Plagiodera versicolora* preyed upon by two groups of *Tenthredo olivacea,* during three successive days.

they finally attacked *P. versicolora,* with the result that the differences in consumption decreased from the beginning to the end of the day. Interestingly, the behavior of both groups of sawflies was less dissimilar at the start of the day 3, even if the predation rate on *P. versicolora* did not reach that on *P. vitellinae.* This suggests that the distaste for *P. versicolora* larvae could decrease after previous experience with these larvae. As the sawflies were collected in a poplar nursery heavily infested by *P. vitellinae,* but in

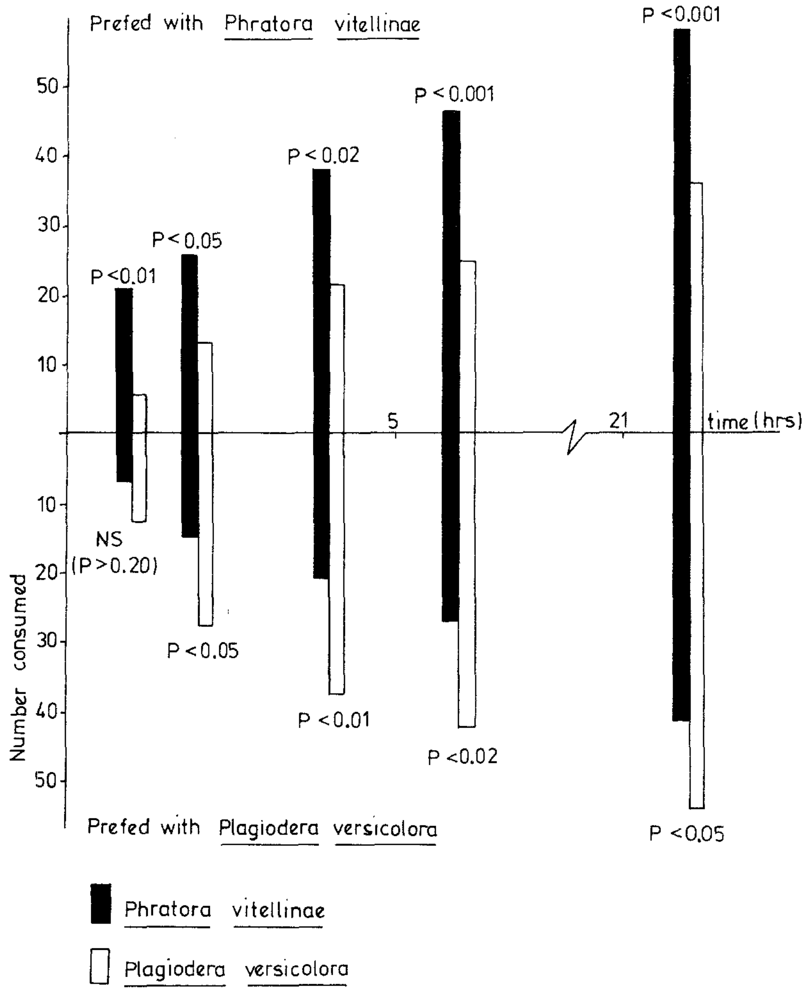

FIG. 3. Differential predation by *Tenthredo olivacea* prefed with the one or the other prey. Black bars: *Phratora vitellinae;* white bars: *Plagiodera versicolora.* Probabilities were calculated by means of the χ^2 distribution.

which *P. versicolora* was absent or rare, their marked preference for the first could be due to some conditioning process rather than being intrinsic.

This latter hypothesis was tested by giving the sawflies the choice between both species (three larvae of each) during the 4th day. The results were striking: the sawflies prefed with *P. vitellinae* larvae showed, as before, a preference for this prey, but those prefed with *P. versicolora* preferred *P. versicolora* (Figure 3). These results demonstrate that prey selection in this predator is not absolute but depends upon previous experience.

DISCUSSION

It is known that conditioning may influence food choice of phytophagous insects (Jermy et al., 1968) and host choice of parasitoid insects (Arthur, 1981). Differential predation due to learning is well known among vertebrates (Tinbergen, 1960; Holling, 1961). The formation of a "search image" has supposedly led to polymorphism in cryptic, nonchemically defended prey species (ref. in Edmunds, 1974). Similarly, vertebrate predators learning to recognize and avoid aposematic, chemically defended prey have been considered as exerting a strong selective pressure towards mimicry.

This is the first time that food conditioning has been demonstrated in an insect predator and for chemically defended prey. We have no indication, so far, that *T. olivacea* actually develops a search image of the larvae on which they prey. The defensive secretion could act as an obvious signal to recognize a specific prey. We were unable, however, to demonstrate that the defensive secretion becomes an attractant or a phagostimulant for the predator. It simply appears that the sawflies were less hesitant when confronted with the secretion after frequent exposure to it. The conditioning process appears to be an habituation, as recently described for some phytophagous insects repeatedly exposed to plant deterrents (Jermy et al., 1982).

So far, we have been unable to experiment with naive insects. Adults are difficult to obtain in the laboratory, due to an obligatory diapause stage. It is not possible to assess the strength of the conditioning when adults are fed from emergence with only one kind of prey. Our experiments indicate, however, that in nature, where diversity of prey is always available, conditioning is reversible. This reversible conditioning seems advantageous for the predator. It allows it to tolerate the chemical defenses of abundant prey, without being strictly dependent on a particular species which could become scarce. It is also possible that conditioning to abundant prey could lower the density of major competitors for the sawfly larvae, which also feed on poplar leaves.

