Wolfgang Völkl · Katrin Vohland

Wax covers in larvae of two Scymnus species: do they enhance coccinellid larval survival?

Received: 19 January 1996 / Accepted: 22 March 1996

Abstract We tested the protective function of larval wax covers in the two ladybird beetle species, Scymnus nigrinus and S. interruptus, against cannibalism, predation and ant aggression, and its importance for the distribution of both species in the field. Cannibalism was generally very low and not influenced by the presence or absence of the wax cover, or by larval size. Fourth-instar larvae of three ladybird species, Adalia bipunctata, Exochomus quadripustulatus and Harmonia quadripunctata, consumed Scymnus larvae – which are much smaller – regularly, independent of the presence or absence of waxes. By contrast, first-instar larvae of the three species had generally little success when attacking Scymnus spp. larvae. Wax-covered S. interruptus larvae survived significantly more attacks by the predacious carabid beetle Platynus dorsalis than larvae without wax cover. Wax-covered S. interruptus larvae and S. nigrinus larvae survived attacks by workers of the ant species Lasius niger and Formica polyctena, respectively, significantly more often than larvae without wax covers. We show that, in the field, Scymnus larvae have higher densities in ant-attended resources than in unattended ones and conclude that both Scymnus species benefit from the ability to feed in ant-attended aphid colonies by a reduced predation risk.

Key words Coccinellidae · Field distribution · Protective waxes · Cannibalism · Ant predation

Introduction

Body coverings of waxes are frequently found among insect taxa. These waxes may have various functions, e.g. reflection of UV radiation (Pope and Hinton 1977), prevention of water transpiration or protection against natural enemies (Eisner 1970). The scale insect Ceroplastes ceriferus Anderson, for example, is protected by its thick wax layer against attacks by the parasitoid Anicetus ceroplastis Ishii (Takabayashi and Takahashi 1993), and larvae of the sawfly species Eriocampa ovata are protected by their cuticular waxes against ant attacks (Eisner 1994). The larvae of some chrysopid species cover themselves with waxes obtained from their homopterous prey to escape ant aggression (Eisner et al. 1978; Mason et al. 1991; Milbrath et al. 1993). The larvae of many coccinellid beetle species are also characterized by the presence of thick wax layers produced by dorsal epidermal cells (Pope 1979). These waxes have also been supposed to protect larvae against natural enemies (Bartlett 1961; Bradley 1973; Pope 1979; Richards 1985) but there are currently no detailed studies on the protective function of coccinellid waxes.

In the present study, we examined the protective function of waxes for larvae of Scymnus nigrinus Kugelann and S. interruptus (Goeze), two specialized univoltine ladybird species. We hypothesized that the potential protective effect of the wax layer might influence the species' distribution in the field: they might be able to exploit resources which otherwise would be difficult to exploit due to predation pressure. The larvae of the Scymnus species studied develop on conifers, especially Scots pine (Pinus sylvestris L.), and tansy (Tanacetum vulgare L.), respectively (Klausnitzer and Klausnitzer 1986; Majerus 1994), where they feed on a variety of aphid species. Scymnus larvae may face various mortality risks when foraging for prey on their host plant. First, larvae may be eaten by conspecifics. Cannibalism is widespread among coccinellids and may cause a considerable mortality – besides egg mortality – especially among younger larval stages (Mills 1982; Osawa 1989; Agarwala and Dixon 1992). Second, larvae are subject to interspecific predation by other ladybird larvae. Interspecific predation occurs among ladybirds as frequently as cannibalism (Evans 1991; Agarwala and Dixon 1992) if larvae of two species live in the same habitat. Both on pines and tansy, other ladybird species are frequently found.
syntopically (Klausnitzer 1967, 1968). The larvae of most of these species – like Exochomus quadripustulatus L. and Harmonia quadripunctata (Poncetopiddan) on pine and Adalia bipunctata L. on pine and tansy – are considerably larger than Scymnus larvae. Thus, Scymnus larvae may be an easy prey for them if aphid food is getting scarce. Another potential predator of _S. interruptus_ may be the carabid beetle, _Platynus dorsalis_ (Poncetopiddan), a common polyphagous predator of aphids in cereal fields and field margins in Europe (e.g. Sunderland 1975; Edwards et al. 1979; Welling 1990). This species climbs on plants up to a height of 30 cm (Griffiths et al. 1983) and may thus occur syntopically with _S. interruptus_ on tansy in field margins. Finally, both Scymnus species share their habitat with ant species which attend some of their aphid prey. On pines, _S. nigrinus_ may frequently interact with red wood ants (_Formica_ spp.) which attend and defend _Cinara_ colonies (Scheurer 1971a; Fossel 1972). On tansy, _Lasius niger_ L. frequently collects honeydew from _Meiopeurus fuscoviride_ Stroyan and _Brachycyrtus cardui_ L. (Klausnitzer 1968). This ant species is known to be aggressive towards aphidophagous insects (Banks 1962; Way 1963; Völkl 1990; Jiggins et al. 1993; Völkl and Mackauer 1993) and may also attack _S. interruptus_ larvae.

We investigated whether the wax cover reduced (a) cannibalism, (b) predation by larvae of other three ladybird species or (c) predation by _P. dorsalis_, and whether it led (d) to an increased survival rate after ant aggression. Finally, we examined whether _S. nigrinus_ larvae reached higher densities on ant-attended resources where they may be protected against predation by other ladybird larvae due to ant aggression towards these ladybirds (Jiggins et al. 1993).

