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APOSEMATISM OF *URESIPHITA REVERSALIS* LARVAE (PYRALIDAE)

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ABSTRACT. A description is presented of the basic biology of *Uresiphita reversalis* and of the characteristics that indicate that the larvae are truly aposematic. Coloration, position and movements on the plants, feeding activity, and the importance of host plant alkaloids are described and discussed. Field observations and experiments with predators indicate that the larvae are distasteful.

Additional key words: *Cytisus monspessulanus*, quinolizidine alkaloids, predation, host plants, feeding stimulants.

Brightly colored insect larvae conspicuous to humans are often assumed to be aposematic, even in the absence of information about the supposed unpleasant attributes. Such is the case with caterpillars of the pyralid moth *Uresiphita reversalis* (Guenée), which feeds on several plant species in the tribe Genisteae (Papilionaceae), including *Lupinus* spp. and *Cytisus* (=*Genista*) spp. (Munroe 1976). While the adult moth is inconspicuous, with brown forewings and orange-brown hindwings, the larvae (after the first instar) are brightly colored and highly exposed on their host plants. The final (fifth) larval instar has obvious black and yellow markings. It is a multivoltine species, commonly found in coastal California but recorded widely in North America. In the San Francisco Bay area, larvae may be found at any time of year, although growth rate varies greatly, and by the end of winter they are rare. There are four to five generations per year, and populations build up in summer, reaching a peak in the early fall. Generation time varies from about five weeks in summer to as long as 25 weeks in winter. A general description may be found in Munroe (1976).

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This study is a characterization of the particular features of *U. reversalis* that may allow it to be labelled aposematic: description of coloration; description of field behavior and degree of exposure on the plant; feeding patterns on the plants in the field, and laboratory experiments on food selection behavior; chemical protection with host plant chemicals; and levels of predation as determined from field and laboratory experiments.

COLORATION

Eggs are laid in batches of up to 70, and the groups of young larvae, together with the damage they impart to leaves, make them conspicuous to humans, even though first instar individuals are green and somewhat cryptic. The later instars have a dark gold background color, and black head and legs. On the sides of every segment are several black tubercles, each with white patches and long white hairs. In addition there are bright yellow patches laterally on each segment (Fig. 1). The intersegmental membranes of individuals are somewhat transparent so that as ecdysis approaches, dull greenish bands alternate with the golden background. Thus, these larvae appear to have coloration patterns that are conspicuous and consistent with aposematism (Rothschild 1972).

POSITION ON THE HOST PLANTS

The positions of larvae on their host plants in the field is first determined by oviposition site. Egg masses found in the field are usually on the abaxial (lower) surface of leaves 2 to 8 cm from the tips of branches or branchlets. When larvae hatch, they usually feed first on the adaxial (upper) surface of the leaf on which the egg mass was laid. They produce copious amounts of silk, and as the leaf shrivels and curls, its edges are loosely bound together by the silk to make a shelter. Larvae remain together in the shelter, coming out only to feed. In the second instar, larvae begin to disperse, often forming two or more subgroups on adjacent branchlets. Contiguous leaflets or leaves are held together with silk to make shelters, from which foraging occurs. Caterpillars rest and molt in these shelters. By the third instar such shelters are no longer used and groups of larvae feed and rest in groups inside a very loose web of silk threads across branchlets.

On *Cytisus monspessulanus*, larvae of *U. reversalis* are almost invariably found on the upper third of the plants, usually on the distal ends of branches. For example, on 4 September 1985, 57 larval groups of different ages were observed on this plant species. All but five groups were within the distal twenty percent of the length of the branch. The remaining five were on young branchlets more proximally. Thus larvae
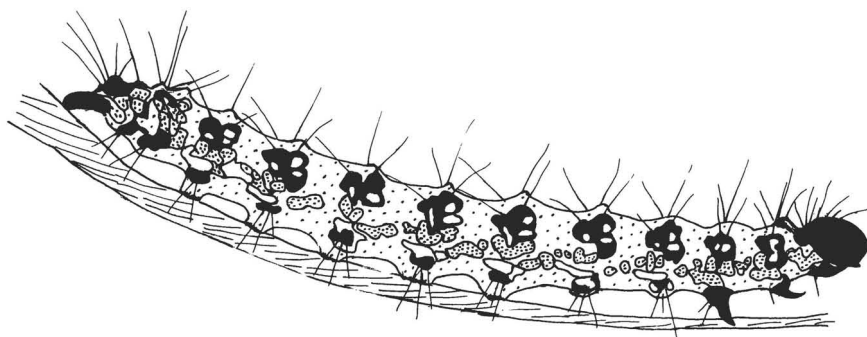
FIG. 1. Drawing of fifth instar larva of *Uresiphita reversalis* to indicate markings. Open areas, white; light dotted area, gold; densely dotted area, bright yellow; black as shown. Hairs are white. Length 25 mm.

