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Dispersal, its influence on reproduction and host-plant finding in the alpine leaf beetle *Oreina cacaliae*

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IMPRIMATUR POUR LA THESE

Dispersal, its influence on reproduction and host plant finding in the alpine leaf beetle *Oreina cacaliae*

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Abstract

The alpine leaf beetle *Oreina cacaliae* was observed to overwinter either in the ground close to their main host plant *Adenostyles alliariae* or, after flight, at distinct overwintering sites away from that host. In spring, beetles flew from overwintering sites to flowering *Petasites paradoxus* on which they fed until the emergence of their main host. This host, *A. alliariae*, contains pyrrolizidine alkaloids (PAs) which the beetles sequester for their own defence. In the flowers of *P. paradoxus* we found the same PA (seneciphylline) as in *A. alliariae* leaves. The observed dispersal polymorphism in *O. cacaliae* might indicate costs associated with flight. Indeed, survivorship of the flyer group was reduced compared to the beetles which did not fly. Without access to food after flight, flying beetles which were smaller in size than non-flyers, larviposited significantly less than non-flyers. Field observations indicated that *O. cacaliae* locate host plants by walking after a random landing in the vegetation, rather than by landing directly on a plant. In windtunnel experiments the beetles were attracted by olfactory cues emitted by their host plants, and distinguished them from non-hosts. Infested host plants, no matter if caused by adult conspecifics, *O. cacaliae* larvae, or artificially with a razor blade, were more attractive than healthy plants. The time that had passed between infestation and experiment was crucial to elicit an enhanced response in *O. cacaliae*. In the spring host *P. paradoxus,* an infestation caused during the night before the experiment increased the attractiveness, whereas in *A. alliariae* only plants that were infested half an hour before the experiment were more attractive than healthy plants. Analysis of volatiles emitted by these plants using gas chromatography yielded quantitative and qualitative differences. Moreover, beetles released at a site surrounded by *A. alliariae* moved to their host plants in upwind directions independent of the wind direction. All experiments performed in both, the field and the windtunnel strongly indicated that *O. cacaliae* localises its host plants by olfaction.

Sommaire

La chrysomèle alpine *Oreina cacaliae* passe les hivers soit dans le sol proche de la plante-hôte principale, *Adenostyles alliariae*, soit à une certaine distance de cet hôte après un vol. Au printemps, les coléoptères quittent les sites où ils ont passé l'hiver et ils s' en volent vers les fleurs de *Petasites paradoxus* où ils mangent jusqu' à l' émergence de la plante-hôte principale. Cet hôte, *A. alliariae* contient des alkaloides pyrrilizidiques (PAs) qui sont séquestrés par les coléoptères pour leur défense. On a trouvé le même PA (seneciphylline) dans les fleurs de *P. paradoxus* que dans les feuilles d' *A. alliariae*. Le polymorphisme de vol observé chez *O. cacaliae* pourrait indiquer des coûts associés au vol. En effet, la survie et la reproduction sont diminués chez les coléoptères qui ont volé et qui n'avaient pas de nourriture après leur vol. Des observations sur le terrain ont indiqué que *O. cacaliae* localise les plantes-hôtes en marchant après un atterrissage par hasard dans la végétation plutôt qu' en atterrissant directement sur la plante. Les coléoptères sont attirés par des odeurs émises par leurs plantes-hôtes et distinguent celles des plantes non-hôtes dans le tunnel de vol. Des plantes-hôtes attaquées, par les larves ou les adultes d' *O. cacaliae* ou artificiellement avec une lame de rasoir, sont plus attractives que des plantes non-attaqués. Au printemps, *P. paradoxus* attaqué pendant la nuit avant l'experience a montré une attractivité plue grande. Pour les *A. alliariae*, les plantes attaquées une demi heure avant l'experience sont plus attractives que les plantes hôtes non-attaquées. Les analyses des odeurs émises par ces plantes par chromatographie gazeuse ont montré des differences quantitatives et qualitatives. Des coléoptères relachés à un endroit entouré d' *A. alliariae* se dirigent toujours contre le vent vers ses plantes hôtes, indépendamment de la direction du vent. Toutes les experiences réalisées, sur le terrain et dans le tunnel de vol, indiquent qu' *O. cacaliae* localise ses plantes-hôtes par olfaction.