**Material and methods**

**Insect material**

Larvae of _S. interruptus_ were obtained from laboratory stock established from adults collected on tansy near the university campus in Bayreuth, Germany, in mid-May 1995. Additional larvae were collected in June 1995 on tansy infested with _Macrosiphoniella tanacetae_ at the university campus in Bayreuth. Larvae of _S. nigrinus_ were obtained from laboratory stock established from adults collected on Scots pine in the vicinity of Bayreuth in April/May 1994 and in May 1995. Additional larvae were collected on Scots pines in the vicinity of Bayreuth in June 1994 and 1995. All larvae were kept singly in small gauze-covered plastic cages (diameter 5 cm, height 12 cm) at 20 ± 1°C, 65–70% relative humidity and 16:8 L:D and fed with a surplus of _Aphis fabae_. Waxy and waxless third- and fourth-instar larvae of _S. nigrinus_ were tested with first- and fourth-instar larvae of _H. quadripunctata_ (L.) colonies on potted pines through an arrangement of sticks. _S. interruptus_ larvae were released individually with a _P. dorsalis_ larva in small plastic cages and observed until the _Scymnus_ larva was attacked by _P. dorsalis_. Each _P. dorsalis_ was used twice, once with waxy larva, and a second time with a waxless larva. To avoid any bias due to the sequence of presentation, we presented waxy larvae first for one beetle cohort (n = 8 beetles), while the second cohort was first tested with waxless larvae. The experiment was stopped after the carabid had either consumed the ladybird larva or the larva had escaped the first carabid attack.

**The influence of the wax layer on cannibalism**

The effect of the wax layer on cannibalism was determined for _S. nigrinus_ with two sets of larvae. Larvae of the first set were waxy, while larvae of the second set were waxless. All larvae did not receive food for 24 h before used in an experiment to standardize their hunger level. Waxy larvae were individually caged in small plastic containers with either a waxless larva or a waxy larva, and any cannibalism was recorded at the end of 6 h. To account for size-specific patterns in cannibalism (Agarwala and Dixon 1992), we used two size classes, small (L1, L2) or large (L3, L4) larvae. The experiment was repeated 10 times for each treatment and size class. We did not account for differences between sibling cannibalism and non-sibling cannibalism (e.g. Osaka 1989) since it was not possible to obtain enough siblings of a particular size class for experiments.

**The influence of the wax layer on predation by ladybirds**

Adults of the predacious ladybirds _H. quadripunctata_, _E. quadripustulatus_ and _A. bipunctata_ were collected on pines and kept in the laboratory until females had oviposited. Egg clusters were kept singly until emergence to obtain larvae of a defined age. Subsequently, larvae were kept singly in small plastic cages at 20°C, 60% relative humidity and 16:8 L:D and fed with a surplus of _Aphis fabae_. Waxy and waxless third- and fourth-instar larvae of _S. nigrinus_ were tested with first- and fourth-instar larvae of _H. quadripunctata_ (n = 10–11) and _E. quadripustulatus_ (n = 12–14), while waxy and waxless third/fourth-instar larvae of _S. interruptus_ were tested with first- and fourth-instar larvae of _A. bipunctata_ (n = 15). First instar larvae of _H. quadripunctata_, _E. quadripustulatus_ and _A. bipunctata_ are somewhat smaller than their offered prey, while fourth instar larvae of either species are considerably larger.

_S. nigrinus_ larvae were caged individually in a small plastic container together with a potential predator for 6 h. We recorded any interspecific predation. First-instar larvae were used in an experiment within 1 day after emergence and before having received any aphid food, while fourth-instar larvae were deprived of food for 24 h before used in an experiment to standardize their hunger level.

**The influence of the wax layer on predation by a carabid beetle**

Adults of the carabid beetle _Platynus dorsalis_ were collected under stones in cereal fields and field margins in the vicinity of Bayreuth, Germany. Beetles (n = 16) were kept in small cages and fed with a surplus of _Aphis fabae_ until used in an experiment. They were deprived of food for 24 h before used in an experiment to standardize their hunger level. Waxy (n = 16) and waxless (n = 16) _S. interruptus_ larvae were released individually with a _P. dorsalis_ larva in small plastic cages and observed until the _Scymnus_ larva was attacked by _P. dorsalis_. Each _P. dorsalis_ was used twice, once with waxy larva, and a second time with a waxless larva. To avoid any bias due to the sequence of presentation, we presented waxy larvae first for one beetle cohort (n = 8 beetles), while the second cohort was first tested with waxless larvae. The experiment was stopped after the carabid had either consumed the ladybird larva or the larva had escaped the first carabid attack.

**The influence of the wax layer on ladybird-ant interactions**

Single nests of _Formica polyctena_ and _Lasius niger_, were established in small terrariums (lxbxh = 70x35x35 cm) and kept at 20°C, 60% relative humidity, 3600 lux and 16:8 L:D within a growth chamber. _F. polyctena_ workers had access to _Cinara pinae_ (Mordv.) and _Cinara pini_ (L.) colonies on potted pines through an arrangement of sticks. _L. niger_ had access to _Aphis fabae_ colonies on creeping thistle and to _Brachycyrtus cardui_ (L.) colonies on...