rest and feed on the upper periphery of the plant, utilizing the plant's youngest foliage. Populations observed on *Lupinus* spp. appear to have similar relative positions.

There is a tendency for young larvae to move outwards, away from the main stems. Egg masses or groups of newly hatched larvae from a laboratory culture were placed about 15 cm from the tips of branches of *C. monspessulanus* in the field during the summer of 1988 and observed at intervals over the next three weeks. In all ten cases, the first damage was to leaves distal to the placing, and over the first five days all feeding was confined to this region. Only after such foliage was severely damaged was there more proximal movement. Thereafter larvae moved out to the tips of branchlets and across (usually up) to other branches. During observations throughout the day, most movement was associated with feeding, but dispersal bouts to new feeding sites occurred within groups at intervals—usually between 0600 and 1000 h and between 1500 and 2000 h (Fig. 2).

Previous work in our laboratory has shown that larvae are attracted to light and that, in choice tests, young leaves are strongly preferred to old ones (Montllor & Bernays unpubl.). These factors may have a role in determining where larvae are found on plants. An additional factor concerns temperature regulation: larvae will be more exposed to the sun when located on the top and the periphery of the plant, and may be assumed under these circumstances to have body temperatures higher than ambient. This provides an important potential advantage for thermoregulation. In the the San Francisco Bay area, this may be especially important because the daily temperature maxima rarely exceed 25°C (in the years 1984 through 1987, the mean number of days each year on which temperatures rose to this level was only 25). Maximum rates of growth and development of the larvae occur at over 30°C in the
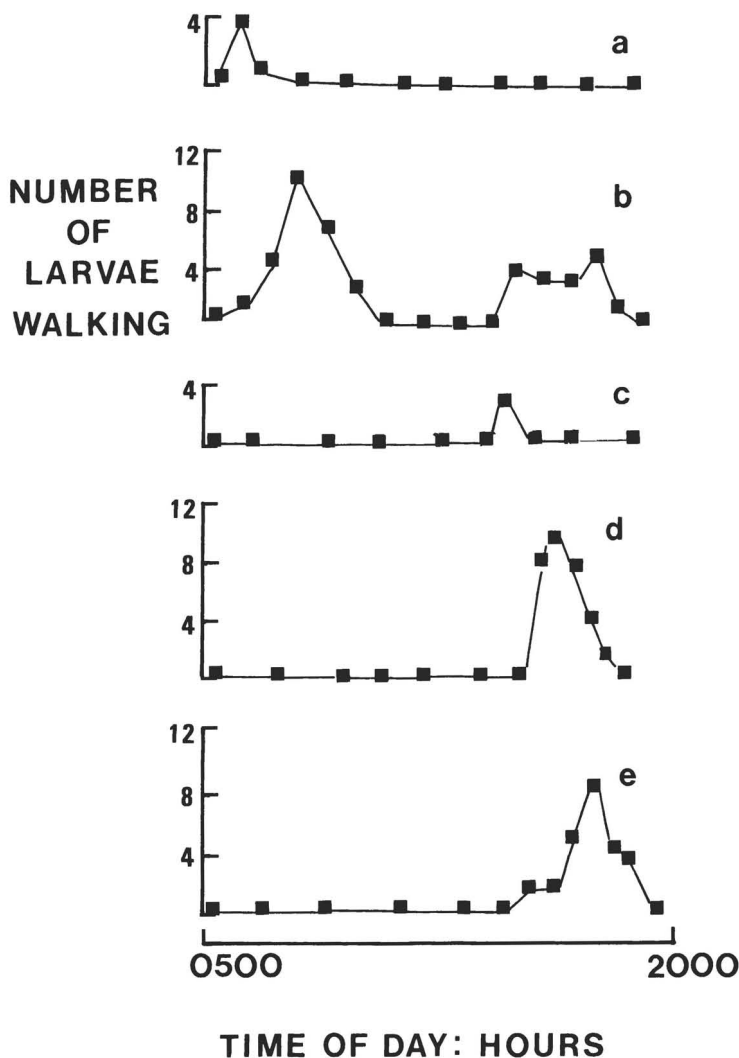
TIME OF DAY: HOURS

