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# Is the alkaloid in 2spot ladybirds (*Adalia bipunctata*) a defence against ant predation?

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**Abstract.** Garden black ants, *Lasius niger* L., in a laboratory colony, attacked three species of live ladybirds found near their nest, killing the smaller two species. A second colony was offered artificial diets containing crushed ladybirds of two species, and the ants' choice of feeding site noted. Both the diets were aversive compared to control, but that containing 7spot, *Coccinella septempunctata* L., was more aversive than the diet containing 2spot, *Adalia bipunctata* L. The implications of this lesser protection for 2spots in terms of the chemical defence of the species are discussed.

**Key words.** chemical defence – alkaloids – predation – Coleoptera – Coccinellidae – *Adalia bipunctata* – *Coccinella septempunctata* – Hymenoptera – Formicidae – *Lasius niger*

## Introduction

Many of the brightly coloured ladybird species are chemically defended. It has been shown that the *Adalia* species, such as the 2spot ladybirds, *Adalia bipunctata*, and the 10spot, *A. decimpunctata*, contain large amounts of an alkaloid, adaline (Pasteels *et al.* 1973a,b; de Jong *et al.* 1991) but are not toxic to nestling blue tits, *Parus caeruleus* (Marples *et al.* 1989; Marples 1993). The closely related 7spot ladybird *Coccinella septempunctata* contains a lower concentration of a different alkaloid, coccinelline (Pasteels *et al.* 1973a; Holloway *et al.* 1991). This is chemically very similar to adaline (Pasteels *et al.* 1973b) but extremely toxic to blue tit nestlings (Marples *et al.* 1989). It is clear that adaline is not a sufficient defence against bird predation, 2spot ladybirds being taken readily in the wild (Marples 1990). This raises the question whether the 2spot, being smaller than the 7spot, is at greater danger from invertebrate predators, and whether it's defences are against, for instance, ants or spiders, or parasites such as *Perilitus coccinellae* (de Jong *et al.* 1991).

In addition to alkaloids, the 2spot and 7spot have been shown to contain pyrazines (Moore *et al.* 1990), a class of volatile chemicals proposed as general 'alerting' signals (Rothschild *et al.* 1984; Guilford *et al.* 1987; Kaye *et al.* 1989; Moore *et al.* 1990). These are not toxic in themselves, but can enhance the ability of birds and rats to learn avoidance of aversive substances such as quinine (Kaye *et al.* 1989; Guilford *et al.* 1987). Pyrazines are also found in ant trails and pheromones (Rothschild & Moore 1987; Moore *et al.* 1990; Woolfson & Rothschild 1990).

When attacked by birds, ladybirds exude an alkaloid rich fluid from their leg joints, a response called 'reflex bleeding' (Cuenot 1896). This brings the alkaloids with their bitter taste, and the pyrazines with their strong smell to the surface as an early warning signal to the attacker.

Ladybirds may be regarded as competitors of ants, as both use aphids as a food source (Moore & Brown 1978), and ants have been observed to attack adult and larval ladybirds in the wild (Banks 1957; Hodek 1973). Garden black ants (*Lasius niger*) drive off or remove ladybird larvae from aphid colonies, dropping them off the edge of the leaf (El-Ziady & Kennedy 1956). Adult ladybirds may die if attacked by several ants, and may be taken back to the ants' nest (Majerus & Kearns 1989). The ladybirds are not defenceless; P.J. de Jong (pers. comm.) observed ants retreating and rubbing their mouthparts against the leaf after contact with a 2spot ladybird. Pasteels *et al.* (1973a) presented *Myrmica rubra* with pieces of dead 2spot ladybird and showed that of the 10 offered, five were taken to the nest to be eaten, of which three were later rejected. The ants became agitated by the presence of the ladybirds and frequently stopped to clean their mouth parts and antennae. When offered 7spots, not one of the seven offered was taken back to the nest. Thus Pasteels' group found some evidence of greater avoidance of 7spot and intermediate levels of protection for the 2spot. Pasteels *et al.* (1973a) also offered a methanol extract of 7spot to the ants, in solution in water, and used a count of the numbers of ants drinking to assess their avoidance.

