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# Searching behaviour of the sevenspotted ladybird, *Coccinella septempunctata* – effects of plant-plant odour interaction

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In the present study the main aim was to investigate the odour mechanisms affecting habitat preferences of *Coccinella septempunctata* (L.). In a field study, the frequency of adult *C. septempunctata* was higher in barley plots containing high densities of the common weeds *Cirsium arvense* (L.) Scop. and *Elytrigia repens* (L.) Nevski. than in control plots with only barley. In olfactometer experiments in the laboratory, adult *C. septempunctata* showed a significantly more positive response to mixed odours of barley and each of the two weeds than to barley alone. Ladybirds responded differently to barley plants that were previously exposed to volatiles from the two weeds. The *E. repens*-exposed barley plant lost its attractivity while the *C. arvense*-exposed barley plants maintained attractivity. As no aphids or pollen resources were present in the plots during the experiment, the results show that *C. septempunctata* responds to the botanical characteristics of the habitat even if no food resources are available. These results strongly suggest that olfactory cues and plant-plant communication from diversified plant stands can be important mechanisms in predator attraction to sites with a complex botanical diversity.

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It is often stated that natural enemies are more effective at controling herbivores in more diverse botanical ecosystems (Letourneau 1987, Vandermeer 1989, Andow 1991). However, the mechanisms behind this are only partly known. This is often used as one of the general explanations of reduced pest problems in mixed cropping (Root 1973, Trujillo-Arriaga and Altieri 1990). An alternative or contributing factor could be increased difficulties for the pest insect to find its host plants (Feeny 1976, Vandermeer 1989).

The searching behaviour of polyphagous predators, exemplified by ladybirds (Coccinellidae), is a combination of randomised movements and responses to a wide range of stimuli (Kesten 1969, Nakamuta 1984, Meiracker et al. 1990) including olfactory cues (Obata 1986, 1997, Ponsonby and Copland 1995, Zhu et al. 1999, Ninkovic et al. 2001). The searching efficiency of herbivores may be reduced in mixed plant stands as a result of the interference of non-host plant volatiles with odours from the preferred host plant (Thiéry and Visser 1987). To what extent this effect is relevant to the searching behaviour of predators is still poorly understood.

Herbivore-attacked plants release chemical signals that serve as important olfactory cues for parasitoids (Grasswits and Paine 1993, Du et al. 1998, DeMoraes et al. 1998, Powell et al. 1998, Guerrieri et al. 1999, Van Loon et al. 2000) and predators (Vet and Dicke 1992, Drukker et al. 1995, Bruin et al. 1992, 1995, Dicke 1999, Sabelis et al. 1999, Ninkovic et al. 2001). Volatiles from an insect-attacked plant may also induce a response in neighbouring, non-attacked plants, making them less attractive to herbivores (Bruin et al. 1992, 1995, Pettersson et al. 1996, Bruin and Dicke 2001, Farmer 2001). Furthermore, some natural enemies can use volatiles emitted by undamaged plants to locate the

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habitats of herbivores (Elzen et al. 1983, Benrey et al. 1997, Takabayashi, et al. 1998). It can be concluded that plant status, as expressed by volatile emissions constitutes a potentially important information source that can modify the behaviour of herbivores as well as their natural enemies (Cortesero et al. 2000).

In the simplest case, volatiles from a specific non-attacked plant may be merely a contribution to the mixture of compounds emitted by other plants in the stand. However, the emitted volatiles may also be messengers for communication with neighbouring plants (allelopathy sensu Molisch 1937, Rice 1984). Coexistence with other plants is the most common type of biotic challenge that a plant individual meets, and there is experimental evidence that communication between undamaged plants also promotes changes in plant-herbivore relations. This has been shown not only for plant-plant communication between different species (Farmer and Ryan 1990) but also for plants of the same species (Pettersson et al. 1999, Ninkovic et al. 2002). However, so far the effects of plant-plant communication between undamaged plants on the third trophic level, *i.e.* predators and parasitoids, have not been thoroughly examined experimentally. The aim of the present study was to investigate the role of odours in pure and mixed plant stands on the foraging behaviour of the seven spotted ladybird, *Coccinella septempunctata* (L.) in a barley field with two weed species.

## Material and methods

### Plant and insect material

For laboratory experiments, adult *C. septempunctata* were collected in the field and kept in cages with potted barley plants (*Hordeum vulgare*, cv. Golf) infested with aphids, *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.). Plants of *Cirsium arvense* (L.) Scop. and *Elytrigia repens* (L.) Nevski. were raised in pots from rhizomes dug up at the field experimental site. Insects and plants were kept in greenhouse at 14 h light, 18–22°C temp. and ca 80% relative humidity.