FIG. 2.   Patterns of dispersal in five groups (a–e) of larvae (groups composed of 83% third instar and 17% second instar) on 22 June 1988, Berkeley, California.

laboratory (Montllor and Bernays unpubl.) as expected by comparison with other lepidopterans (e.g., Stamp & Bowers 1988).

Larvae are almost invariably found in groups. Initially, this reflects the fact that eggs are laid in masses on the plant. Of the egg masses found in the field during 1985–87, numbers of eggs varied from 23 to 70 (median 37, n = 13). Typically, groups of around 15–20 second and third instar larvae are found, while groups of fourth instars and fifth instars are smaller. It is not clear whether the larvae are actively gre-

garious, or whether the stimuli for movement and direction of movement cause similar displacements. In any case, the exposed positions in which larvae are found, their clumped distribution, and their low rates of movement are consistent with their being aposematic.

FEEDING

Host Plants

Survival and growth of larvae on a range of potential host plants (host records and related plants) in the San Francisco Bay area were tested by giving twenty individual larvae single species diets in small cages in the laboratory and examining survivorship and development rates. These experiments were run simultaneously at laboratory temperatures of 24–26°C and a 12:12 (L:D) cycle. Food was changed daily. There was no feeding and rapid mortality on *Medicago*, *Trifolium*, *Vicia*, and *Pickeringia*. There was limited feeding but no pupation on *Laburnum* and *Ulex*. However, larvae fed extensively on *Cytisus scoparius*, *C. striatus*, *Lupinus arboreus*, and *C. monspessulanus*. These data together with data on rearing from museum collections indicate that the main hosts of *U. reversalis* in California are species of *Lupinus*, with some specimens from *Cytisus*. These plants all contain quinolizidine alkaloids (Kinghorn & Balandrin 1984).

When given a choice in the laboratory of leaves and/or leaflets from *Cytisus monspessulanus* or *Lupinus arboreus*, presented in an alternating array in small cages over two hours, fifth instar larvae collected from either plant in the field significantly preferred the lupine (n = 20, Wilcoxon's Signed Rank Test, P < 0.01). The basis for host plant preference is not yet known, but is assumed to involve quinolizidine alkaloids, as a wide variety of these occur differentially in the hosts (Johnson et al. 1987, Johnson & Bentley 1988, Kinghorn & Balandrin 1984, Wink 1987); for example, in a single sample of *C. monspessulanus*, 27 different alkaloid structures were identified (Wink pers. comm.).