General Introduction

The alpine leaf beetle *Oreina cacaliae* belongs to the Chrysomelidae, a family of phytophagous insects, which is quite host specific (Mitchell, 1988). Because of their exposed location on the leaf surface and their relative immobility, they are particularly vulnerable to predators and parasitoids (Price, 1984). Additionally, their relatively low energy conversion rates (Southwood, 1973) require that they spend long periods of time feeding. Therefore, it is not surprising that a number of defensive mechanisms have evolved in the Chrysomelidae. Additionally they are mostly specialised feeders on toxic host plants. Larvae and adults of *O. cacaliae* sequester the *N*-oxides of toxic pyrrolizidine alkaloids (PAs), (preferentially seneciphylline *N*-oxide) in their elytral and pronotal defensive secretion. They obtain these substances from their host plant *Adenostyles alliariae* (Ehmke *et al.*, 1991; Ehmke *et al.*, 1999). The use of presynthesized secondary compounds available in the host plant minimises the cost of chemical defence of an otherwise high metabolic cost of allomone biosynthesis (Pasteels *et al.*, 1988). The protection obtained from the sequestered PAs has been shown to be even more effective against predation by naive birds than the protection provided by autosynthesized cardenolides in another *Oreina* species, (Rowell-Rahier *et al.*, 1995). Each herbivorous insect, in the course of its evolution has become associated with particular host plants, which are nutritionally and ecologically adequate. *O. cacaliae* feeds on *Petasites paradoxus* in spring, and larviposits and feeds on *A. alliariae* later in the season. The larvae develop to the fourth instar during summer, dig in the soil in autumn where they overwinter and develop into a new generation of adults in July. Those new emerging adults can be distinguished from the adults that had overwintered in the adult stage by their soft elytras.

Maintenance of the associations between herbivorous insects and host plants requires host plant recognition (Dethier, 1982). Since any given plant possesses definite qualities that characterise it (such as form, colour, texture, water content, and hundreds of chemical compounds), recognition of that plant by a herbivore depends not only on the nature of these features but also on the characteristics of the herbivore's sensory system, and its detecting capabilities (Dethier, 1969). It has been shown that the sense of smell is of particular importance in host plant recognition of monophagous and oligophagous insects (Weissbecker *et al.*, 1997). Olfactory receptor systems have evolved in phytophagous insects enabling them to perceive plant volatiles. The host plant odour recognition of an insect can be tested in olfactometers in the lab. In addition, the composition of the volatile blend emitted by a plant can be determined by various chemical analytical techniques.

The ability to disperse by flight is an important feature of insects and has played a key role in their evolutionary success (Zera & Denno, 1997). Insects have been able to exploit habitats they otherwise could not use, as well as to increase their flexibility of response to environmental contingencies (Rankin & Burchsted, 1992). The spatial structure of the environment strongly affects the foraging strategies of insects. Their great mobility enables them to look for food items within a patch and also to move between patches or habitats that may be widely dispersed (Hassell & Southwood, 1978). Simulation models have demonstrated the importance of dispersal as a major stabilising factor, without which the population would quickly become extinct (Roff, 1974).

In many insects, dispersal by flight occurs prior to reproduction, and the ability to fly is often lost once reproduction begins. Thus, dispersal is viewed as an evolved adaptation, characteristic of a particular stage in the ontogeny of the insect, rather than a spontaneous response to current adversity (Harrison, 1980). The widespread occurrence of flight polymorphisms with distinct phenotypic morphs strongly suggests that fitness costs are associated with the ability to fly (Roff, 1984). The energy used to construct wings and flight muscles is simply not available for reproductive investment (Zera & Denno, 1997). To determine the possible cost of flight survival and reproduction between flying and non-flying beetles were compared. The fact that flyers emerge earlier than non-flyers in spring and feed on the spring host *P. paradoxus*, whereas non-flyers overwinter in the soil near to the patches of the main host *A. alliariae* allows to distinguish flyers easily from non-flyers in the field.

