ANT TRAIL: A HIGHWAY FOR *Coccinella magnifica* REDTENBACHER (COLEOPTERA: COCCINELLIDAE)

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Red Wood Ant species chemically mark .trails connecting their nest to aphid colonies. The trail pheromones are produced in the ant's hindgut. The large trails are maintained thanks to visual clue and because new pheromone marks are regularly laid down. As a consequence, Red Wood Ant's workers create a network of trails equivalent to hundreds of meters around every nest mound. *Coccinella magnifica* is an aphidophagous ladybird species living only in the vicinity of Red Wood ant's nests. It preys upon aphid colonies regardless of ant's presence. We show here that, in the laboratory, *C. magnifica* can follow a recruitment trail laid down by *Formica polyctena* under artificial conditions with its hindgut's pheromone. This is confirmed by field observations of ladybirds walking along natural Red Wood Ant's trails. As *C. magnifica* appears to be a specialist, this behaviour is adaptive to efficiently locate prey.

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INTRODUCTION

In most temperate forests, lives a well-known ant species: the red wood ant (*Formica rufa sensu lato*) which is a taxonomical complex including some ecological species separated by their habitat preferences (YARROW 1955; SEIFERT 1996). It lives in large colonies whose nests are built as a hillock of dead plant fragments. This mound can reach 1.5 to 2 meters high and shelter hundreds of thousands ant individuals (HÖLLDOBLER & WILSON 1990). The other striking aspect of that ant are their wide foraging trails, linking the nest to food resources scattered in the surrounding (SKINNER 1980). Trails are marked by workers which use chemical pheromones probably produced in the hindgut and in other glands (GABBA & PAVAN 1970; PARRY & MORGAN 1979; ATTYGALE & MORGAN 1984). Moreover, visual cues have been demonstrated to play an important role in red wood ants orientation.

Ants are largely known as invertebrate predators but they also gather honeydew and occasionally prey upon aphids dwelling in trees and shrubs (e.g. WAY 1963; SKINNER 1980).

What are the relationships between ants, aphids and aphid predators?

Since more than half a century, it is largely admitted that ants increase attended aphid populations by cleaning the honeydew droplets glued on the colony and by protecting them against intruders, especially ladybirds, lacewings, bugs and syrphids (e.g. BANKS 1962; WAY 1963; HÖLLDOBLER & WILSON 1990).

Ladybirds found in red wood ant's habitat are mainly woodland-specialised species whose adults and larvae feed on tree aphids. In comparison with open areas, aphid resources are more dispersed vertically in the forest vegetation stages. All these hotspots of attended aphids are scattered in a three-dimension maze of branches, which create a more complex situation for food location than a two-dimensions system in herbaceous vegetation. Some ladybirds species encountered in woodland are: *Myzia oblongoguttata* L. in deciduous ones, or *Myrrha octodecimguttata* L. in pine forests, or *Coccinella magnifica* Redtenbacher in mixed forests, which is morphologically very similar to *Coccinella septempunctata* L. but scarcer because of its localised distribution (MAJERUS 1994). Some of these species are occasionally observed on ants trails, being brought back to the nest. Field observations revealed that *M. oblongoguttata* and *Anatis ocellata* L. are sometimes observed as prey, carried by *F. rufa* L. on foraging trails (SLOGGETT 1999). In that cases, ladybirds have been killed by ants or found already dead. *C. magnifica* is the only species usually observed alive on trails, walking among ant workers (SLOGGETT 1999, pers. obs.). This species is known to live around *F. rufa*'s colonies (DONISTHORPE 1920a, b; MAJERUS 1989), and it is suspected to benefit from ant-attended colonies, by preying upon it, in spite of the ant aggressive behaviour.

C. magnifica has evolved as a specialised predator, adapted to avoid ants aggressiveness. Though, we ignore whether this apparent immunity is due to the use of appeasement chemicals produced by the ladybird or only to behavioural adaptations. Since it is clearly established that adults and larvae of *C. magnifica* eat attended aphids, there is no information attesting that it also follows ants trails to enhance its food location efficiency. If it was the case, this adaptation, combined with an apparent immunity toward ants, could represent a strong selective advantage.

We want to test the fact that *C. magnifica* is found along *F. rufa*'s trails not as a prey but as an intruder.

We report here the results of two experiments using a *Formica polyctena* Förester colony kept in laboratory and two species of ladybirds, *C. magnifica* and *C. septempunctata* .