Individual fourth or fifth instar larvae presented with an alternating array of young trifoliates and more mature single leaflets (with similar areas) of *C. monspessulanus* in Petri dishes in two-hour laboratory tests preferred young leaves over old leaves (n = 20, Sign test, P < 0.001). The basis for this preference is also not known, but by analogy with other phytophagous insect species it can be expected that the tender young leaves, rich in nitrogen, provide a better diet. Young leaves are also particularly rich in quinolizidine alkaloids (Montllor unpubl.), consistent with the assumption of the importance of these compounds to larval preference.
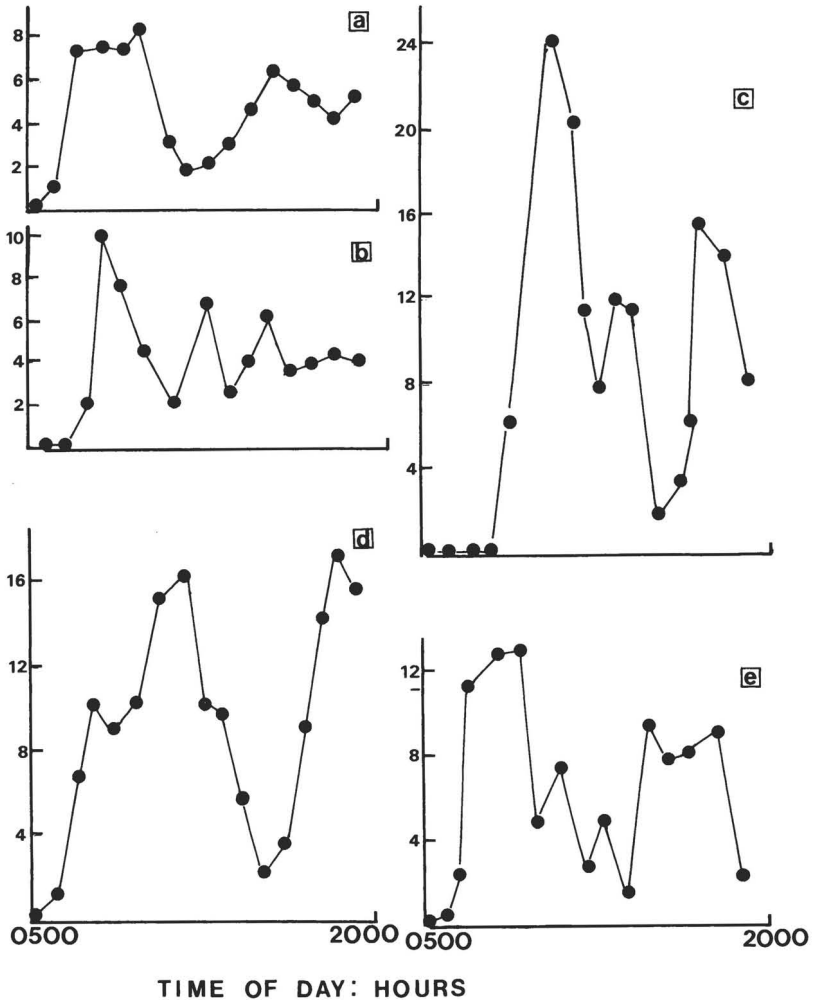
LARVAE FEEDING: %

FIG. 3.   Patterns of larval feeding throughout the daylight hours on five separate days during summer 1988, Berkeley. a, b = first instar; c, d = second instar and third instar; e = third instar and fourth instar.

Feeding Patterns in the Field

Field observations indicate that larvae of *U. reversalis* feed primarily in the day. In July 1988, five groups were observed from dawn until dusk on three separate days; in June five more groups were similarly observed on two days. The numbers of individuals feeding were monitored at intervals. At first light, young larvae began feeding within 30 minutes on four of the five occasions. On the fifth occasion, early

TABLE 1.   Effects of sparteine and cytisine on the palatability of sucrose-impregnated glass fiber filters[1]: + = trend but not significant, + + = significantly preferred at P < 0.05, + + + = significantly preferred at P < 0.01. At each concentration n = 14–19.

	Concentration of alkaloid (% dry weight)				
	0.1	0.5	1.0	2.0	All concs pooled
Sparteine	+	+	+	+	+ +
Cytisine	+ + +	+ +	+ + +	+ + +	+ + +

[1] 5% dry weight of sucrose, larvae presented with a choice of alkaloid-treated (alkaloid plus sucrose) or control discs (sucrose only). Preferences tested using Wilcoxon's Signed Rank Test.

morning temperatures were similar (11–14°C and no direct sun), but the conditions were very foggy and the plants wet; feeding began only after the plant surface dried off at about 0900 h. Although there were morning peaks of feeding, there were no other obvious patterns relating to time, temperature, or sunshine (Fig. 3). Overall, it is clear that, as typical aposematic caterpillars, *U. reversalis* larvae feed actively throughout the day. This could be chemically significant; similar alkaloids are known to vary in concentration throughout the day in *Lupinus albus* (Wink & Witte 1984).

Host Plant Alkaloids

Two host plant alkaloids common to some species of *Cytisus* and *Lupinus*, sparteine and cytisine, were tested as potential phagostimulants for larvae of *U. reversalis*. Individual larvae from a laboratory culture were given a binary choice of glass fiber filters (GF/A) impregnated with sucrose (5% dry weight) and similar glass fiber filters with the sucrose plus sparteine or cytisine at one of several different concentrations. Experiments were at 25°C with the filters in Petri dishes. Amounts eaten were visually estimated after 24 h. The alkaloids were shown to be phagostimulatory (Table 1), but they were not equally effective as feeding stimulants, and it is conceivable that the actual profile of quinolizidine alkaloids influences acceptability.