Host-plant finding relates to the insect's spatial manoeuvres while on the ground or in the air, and is affected by the spatial distribution of plants and other vegetational characteristics (Visser, 1988). The anemotactic system appears to be the most powerful mechanism of guidance to an odour source and it is probably the main mechanism allowing host finding from several meters away, unless the source is conspicuous enough for a visual response (Kennedy, 1977). Odour released into the air is transported by the wind. Close to the source the odour molecules spread by molecular diffusion and further away by turbulent eddies. As a consequence, the odour concentration gradient close to the source is disrupted further downwind. The odour released from a point source appears at a fixed point downwind as a series of odour bursts (Murlis & Jones, 1981). The mechanism for maintaining an upwind course in an odour plume has been investigated in a variety of insects, suggesting that mechanisms of walkers and flyers are basically the same, although complicated somewhat by the third spatial dimension in flyers. Perception of a stimulating chemical provokes upwind orientation, and perception of the plume boundary, (i.e. a change in odour concentration) causes turns to the opposite track, keeping a walking or flying insect within a plume (Bell & Tobin, 1982).

References

Bell, W. J. & T. R. Tobin, 1982. Chemo-orientation. Biological Review 57: 219-260.

Dethier, V. G., 1969. Whose real world? American Zool. 9: 241-249.

Dethier, V. G., 1982. Mechanism of host-plant recognition. Entomologia Experimentalis et Applicata 31: 49-56.

Ehmke, A., M. Rowell-Rahier, Jacques M. Pasteels & Thomas Hartmann, 1991. Sequestration of ingested (^{14} C)Senecionine N-oxide in the exocrine defensive secretions of chrysomelid beetles. Journal of Chemical Ecology 17: 2367-2379.

Ehmke, A., M. Rahier, J. M. Pasteels, C. Theuring & T. Hartmann, 1999. Sequestration, maintenance, and tissue distribution of pyrrolizidine alkaloid-*N*-oxides in larvae of two *Oreina* species. Journal of Chemical Ecology 25: 2385-2395.

Harrison, R. G., 1980. Dispersal polymorphism in insects. Annual Review of Ecology and Systematics 11: 95-118.

Hassell, M. P. & T. R. E. Southwood, 1978. Foraging Strategies of Insects. Annual Review of Ecology and Systematics 9: 75-98.

Kennedy, J. S., 1977. Olfactory responses to distant plants and other odour sources. In: H. H. Shorey & J. J. McKelvey Jr (ed), Chemical control of insect behaviour, Wiley-interscience, New York. pp. 67-91.

Mitchell, B. K., 1988. Adult leaf beetles as models for exploring the chemical basis of host-plant recognition. Journal of Insect Physiology 34: 213-225.

Murlis, J. & C. D. Jones, 1981. Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources. Physiological Entomology 6: 71-86.

Pasteels, J. M., M. Rowell-Rahier & M. J. Raupp, 1988. Plant-derived defence in Chrysomelid beetles. In: B. P. & L. D. K. (eds), Novel Aspects of Insect-Plant Interactions, pp. 235-272.

Price, P. W. 1984. Insect Ecology, 2nd ed. Wiley, New York.

Rankin, M. A. & J. C. A. Burchsted, 1992. The cost of migration in insects. Annual Review of Entomology 37: 533-559.

Roff, D. A., 1974. The analysis of a population model demonstrating the importance of dispersal in a heterogeneous environment. Oecologia 15: 259-275.

Roff, D. A., 1984. The cost of being able to fly: a study of wing polymorphism in two species of crickets. Oecologia 63: 30-37.

Rowell-Rahier, M., J. M. Pasteels, A. Alonso-Mejia & L. P. Brower, 1995. Relative unpalatability of leaf beetles with either biosynthesised or sequestered chemical defence. Animal Behaviour 49: 709-714.