MATERIAL AND METHODS

Tests with a naturally-laid trail

In the first experiment, we used a naturally-laid ant trail which is obtained as follows. A nest is connected by a bridge to an area with a sugar solution. When the recruitment of workers was important, a cardboard sheet, on which a grid was traced with a pencil, was place on the bridge (shaded squares on Fig. 1). Only a row of squares was accessible to the ants, the others were covered with two smaller cardboard sheets. Workers walked for two hours on this row when travelling back and forth between the nest and the sugary solution. Then, the cardboard sheet was uncovered and deposited in an arena where the tests were performed with *C. magnifica* adults.

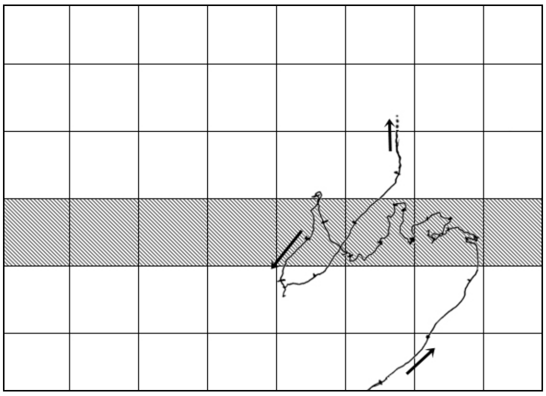

Fig. 1. Cardboard sheet on which a row of squares (shaded) have been marked by trail pheromones laid by foraging ants. An sample of a path walked by a ladybird is figured.

Marked and non-marked squares on which the ladybird walked were counted during two minutes. The comparison between ladybird's path and the rows of marked square was obtained by using a similarity index SI (1) (VERHAEGHE 1982), indicating if there is a preference for marked squares:

$$S.I. = \frac{Nc^2}{Na \times Nb} \qquad (1)$$

where Nc = number of marked squares crossed by the ladybird, Na = total number of marked squares and Nb = total number of squares crossed by the ladybird.

Occupation time of each square was also measured as an estimation of walk speed.

Each adult was firstly tested with a marked cardboard and then presented with an unmarked cardboard. There were 9 replicates with the ant trails and 8 with a clean cardboard.

Tests with an artificial circular trail

In the second experiment, we aimed at confirming our first results and obtaining more precise information about the nature of the observed ant-trail. We made an artificial circular trail, by dissecting ants, extracting the gland which produces the trail pheromones. Ant workers were collected on the foraging trails from the laboratory nest and dissected one by one under water, with ophthalmologic dissection tools. When one hindgut was isolated, it was transferred to 75 ml water and crushed. The results of five dissections were gathered and deposited on a strong filter paper with a Stadler® metallic pen. This method, described by Pasteels and VERHAEGHE (1974), has already been used in numerous trail-following experiments with ants and other invertebrates (CAMMAERTS et al. 1990; LENOIR et al. 1991; QUINET & PASTEELS 1995).

The 12-cm diameter circular trail on a filter paper was put in a circular arena of 25-cm in diameter and left for 10 minutes before the beginning of the tests. Two identical sequences of tests have been performed with the same trail: (1) four ants observed during two minutes; (2) four ladybirds (*C. magnifica* or *C. septempunctata*) during four minutes; (3) four ladybirds of the second species (*C. septempunctata* or *C. magnifica*) during four minutes; (4) four ants observed again during two minutes to check out whether the trail is still active. The first sequence took place 10 min after the trail has been laid down and the second 70 min later.

The artificial trail was compared to a control trail obtained with pure water. The sequence of tests was replicated 5 times with the trail and 4 times with the control.

All these observations were filmed and analysed afterward. For each sequence, the detection rate was measured and the "following score" was noted. The detection rate is the number of times an animal crossed the trail, stopped and eventually followed it. The "following score" is the number of 10° arcs consecutively followed by an insect. This score was log-transformed. Both the detection rates and the "following scores" are presented by their mean. The statistical difference between detection rates is obtained by comparing the proportion of defectives (detection of trail or not) during all tests. The result is a binomial distribution on which we calculated a value for z (NIST/ SEMATECH 2003), representing the significance level for the difference between the two proportions (test vs. control). The differences between following scores were tested with a Median test.