The host plant alkaloids are bitter to humans and are also known to be poisonous to mammals, including livestock, causing vomiting, convulsions and death (Kinghorn & Balandrin 1984). These compounds are likely to have similar effects on birds. Actively feeding larvae, having a preference for foliage with the highest levels of alkaloids, will contain high doses of the toxins from the gut contents alone. In addition, larvae transfer ingested alkaloids to the cuticle, although pupae contain no detectable alkaloids (Montllor et al. in press). It seems likely that, in common with a number of other species of Lepidoptera feeding on alkaloidal plants, larvae gain protection from the ingested alkaloids. Other cases of protection from predation due to ingestion of host plant
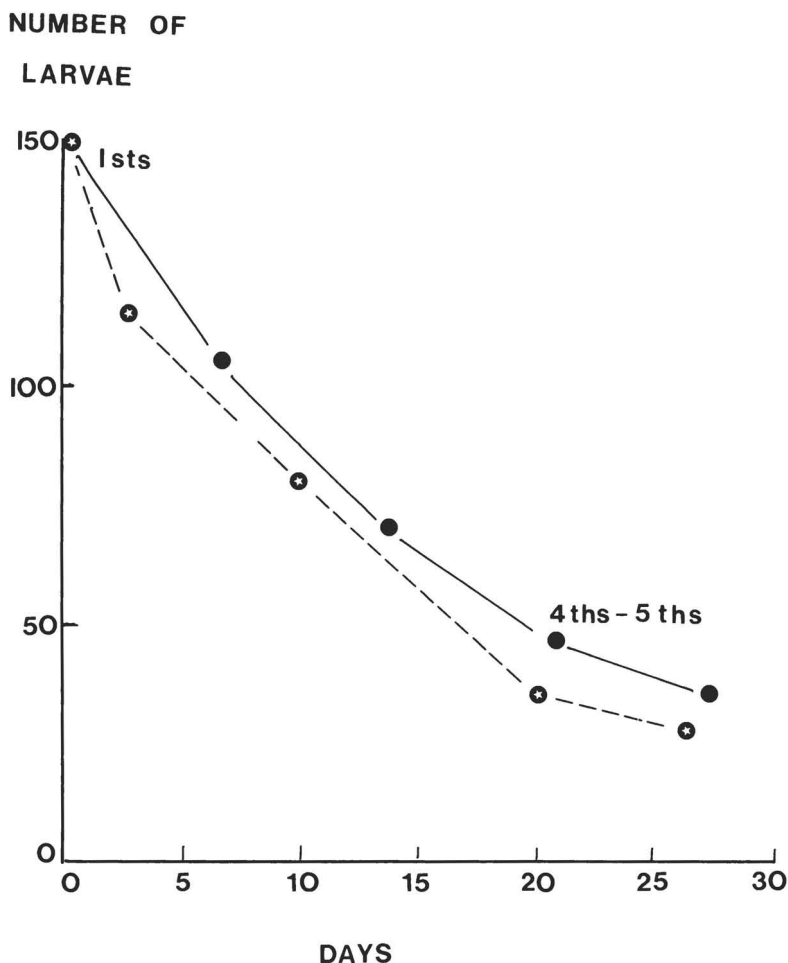
NUMBER OF

LARVAE

FIG. 4.   Survivorship curves of two cohorts of 150 larvae from the time of hatching. Total number of larvae from 5 original egg masses per cohort were counted on each date. Observations were made during the period May–July 1988.

alkaloids involve mainly pyrrolizidine alkaloids (Rothschild 1972, Schneider 1987). This seems to be the first example of the use of quinolizidine alkaloids for protection by caterpillars.