Southwood, T. R. E., 1973. The insect/plant relationship. An evolutionary perspective. In H. F. van Emden (ed.), Insect/Plant relationships. Symposium of the Royal Entomological Society of London, Vol. 6. pp. 3-30. Blackwell Scientific, Oxford.

Visser, J. H., 1988. Host-plant finding by insects: orientation, sensory input and search patterns. Journal of Insect Physiology 34: 259-268.

Weissbecker, B., S. Schütz, A. Klein & H. E. Hummel, 1997. Analysis of volatiles emitted by potato plants by means of a Colorado beetle electroantennographic detector. Talanta 44(12): 2217-2224.

Zera, A. J. & R. F. Denno, 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42: 207-31.

Outline of the thesis

Dispersal by flight, possible costs of flight and mechanisms of host plant recognition were studied in the alpine leaf beetle *Oreina cacaliae*.

In **Chapter 1** the occurrence and the flight behaviour of *O. cacaliae* in Val Ferret (Valais) during three successive years is described. Beetles were marked at different places throughout three years, to determine their dispersal ability.

Chapter 2 investigates the possible costs of flight. Flyers could have to pay for their flying ability with a reduced survivorship and a reduced reproductive success. Morphological differences between flyers and non-flyers might exist.

In **Chapter 3** the attractiveness of already infested host plants for *O. cacaliae* was tested in the windtunnel. We wanted to test if the time that has passed between injury and experiment is important and if infestation caused by larvae or adults of *O. cacaliae* elicits a different response than plants injured artificially with a razor blade. The odours emitted by the healthy and injured plants were collected and compared using gas chromatographic analyses.

In **Chapter 4** the importance of olfaction for the host finding behaviour of *O. cacaliae* in the field was tested. We released marked beetles in the middle of a stone-covered path, three times while the wind blowing from the north and once with wind blowing from the south. If vision was important for the host finding behaviour of the beetle, the beetles distribution on host plants, should not differ between experiments because the visual surrounding was always the same. If olfaction was important, the beetles should rather choose plants located upwind from the release point. The latter was the case.

I

DISPERSAL BY FLIGHT AND HOST PLANT RECOGNITION AFTER SPRING EMERGENCE IN THE ALPINE LEAF BEETLE *OREINA CACALIAE*

Key words: dispersal by flight, spring emergence, mark-recapture, host-plant location, olfaction, plant odour, volatiles, behaviour, windtunnel, PAs.

ABSTRACT

Throughout two complete seasons the movement patterns of the alpine leaf beetle *Oreina cacaliae* were monitored and its host-plant odour preference was tested. The beetles are confronted with the problem to locate host plants in spring, after overwintering in the soil, and to complete their life cycle within the short season of the alpine environment. Beetles that take flight in spring emerge earlier than non-flying conspecifics. Mark-recapture experiments showed that the beetles flew from overwintering sites to the flowering spring host *Petasites paradoxus* where they started feeding. Later, as soon as the plant emerged, the beetles switched to their principal host plant, *Adenostyles alliariae*, on which they larviposit. The flyers emerge before their main host plant *A. alliariae*, that contains pyrrolizidine alkaloids (PAs), which the beetles sequester for their own defence. They appear to overcome this problem by feeding on early emerging *P. paradoxus* flowers in which we found the same PA (seneciphylline) as in the *A. alliariae* leaves. Field observations indicated that *O. cacaliae* is a poor flyer and take-off directions were strongly influenced by the prevailing wind direction. The host plant localisation was observed to occur by walking after a random landing in vegetation, rather than by landing directly on a plant. Wind tunnel experiments should show whether walking *O. cacaliae* are able to discriminate between plant volatiles emitted by either host or non-host plants. In spring, the flowering host *P. paradoxus* was preferred over the flowering non-host *Tussilago farfara*. Only the flowering state of *P. paradoxus* was attractive while the plant lost its attractiveness after changing to the vegetative state. Later in the season, the vegetative state of the principal host *A. alliariae* was preferred over vegetative *P. paradoxus*. An additional test with a leaf model, emitting no plant odour, suggested that visual cues are of lesser importance. The results show that *O. cacaliae* is able to discriminate between odours emitted by host and non-host plants even though all plants that were tested are taxonomically closely related, belonging to the same subtribe Senecioninae of the Asteraceae.