### Field experiment

Field experiments were made in June and beginning of July 2000 in a ca 50 ha field of spring sown barley (cv. Anabell). The experimental site was located 35 km North of Uppsala (Sweden) and managed in accordance with conventional farming practices in the region. This means a seed rate of 200 kg/ha, and fertilisation corresponding to 90 kg N/ha. The growing conditions and crop development in the field were very homogenous, but small weed aggregations occurred in different parts of the field due to failing weed control. Centres of circular plots with a diameter of 4 m were marked out in the field with sticks. Three categories of plots were selected, one with only barley and no weeds (10 plots), one with a mixture of barley and *C. arvense* (11 plots) and one with barley and *E. repens* (11 plots*)*. The plots were chosen in accordance with common agronomic practice to have approximately the same amount of green biomass, similar crop status and comparable growing conditions. The plots were separated by at least 20 meters but combined in groups with all three categories. The weed infestation was estimated in the 'weed plots' and found to be 20-50% of soil cover. Starting when the barley crop was at the two leaf stage, six estimates of the frequency of adult *C. septempunctata* in the plots were made over the course of four weeks. At the same time a check was made for aphid infestations and flowering (pollen) of plants that might interfere with the experimental aims. The weed species considered in the experiments did not reach a flowering stage during the experiment and no obvious alternative food source was noticed in the plots. The aphid incidence was extremely low in the experimental region during 2000 and aphids appeared in the experiment at very low frequences only during the last two observations made. The sensitivity of the ladybirds to prevailing weather conditions made it necessary to select days with good weather for the sampling. The sampling was chosen to coincide with an active searching period of adult *C. septempunctata*. A 2 m long string was fastened to the stick in the centre of the plot and the observer used this to identify the periphery of the plot while making the observations.

Statistical analysis was done on counts of ladybirds in the different plots at different times. This type of data can be modelled using a generalised model assuming a Poisson distribution (McCullagh and Nelder 1989) and here repeated measurements were made at the same plots. This was accounted for by using a repeated measures design, which was analysed using the Glimmix macro of the SAS (1997) system; Wolfinger and O'Connell (1993). Dependence over time was modelled using a spatial power model, where the correlation between occasions was assumed to decrease with the time span between occasions. Least square means of the model parameters were calculated and compared between treatments.

### Plant exposure in laboratory experiments

Responses of adult *C. septempunctata* to odours were tested both to barley and mixtures of barley and weed odours, and then to odours from barley plants that had been exposed to air from one of the two weeds. Barley plants with no specific exposure but otherwise treated identically to other test plants were used as controls in the olfactometer tests. Barley plants used in the experiments were at a three leaf stage and the weed plants used in the exposure treatments were chosen to approximately represent the same amount of biomass. The exposure of barley plants to air with volatiles from the weeds was done in a twin chamber arrangement connected to a vacuum pump (Pettersson et al. 1999). Air was led from one chamber (A, with a weed plant) to the next (B, with a barley plant) and then vented outside the greenhouse. The air flow through the system was tested with salmiak smoke and the air in the system was completely changed after two minutes. A treatment time of five days was used, based on previous studies (Pettersson et al. 1999).

## Olfactometry experiment

The influence of host plant odours on the preferences of adult *C. septempunctata* observed in the field was studied in a laboratory test using a two-arm olfactometer. This apparatus consisted of an arena (6 × 6 cm) with two conical, extended arms (arm length 7 cm) to which odours could be introduced. The arms were closed at the ends with a net preventing the test animals from reaching the attached odour source in the arm. The arena was cut out in 2 cm foam plastic with closed cells and placed between two layers of 3 mm perspex. Air was drawn out from the centre of the arena creating two arm zones, each overstreamed by the odour from the source attached, and a central arena zone in the middle of the arena. Air flow through the system was set to 3 ml/s measured at the arm inlets and was controlled with flow meter and a valve. The test insects were randomly chosen from a stock culture of field collected beetles and each individual was used only once. In these experiments the outlets from twin cages with different plant arrangements were directly connected to one of the olfactometer arms. As a standard control, a cage with approximately the same amount of untreated barley plants was used and connected to the other arm. One test insect was placed in the olfactometer and its position in the arena was registered 10 times at 2 min intervals, which was long enough to permit an adult coccinellid to move from one end of the arena to the other. After an adaptation period of 10 min, a vacuum pump was started, drawing air through the tubes and arms towards the centre of the arena. The accumulated number of visits in the arm zones after ten observations was regarded as one observation. If the test individual did not move between two consecutive observations the series was interrupted and a new series of 10 observations was started with a new insect. Data were analysed with Wilcoxon Matched Pairs statistics.