PREDATION

Field Observation in Relation to Invertebrate Predators

In two separate field sites in Berkeley, five groups of newly hatched larvae were placed on appropriate branches of single *C. monspessu-*
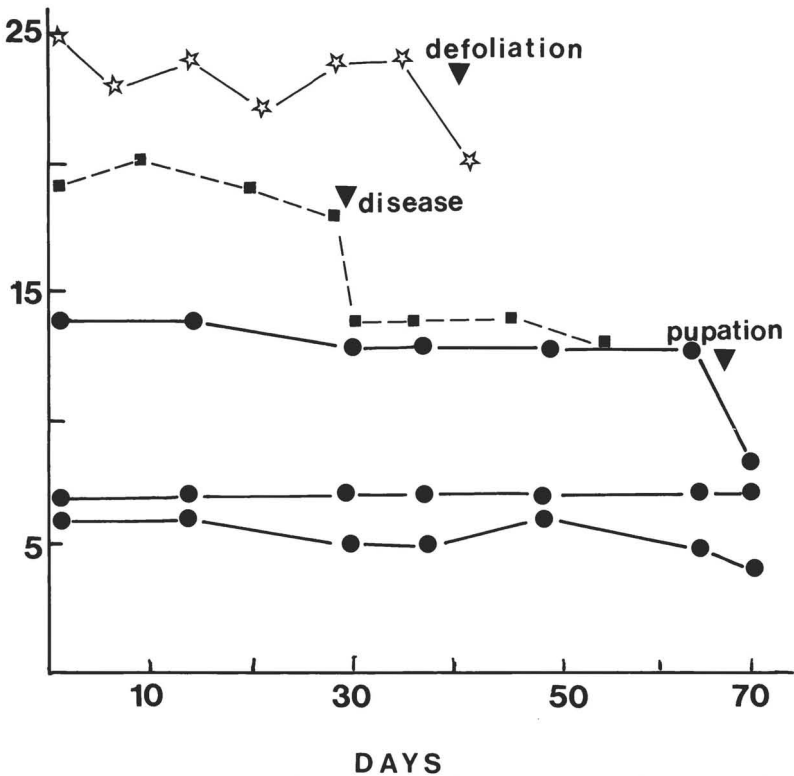
NUMBER IN GROUP

FIG. 5. Survivorship curves of five fifth instar larval groups. Declines in numbers marked with an arrow could be attributed with certainty to defoliation of host plant, virus disease, and pupation as indicated. The otherwise constant numbers imply a lack of predation. Observations were made during the period October–December 1986.

lanus plants in summer 1988. At each site, continuous observations were made from dawn to dusk on activities of the larvae, and any predation attempts on them noted. Similar observations were made on two subsequent occasions during the second and third instars at one site, and on four occasions (during second, fourth, and fifth instars) at the other site. Total counts were made at both sites on four dates during development. Each group had about 30 larvae at the start, and mortality of early instars was high (Fig. 4). This was in marked contrast to the data for fifth instars that was noted in an earlier year (Fig. 5). During the periods of observation, Hemiptera (Anthocoridae) fed on first and second instar caterpillars, and Hymenoptera (Vespidae) removed middle instars. In one of these experiments, 80 larvae (53%) were lost in 17 days, giving an average daily loss of 4.4 larvae. About 40% of the loss

TABLE 2. Numbers of invertebrate predators relevant for *U. reversalis* collected by beating bushes of the host plant, *Cytisus monspessulanus*.

Sample date	Numbers per 60 bushes (6 sites)			
	Salticidae	Thomisidae	Heteroptera	Chrysopidae
18 July 1988	29	13	5	4
27 July 1988	22	6	3	0
18 August 1988	17	4	4	0

could be accounted for by observed predation levels. In the second experiment, 15% of loss could be similarly accounted for.

During the period of field experimentation, censuses of potential predators were made by beating bushes, collecting the arthropods, and testing the predators among them with first and second instar caterpillars of *U. reversalis* as prey. Ants and coccinellid beetles did not attack larvae that they were confined with in small containers in the lab, while neuropterans (*Chrysopa*) and heteropterans (Anthoridae, Nabidae) did. Spiders in the families Salticidae and Thomisidae were deemed relevant predators from daily field observations, and both of these attacked larvae in the lab in no-choice tests. The census data indicate that bushes of *Cytisus* averaged 0.4 to 0.8 relevant predators per bush (Table 2). To test whether invertebrate predation is a significant cause of mortality, we conducted field experiments in which some groups of larvae were protected from predation.