Despite their spectacular chemical defense, chrysomeline larvae are preyed upon by 42 European insects belonging to 17 families and five orders (Fabre, 1891; Clausen, 1940; Jolivet, 1950). If habituation leading to prey preference proved to be frequent, a strong selective pressure would exist for the chemical polymorphism of defensive secretions commonly observed in insects (ref. in Pasteels et al., 1983a). It would be premature, however, to draw general conclusions from experiments with a single predator: in phytophagous insects, it has indeed been demonstrated that repeated exposure to feeding deterrents can lead either to habituation or aversion learning (Jermy et al., 1982).

It should be stressed that mimicry and polymorphism in chemical defense, which lies beyond external appearance, are in no way mutually exclusive. These two strategies could be used simultaneously by the same species: mimicry against vertebrate predators, long-lived and with a high learning capacity, and chemical polymorphism against arthropod predators able to become tolerant to defensive compounds. In fact, the two prey species studied here may well be using both strategies simultaneously.

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REFERENCES

Arthur, A.P. 1981. Host acceptance by parasitoids, pp. 97–120, *in* D.A. Nordlund, R.L. Jones, W.J. Lewis (eds.). Semiochemicals. Wiley, New York.

Blum, M.S., Brand, J.M., Wallace, J.B., and Fales, H.F. 1972. Chemical characterization of the defensive secretion of a chrysomelid larva. *Life Sci.* 11:525–531.

Blum, M.S., Wallace, J.B., Duffield, R.M., Brand, J.M., Fales, H.M., and Sokoloski, E.A. 1978. Chrysomelidial in the defensive secretion of the leaf beetle, *Gastrophysa cyanea* Melsheimer. *J. Chem. Ecol.* 4:47–53.

Clausen, C.P. 1940. Entomophagous Insects. McGraw-Hill, New York, pp. 19–20.

Edmunds, M. 1974. *Defence in Animals.* Longman, New York, pp. 7–61.

Fabre, J.H. 1891. Souvenirs Entomologiques, Vol. 4. Delagrave, Paris, pp. 173–190.

Holling, C.S. 1961. Principles of insect predation. *Annu. Rev. Entomol.* 91:293–320.

Jermy, T., Bernays, E.A., and Szentesi, A. 1982. The effect of repeated exposure to feeding deterrents on their acceptability to phytophagous insects, pp. 25–32, *in* J.H. Visser and Z.K. Mink (eds.). Proceedings 5th International Symposium Insect–Plant Relationships. Pudoc, Wageningen.

Jermy, T., Hanson, F.E., and Dethier, V.G. 1968. Induction of specific food preference in lepidopterous larvae. *Entomol. Exp. Appl.* 11:211–230.

Jolivet, P. 1950. Les parasites, prédateurs et phorétiques des Chrysomelidae (Coleoptera) de la faune franco-belge. *Bull. Inst. R. Sci. Nat. Bruxelles* 26(34):1–39.

Pasteels, J.M., Braekman, J.C., Daloze, D., and Ottinger, R. 1982. Chemical defence in chrysomelid larvae and adults. *Tetrahedron* 38:1891–1897.

Pasteels, J.M., Grégoire, J.C., and Rowell-Rahier, M. 1983a. The chemical ecology of defense in arthropods. *Annu. Rev. Entomol.* 28:263–289.

PASTEELS, J.M., ROWELL-RAHIER, M., BRAEKMAN, J.C., and DUPONT, A. 1983b. Salicin from host plant as precursor of salicylaldehyde in defensive secretion of chrysomeline larvae. *J. Physiol. Entomol.* 8:307–314.

PASTEELS, J.M., ROWELL-RAHIER, M., BRAEKMAN, J.-C., and DALOZE, D. 1984. Chemical defences in leaf beetles and their larvae (Chrysomelinae, Chrysomelini): The ecological, evolutionary and taxonomic significance. *Behav. Ecol. Syst.* In press.

ROWELL-RAHIER, M., and PASTEELS, J.M. 1982. The significance of salicin for a *Salix* feeder, *Phratora* (= *Phyllodecta*) *vitellinae*, pp. 73–79, *in* J.H. Visser and A.K. Mink (eds.). *Proceedings 5th International Symposium Insect–Plant Relationships.* Pudoc, Wageningen.

SUGAWARA, F., KOBAYASHI, A., YAMASHITA, K., and MATSUDA, K. 1978. Identification of octadecyl acetate and (2)-11-eicosenyl acetate, major components of the defensive secretion of *Gastrophysa atrocyanea* Motschulsky. *Agric. Biol. Chem.* 42:687–688.

TINBERGEN, L. 1960. The dynamics of insect and bird populations in pine woods. *Arch. Neerl. Zool.* 13:259–473.

WAIN, R.L. 1943. The secretion of salicylaldehyde by the larvae of the brassy willow beetle (*Phyllodecta vitellinae* L.) Annual Report Long Ashton (Bristol) Agricultural and Horticultural Research Station for 1943, pp. 108–110.

WALLACE, J.B., and BLUM, M.S. 1969. Refined defensive mechanisms in *Chrysomela scripta. Ann. Entomol. Soc. Am.* 62:503–506.

WHITEHEAD, D.R., and DUFFIELD, R.M. 1982. An unusual specialized predator prey association (Coleoptera, Coccinellidae, Chrysomelidae): Failure of a chemical defense and possible practical application. *Coleopt. Bull.* 36:96–97.