INTRODUCTION

The alpine, middle-european *Oreina cacaliae* (Schrank, 1785) (Coleoptera: Chrysomelidae) is confronted with the problem of a long winter and a short season when the beetles are active. Larvae and adults of *O. cacaliae* have been reported to feed on plants of the Asteraceae including the genera *Adenostyles* and *Senecio* (Kühnelt, 1984; Jolivet, 1986). *A. alliariae* is the principal host plant of *O. cacaliae* at the field site near La Fouly (1500 m above sea level) where host plants of the genus *Senecio* do not occur. It is on *A. alliariae* that the beetles larviposit in early summer. Larvae and adults of *O. cacaliae* sequester in their elytral and pronotal defensive secretion the *N*-oxides of pyrrolizidine alkaloids (PAs), preferentially seneciphylline *N*-oxide, that they obtain from *A. alliariae* (Ehmke *et al.*, 1991; Ehmke *et al.*, 1999). These beetles were shown to be protected from predation by naive birds (Rowell-Rahier *et al.*, 1995). *O. cacaliae* larvae feed on *A. alliariae* until they are fully grown and subsequently dig into the soil to overwinter before pupating the following summer. Until now, adult beetles were thought to overwinter in the soil next to patches of *A. alliariae.*

The ability to fly has made insects the success they are, enabling them to exploit habitats they otherwise could not use and to increase their flexibility in the response to environmental contingencies (Rankin and Burchsted, 1992). Preliminary field observations suggested different overwintering strategies involving dispersal by flight and the use of a spring host plant, before the emergence of their main host. The most direct means to determine the dispersal by flight and behaviour after spring emergence is by recapture of marked beetles. Most studies on flight activity are performed under artificial circumstances with the insect being fixed to a pin or tethered to a flight mill. Our field observations and mark-recapture studies were aimed to discover the flight behaviour of *O. cacaliae* in the field and to see how they locate suitable hosts in spring.

In the current study we investigated whether the beetles use plant odours to locate their host plants and to distinguish them from non-hosts. We used the Asteraceae *Tussilago farfara* and *Petasites paradoxus,* both emerging in the flowering stage, for our experiments, because they are the only perennial herbs present when *O. cacaliae* emerges early in spring. Later in the season we tested the attractiveness of the main host *A. alliariae.* All plants tested in the choice experiments are taxonomically closely related belonging to the subtribe Senecioninae of the family Asteraceae (Nordenstam, 1977). An experiment with a leaf model with exactly the same size and shape as a real leaf and the colour matched by colour photocopy, was conducted to determine the importance of vision in host plant recognition by *O. cacaliae.* Host plant odours have been shown to be important for the attraction of several Chrysomelidae. The Colorado potato beetle (*Leptinotarsa decemlineata*) was shown to move upwind in a windtunnel even in the absence of host plant odours but host-plant odours enhanced this anemotactic response (de Wilde *et al.*, 1969). Visser (1976) confirmed this result by offering potted potato plants as odour sources in a windtunnel and defined the observed behaviour of *L. decemlineata* as odour-conditioned anemotaxis. Two species of *Diabrotica* are attracted by volatiles of corn silks (Prystupa *et al.*, 1988). The attractiveness of host plant odour can be blocked by blending the odour of potato with the one of a non-host plant like tomato or cabbage (Thiery and Visser, 1986; Thiery and Visser, 1987). The flea beetle, *Phyllotreta cruciferae* (Chrysomelidae) was not attracted to its principal host plant, oilseed rape (*Brassica napus*), when it was offered together with a less important host plant in a two-choice olfactometer test (Peng and Weiss, 1992).

We studied early season dispersal by flight of *O. cacaliae* in the field using mark-recapture studies and the influence of wind on take-off direction. In addition, the importance of host plant odours (and vision) in the localisation of a suitable host plant by *O. cacaliae* after spring emergence was determined in several windtunnel experiments with host and non-host plants.