# Results

## Field experiments

The average numbers of *C. septempunctata* observed in different categories of plots are summarised in Fig. 1. A generalised linear mixed model with Poisson distribution, a log link and a spatial power structure of the between-occasion correlations had a good fit to the data (Deviance/d.f. = 1.09). The plots with only barley had significantly lower numbers of ladybirds than both plots of barley-*E. repens* mixture ($p < 0.0005$) and plots of barley-*C. arvense* mixture ($p < 0.0008$) during the observation period. There was no significant interaction between treatment and day ($p = 0.88$). The difference between plots with *E. repens* and plots with only barley was significant ($p < 0.0005$) as was the difference with plots *C. arvense* ($p < 0.0008$). The difference between the two weed infested plots was not significant ($p = 0.88$).

## Laboratory experiments

With barley plants as controls in the olfactometer experiments, no difference between ladybird preferences was found to odour of comparable amounts of biomass of the two weeds in the olfactometer tests (Fig. 2a and 3a). A significant arresting/attracting effect on ladybird walking behaviour was apparent when compared with untreated barley odour (Fig. 2b and 3b). Barley plants treated with volatiles from *C. arvense* retained their attractivity to adult *C. septempunctata* for some time (Fig. 3c) whereas barley plants treated with volatiles from *E. repens* lost this capacity immediately when separated from the *E. repens* plant (Fig. 2c).

# Discussion

The results show that odour cues may play a role in focusing ladybirds to places with botanical diversity, and reveal a new dimension of volatile-mediated

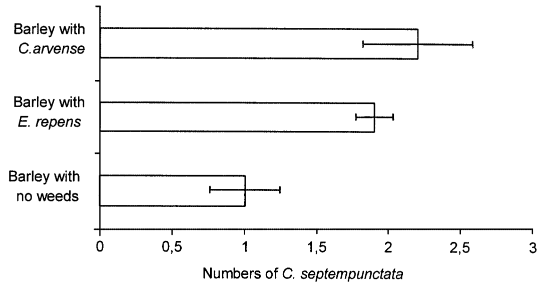

Fig. 1. Least square means of *C. septempunctata* observed in the different categories of plots over a 25 day period, based on six separate observations. Statistics are explained in the text. Error bars indicate ± s.e.m.

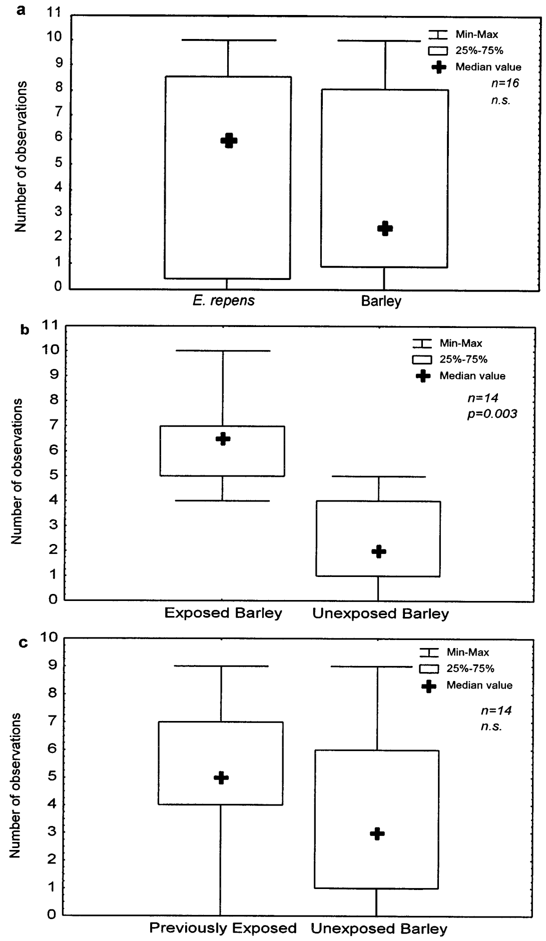

Fig. 2. Olfactometer responses of adult *C. septempunctata* to odours from different combinations of *E. repens* and barley. (a) Comparison between barley and *E. repens*; (b) mixture of odours from barley and *E. repens* compared with only barley; (c) odour from barley previously exposed to air from *E. repens* compared with untreated barley. The median is indicated by the + symbol and each box encloses the middle 50% of the data. The minimum or maximum number of visits by tested insects is represented by the whiskers (vertical lines); n = number of insects tested; p = probability by Wilcoxon matched pairs test; n.s. = no significant difference.