Survivorship of *U. reversalis* larvae placed on branches of *C. monspessulanus*, with or without net bags over the branches, was compared. In April–May 1988 approximately 30 neonate larvae were placed on each of three plants at each of five sites in the San Francisco Bay area. Each plant had a protected and an unprotected group. A similar experiment was done in June–July 1988 on a total of 21 plants at six sites. In all cases, survivorship was considerably higher on bagged branches (Table 3), and although there may be other unknown mortality factors, it is likely that the exclusion of predators was the primary reason for the difference. Fifth instar larvae in five natural groups at three sites in Berkeley were monitored in fall and winter 1986. These represented the last generation of the year and persisted as fifth instars over 5 to 12 weeks because of low temperatures. In all cases the population declines could be attributed to causes other than predation (Fig. 5), in spite of the exposed positions of the larvae and the abundance of birds. Rejection behavior of birds was observed. On two occasions during four all-day observations, a bird was observed to pick up a caterpillar, subsequently drop it, and proceed to rub its beak on the branches of a tree. Aposematism is most commonly associated with protection from

TABLE 3. Survivorship of larvae in the field with or without protection from predators. In all four comparisons, survival was significantly greater in the protected groups (Chi Squared Test, P < 0.01).

	Number of groups (total number of larvae)	Mean % surviving (±SD)	
		1–2 weeks	3 weeks
Spring 1988			
Protected	15 (482)	76 (15)	36 (15)
Unprotected	15 (499)	39 (4)	9 (8)
Summer 1988			
Protected	21 (530)	61 (18)	37 (23)
Unprotected	21 (503)	38 (17)	18 (14)

predation by birds, and our limited field observations indicate that birds do not prey on larvae of *U. reversalis*.

Predation Experiments

The possible defense against invertebrate predators was tested by comparing the relative acceptability of *U. reversalis* to two invertebrate predators in a situation where food deprivation was clearly not driving indiscriminate predation. In the first case, the vespid wasp *Mischocyttarus flavitarsus* (Saussure), a generalist predator, was employed (Bernays 1988). Naturally hunting wasps from nests in a large greenhouse were allowed to forage in open arenas (1m × 1m × 1m) in which caterpillars of *U. reversalis* feeding on *C. monspessulanus* were available at the same time as *Trichoplusia ni* (Hübner) (Noctuidae), *Estigmene acrea* (Drury) (Arctiidae) or *Heliothis virescens* (F.) (Noctuidae), each feeding on its own host plants in close proximity. In each of these three experiments, laboratory-reared larvae of *U. reversalis* and the alternate species were presented at similar sizes and densities. In each case the alternative prey were always attacked early in the experiments.

TABLE 4. Numbers of caterpillars taken by the wasp *Mischocyttarus flavitarsus* (Vespidae) during choice trials. Experiments were terminated after approximately half of either prey species was removed. In each of the three experiments the differences in acceptability are significantly different (Chi Squared Test, P < 0.001).

	Numbers of size-matched caterpillars	
	Nos. at start	% taken
1. *Estigmene acreae* (Arctiidae)	20	50
Uresiphita reversalis	20	0
2. *Heliothis zea* (Noctuidae)	25	52
Uresiphita reversalis	25	0
3. *Trichoplusia ni* (Noctuidae)	22	59
Uresiphita reversalis	30	0

Experiments were stopped when approximately half of one species was taken. In no case were any *U. reversalis* larvae taken before half of the alternate species was removed (Table 4).

Experiments on predation by Argentine ants, *Iridomyrmex humilis* (Mayr) (Formicidae: Dolichoderinae), also showed that *U. reversalis* is extremely unacceptable relative to many other species of caterpillars (Bernays & Cornelius 1989).

CONCLUSIONS

Uresiphita reversalis larvae have all the characteristics of aposematic caterpillars. They are brightly colored, relatively inactive, found in groups, and they feed actively during the day. Their favored food is rich in toxic quinolizidine alkaloids, which are phagostimulants. Alkaloids that are not excreted after ingestion are deposited in the cuticle. Larvae are apparently distasteful to at least one avian species, wasps and ants.

Predation data are limited, but on the basis of the observations presented here, it appears that in spite of being chemically defended, *U. reversalis* larvae do suffer extensive predation. Losses to invertebrate predators apparently account for a significant proportion of the mortality in the early instars. Arthropods with piercing and sucking attacks seem to be the dominant predators, perhaps circumventing any protection provided by the alkaloidal cuticle. The early larval instars are much more vulnerable to predation than the last larval instar. Thus, chemically defended aposematic species may nonetheless suffer high mortality from predation.

ACKNOWLEDGMENTS

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