I here present a small preliminary study in which the reaction of ants to ladybirds near their nest was observed. I then describe a main experiment designed to apply the quantitative procedures in the latter part of Pasteels' experiment to a comparison in acceptability of 2spot and 7spot to ants.

## Preliminary study

I offered a laboratory colony of *L. niger* live 2spot, 10spot and 7spot ladybirds. The ants immediately attacked the ladybirds, repeatedly stinging them, despite exhibiting the mouth wiping described above. The attack appeared to have no effect on the 7spots, but the 2spots and 10spots folded their legs under them so the edge of their elytra reached the ground, preventing the ants from getting at their more vulnerable underside. Such defensive behaviour has also been reported by Majerus & Kearns (1989). Surprisingly no reflex bleeding in response to ant attacks was seen either in these laboratory experiments or by de Jong in the wild. In contrast, El-Ziady & Kennedy (1956) report that attacked larvae do reflex bleed, repulsing the ants for a short period only, before renewed attack.

The ants in my preliminary study continued to attack for an extended period, and both the 10spot and 2spot ladybirds subsequently died. In the wild they may be more able to escape death by retreating from the ant nest further than my arena allowed. The 7spots remained apparently unharmed despite being left overnight in the ant arena. By the morning the ants were no longer responding to the 7spots. Whether the 7spots' apparent protection from ant attack was chemical or purely physical, being larger and with a tougher exoskeleton than 2spots, is not known.

Pasteels *et al.* (1973a) gave some evidence that alkaloids are not the only protective chemicals present. He offered the ants three species of ladybird which did not contain alkaloids, only one of which, *Aphidecta obliterata* proved acceptable, some of each of the other two species, *Rhizobius litura* and *Subcoccinella 24-punctata* being rejected uneaten.

## Main experiment

### *Method*

Part of a *L. niger* colony was taken from a garden in Cardiff, England, including about 200 workers and a few larvae, pupae and eggs. They were put into an arena with 4 cm high sides coated with a solution, 'fluon', a teflon-like paint which the ants could not climb. A nesting chamber with a little soil was provided. The soil was moistened daily, and water was available from a small dish. A sugar solution in a second dish provided food during a two week settling period prior to the experiment. After this, the food dish was removed and thereafter food was available only during and just after the daily experimental trials.

Ladybirds were presented to the ants incorporated into an artificial diet. Twenty whole ladybirds were ground with a stiff dough, normally used for feeding insectivore birds (Marples *et al.* 1989). Any variation between beetles, or within a beetle in the distribution or amount of defence chemicals was thus evened out across the dough. The ladybird dough was made into 15 mg pellets, each containing the equivalent of half a 2spot ladybird by weight, or the same weight of 7spot, about 1/6 of a beetle. Thus the ants' response to the defence systems could be compared between the ladybird species as the natural differences in concentration of the alkaloids and pyrazines were preserved, even though whole beetles were not presented. The 'control' pellets were made with the equivalent weight of mealworm larva. The pellets were made before the experiment and stored frozen. Freezing would not destroy the alkaloids (pers. obs.), nor would it destroy any pyrazines present (Rothschild pers. comm.). The feeding sites were drawn on the arena floor as circles 2 cm in diameter.

**Table 1** The combinations of pellet types offered to the ants in each stage of the experiment, with the duration of each stage

| | Pellet type | Duration |
|---|---|---|
| Stage 1 | Mealworm *vs* 2spot | 12 days |
| Stage 2 | Mealworm *vs* 7spot | 6 days |
| Stage 3 | 2spot *vs* 7spot | 6 days |
| Stage 4 | Mealworm *vs* 2spot *vs* 7spot | 9 days |

The ants were given a daily feeding trial in which a pellet was placed in each feeding site and moistened with a drop of water. After 15 min 'search time' the number of ants on each site was recorded every 2 min for 20 min. At the end of each 2 min interval any ant within or partly within the circle was counted. Most ants fed for less than 2 min, so readings would usually be of different ants. Occasionally ants settled to very long feeding bouts and were counted twice. Thus counts do not refer to total numbers of ants choosing, but to an acceptability of the food source.