tritrophic (plant-predator) interactions. Our results indicate two possible modes of action behind the effects of increased botanical diversity on ladybird behaviour. The first is supported by the *E. repens*-barley combination results, and indicates that a more complex mixture of plant volatiles per se is more attractive/arresting than a less complex mixture. The second involves plant-plant communication and is supported by the results from the *C. arvense*-barley combinations. It is more complex and presupposes that interaction between plants results in a physiological change in the barley plants. The second mechanism could be related to competitive plant interaction since competition with other plants is the most common biotic challenge a plant individual meets with. This mechanism would be in line with previous findings on tomato plants (Farmer and Ryan 1990) demonstrating that interplant communication can occur between the leaves of one plant species and another. Allelopathic mechanisms have also been shown to affect aphid host plant acceptance (Pettersson et al. 1999, Ninkovic et al. 2002). This is in line with findings that general abiotic plant stress has a positive effect on the searching behaviour of natural enemies (Takabayashi et al. 1994) and contributes to the understanding of mechanisms of general habitat location in adult coccinellids.

Polyculture may favour some generalist predators, but is more likely to reduce the efficiency of specialist predators (Sheehan 1986). There is a need for further

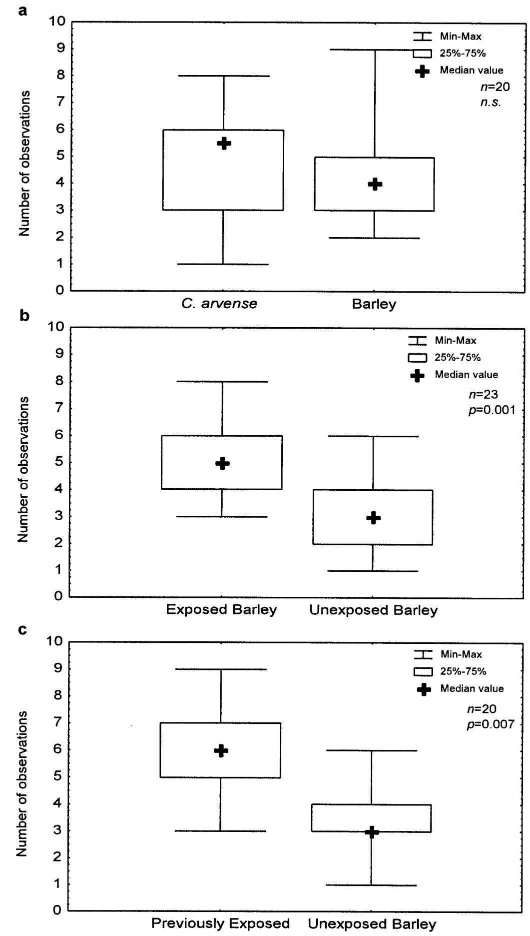

Fig. 3. Olfactometer responses of adult *C. septempunctata* to odours from different combinations of *C. arvense* and barley. (a) Comparison between barley and *C. arvense*; (b) mixture of odours from barley and *C. arvense* compared with only barley; (c) odour from barley previously exposed to air from *C. arvense* compared with untreated barley. The median is indicated by the + symbol and each box encloses the middle of 50% of the data. The minimum or maximum number of visits by tested insects is represented by the whiskers (vertical lines); n = number of insects tested; p = probability by Wilcoxon matched pairs test; n.s. = no significant difference.

knowledge on habitat location mechanisms of coccinellids (Sloggett and Majerus 2000). The response to plant odours in the present experiments may be a response to a particular habitat which is followed later by a more specific searching procedure in which identification of targets such as attacked plants, pollen sources etc. plays a role (Ninkovic et al. 2001).

*C. septempunctata* is considered to be an aphid specialist but it is very polyphagous with a broad menu and can thus be seen as representative of polyphagous predators. The present results contribute to improved understanding of the searching behaviour of this group, whose importance has often been stressed in discussions on pest problems and mixed cropping. The demonstrated effect on predator searching behaviour is also relevant to discussions on the importance of natural enemies, and for a better understanding of the importance of biodiversity in natural botanical communities.

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