RESULTS

Tests with a naturally-laid trail

As the trail is deposited by ants, we assume that the right pheromones are present on the cardboard sheet. In comparison with the control, *C. magnifica* walked more often ($S.I._{Test} = 0.4219$; $S.I._{Control} = 0.0417$; Mann-Whithney U = 109.0; $P<0.01$, Fig. 2) and slower (Median occupation time: marked squares: 2.313 sec; covered squares: 1.259 sec; Mann-Whithney U = 126.0, $P< 0.001$ Fig. 3) on trail-marked squares. These results are consistent with an olfactory detection and orientation of *C. magnifica* thanks to *F. polyctena* trail pheromones.

Tests with an artificial circular trail

Over the course of the experiments the quality of the trails deteriorated so that the detection rate of ant F5 was not different in the presence or absence of the pheromone (Table 1). *C. magnifica* show a strong tendency to respond positively to the pheromone and not to the control. On the contrary, the detection rate of *C. septempunctata* is not modified by ant trails.

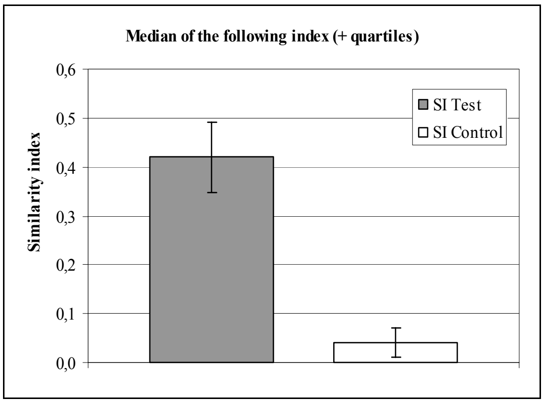

Fig. 2. Following index performed by *C. magnifica* on a marked sheet (in grey) and on control (in white).

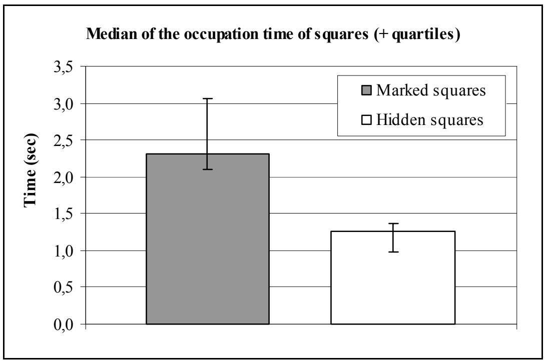

Fig. 3. Occupation time of squares, used as an estimation of walk speed on marked (in grey) and non-marked (in white) squares.

Table 1.
The detection rates of F. polyctena (F2, F3, F4 and F5), C.magnifica (CM_1 and CM_2) and C. septempunctata (C7_1 and C7_2) measured in two sequential experiments

Sequence		*Detection rate (%)*		
		Test (N=)	*Control (N=)*	*z=*
Nr. 1	F2	60 *(225)*	45.99 *(137)*	4.28 (***)
	CM_1	45.78 *(166)*	36.14 *(166)*	2.58 (*)
	C7_1	56.25 *(64)*	51.19 *(84)*	0.81 (N.S.)
	F3	62.11 *(190)*	28.26 *(92)*	2.74 (**)
Nr. 2	F4	57.22 *(180)*	48.62 *(181)*	2.31 (*)
	CM_2	41.38 *(145)*	27.52 *(109)*	3.74 (***)
	C7_2	35.58 *(104)*	37.93 *(58)*	-0.61 (N.S.)
	F5	50.48 *(198)*	51.48 *(154)*	-0.61 (N.S.)

For all the insects tested, there was no difference between the "following scores" measured with and without pheromone.

DISCUSSION

In the laboratory, *C. magnifica*, is able to follow a natural foraging trail delineated by pheromone spots.

Artificial trails made up of hindgut extracts are less efficient than natural trails. They, however, trigger detection and trail-following by ants and *C. magnifica*.

Our preliminary results might be confirmed by testing an extract of 10-hindguts instead of 5, expecting a response twice bigger with ants and *C. magnifica*.

Knowledge about pheromones produced by Dufour, poison and mandibular glands are quiet scant in the literature, so that we do not know their respective role in the formation of *F. rufa* foraging trails. Additional tests must be performed to check whether one or more of these glands could be used to enhance trail-following efficiency.

The fact that *C. magnifica* is able to follow ants trails is adaptive because these ladybirds are able to discover and exploit large aphid colonies attended by ants.

This study leads to another question: does the trail pheromone intervenes in mate encountering, selection of oviposition sites and egg laying?

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