During stages 1, 2 and 3 the treatments were presented at a constant site for the first half of the trials, then the sites were swopped. This was to counter any site preference unrelated to the treatments. In stage 4, when all three types of pellet were offered together, each pellet type was presented for three trials on each site. In a previous experiment the positions had been swopped daily but this appeared to be too often for the ants to learn to avoid either site. The combinations of pellets offered to the ants in each stage of the experiment are shown in Table 1.

After each trial the ants were given a good pellet containing neither mealworm nor ladybird for about 1 h, which fed the colony even if both treatments were inedible to them. They had no access to food for the remainder of the 23 h between trials. The ants did not appear to be carrying food back to the nest, but small particles may have been transported unnoticed.

## Results

The day's total number of ant visits spent at each site and treatment are presented in Figure 1. In almost all trials the control treatments were visited most and the 7spots least. An interesting exception to this is the first occasion when three treatments were offered (Fig. 1D). On this occasion the treatment on the new site (the 7spot) was visited the most. It is possible that the high level of pyrazines in the 7spot, detectable at a distance but too general a signal to be identified as coming from any particular species (Moore *et al.* 1990), attracted the ants to the new site until they had sampled it and found it to be aversive. By the second trial it was the least visited site.

A Mann-Whitney U-test (Sokal & Rohlf 1981) performed on the totals which visited the 2spot and control treatments in stage 1 (Fig. 1A) shows that significantly more ants fed at the control than the 2spot food ($U = 197.5$; $df = 11, 11$; $p < 0.01$). A two-way G-test (Sokal & Rohlf 1981) performed on the number of ants in each of the two-treatment comparisons shows a very highly significant treatment effect ($G = 122$; $df = 2$; $p < 0.01$) mostly due to the ants avoiding the 7spot treatment. An analysis comparing the 7spot treatment

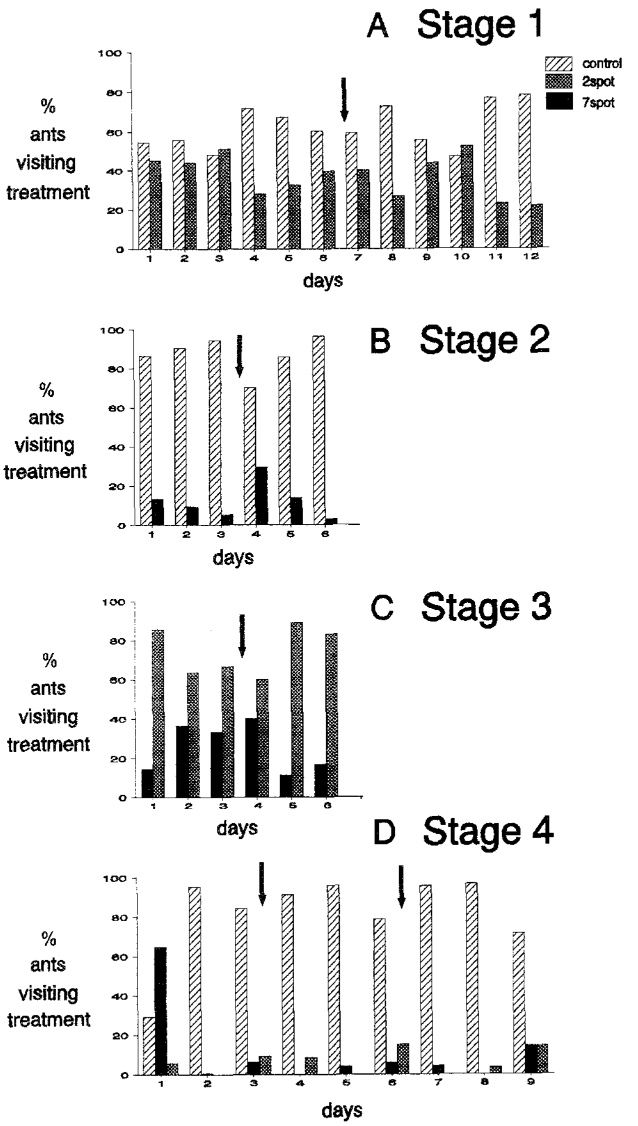

**Fig. 1** The percentage at each site of each day's total number of ants scored feeding. The sites on which the treatments were presented were swopped at the points indicated by the arrows

with both the others accounts for most of the variance ($G = 117.23$; $df = 1$; $p < 0.01$). The ants avoided the 7spot equally whatever the alternative food, similar proportions visiting it whether the alternative was control (Fig. 1B) or 2spot (Fig. 1C) ($G = 3.24$; $df = 1$ NS). Had they been avoiding both 2spot and 7spot equally, a smaller proportion of the ants would have visited 7spot when the alternative food was the control treatment than when it was 2spot.

There was no significant site effect in these three stages of the experiment. However, when the sites were swopped in stage 2 (Fig. 1B), the acceptance of control reduced and of 7spot increased on the first day after the swop. This effect was not seen in the other data sets, but may indicate a latency before some of the ants noticed and responded to the new position of their preferred treatment.

When all three treatments were presented together (Fig. 1D) the ants strongly preferred the control treatment and this caused a very significant treatment effect ($G = 207.4$; $df = 2$; $p < 0.01$). The interactions between the treatment with site and with replicate were also significant but far smaller (site $G = 11.1$; $df = 4$; $p < 0.05$; replicate $G = 20.6$; $df = 4$; $p < 0.01$) and were probably largely due to the high acceptance of 7spot on the first day, referred to above.

## Discussion

The experiment was designed to determine whether ants are repulsed by preparations of either 7spot or 2spot ladybird and to compare their reactions to each. The results support the findings of Pasteels *et al.* (1973a) that 7spot is aversive to ants and 2spot partially aversive. Direct comparison of these two species showed clearly that avoidance of 2spots was less than for 7spots. When 2spot and 7spot treatments were presented together and without a control pellet, fewer ants came to feed compared with the numbers recorded when control food was present. However, of those which did eat, the proportion which preferred 2spot to 7spot was as great as when the alternative was control.

This acceptance of the 2spot in the absence of more palatable food suggests a lower efficacy of chemical protection in this species, despite the presence of a higher concentration of alkaloid compared to 7spot (de Jong *et al.* 1991). Perhaps the higher concentration of pyrazines in the 7spot (Moore *et al.* 1990) enhances the effect of the 7spot alkaloid and speeds avoidance learning. More effective memory of the 7spot toxin would make the ants avoid the 7spot more, even if the two ladybird species were equally toxic.

It appears from the preliminary study described above that the 2spot is physically more vulnerable to attack by ants than the 7spot is. It is therefore surprising to find them also less chemically protected. Why should the 2spot synthesise a two part defence which is relatively ineffective? The chemical conversion between adaline and coccinelline is simple (Pasteels *et al.* 1973b), and there is even some evidence of trace amounts of coccinelline in 2spots (H. de Vos unpubl.; Marples 1990), although this needs to be confirmed. If these trace amounts are indeed present, it suggests that the biochemical pathways needed to produce coccinelline may be present. If this is so, it is unclear why the 2spot does not synthesis effective quantities either of this alkaloid, or of the pyrazines. Is there another chemical element, present in 7spots, but absent in 2spots, which is actually responsible for ant deterrence? This suggestion is supported by Pasteels' finding (Pasteels *et al.* 1973a) that even some ladybirds which lack alkaloids were aversive to ants.

In the wild, alternative food is usually available, so the low level of chemical protection conferred by adaline may be sufficient to deter ants (and other predators) from attacking. Ants would usually meet ladybirds on and near aphid colonies, where alternative food is plentiful. Under these circumstances the 2spots would not face any significant risk of predation by the ants, although they would probably still be driven off the aphid colonies, and as larvae this could be fatal.

No repulsion was seen in a previous experiment, when the sites for feeding were alternated daily, and a disturbance of the foraging patterns sometimes occurred when the sites were swopped. Ants lay pheromone trails to large food sources (Wilson 1975) which would have persisted from one day to the next. They may have followed these, rather than responding immediately to the stimulus presented. However, as I observed that individual ants sampled both sites during their 15 min 'search time' before the trial, it is perhaps surprising that they did not change their behaviour in response to the new conditions more quickly. It may be that some individuals sample new sites and respond immediately to the stimulus they find, and others continue in their established behaviour, finishing up the original source of food.

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