MATERIAL AND METHODS

Field work on dispersal by flight

All field work was done in the Swiss Alps near La Fouly in the Val Ferret (Valais) at 1500 m above sea level (45.56.10 latitude, 7.05.95 longitude).

Take-off direction. Whenever beetles were observed while taking-off spontaneously, from either an overwintering site or from a *P. paradoxus* patch, their take-off direction (with respect to magnetic north) was noted together with the windspeed (m/s) and the prevailing winddirection. Only at three sunny days (18th of March, 22nd of April and 12th of May) were beetles observed taking-off. Wind speed was measured with a portable anemometer (50518, Bioblock Scientific, Frenkendorf, Switzerland) 70 cm above ground and direction was noted with the aid of a compass.

Beetle distribution. The beetles' distribution after dispersal by flight was observed at a sunny day in the field. On the 22nd of April 1998 all beetles found along the walking paths of 1.2 km length were recorded. All beetles started their flight at the overwintering places R1 or R2. At this time of the year *P. paradoxus* had emerged at three places in the area only, and there was a considerable snow cover over the rest of the study area.

Flight distance. Beetles were marked with 2 to 7 spots of correction fluid (Tipp-Ex) on the elytra at unique positions enabling us to identify their release point upon recapture. The marking code could easily be read without disturbing the insect. Once marked, the beetles were released immediately at their original positions. During two consecutive years, 1997 and 1998, the main host plant patches in the study area were checked weekly during 3 to 4 h for marked beetles, in order to follow the movement of individuals over time across the field area. Once 1998 two days were spent to search for marked beetles in the whole area measuring 4 km^2. Of the 2521 beetles marked in the field, 610 were marked at the overwintering sites early in spring (between 13th of March and 22nd of April), while 1008 and 460 were marked, in *P. paradoxus* (between 22th of April and 13th of May) and *A. alliariae* (after 20th of May) patches, respectively. On *P. paradoxus* 3 km further up the valley another 390 beetles were marked. In autumn (between the 25th of August and the 23rd of September) we marked 33 beetles at the overwintering place soil and 20 beetles at the overwintering places R1 and R2. Meteorological data were obtained from the meteorological station at Evolène 2.5 km away from our study area in a parallel valley with the same exposition. To measure the temperatures under rotten leaves of *P. paradoxus* a Grant 1200 Datalogger (Tectron AG, Wald, Switzerland) was used.

Laboratory studies

Insects. Adults of *O. cacaliae* were collected weekly from *P. paradoxus* in spring and from *A. alliariae* in summer, near La Fouly (VS) in the Swiss Alps. To prevent stress, beetles were transported to the laboratory in cooled containers. The beetles were maintained in glass jars with perforated lids in an incubator at a day temperature of 16°C and a night temperature of 12°C under a light regime of 16L: 8D. Beetles were used for windtunnel experiments within three days after collection and sexed using sexual polymorphism of the tarsi (Lohse and Luche, 1994).

Host plants. Plants used in windtunnel experiments were dug out weekly at, or near the sites where beetles were collected. They were immediately potted in ceramic pots of 15 cm diameter and brought to the laboratory, where they were placed near a window and kept at room temperature until use. *P. paradoxus* first flowers and after about a month the flower decays and the plant starts to produce leaves. In 1998, *P. paradoxus* was flowering from the 20th of April to the 20th of May, approximately. To obtain vegetative and flowering stages of *P. paradoxus* at the same time, the flowering plants were collected at a higher altitude in the valley, while the vegetative stages were found further down.

Wind tunnel. All behavioural studies were conducted in a plexiglas windtunnel measuring 0.60 x 0.60 x 1.80 m. Visual surroundings were made homogeneous with a white curtain that formed a tent around the windtunnel. A light source outside this tent, above the upwind end of the windtunnel, provided a light intensity of 60 lux inside the windtunnel. This weak light intensity was chosen to prevent the beetles from flying. We made sure that the light intensity was the same at both sides of the windtunnel. The air was pulled through a charcoal impregnated fabric that cleaned the air at the tunnel entrance. The filter and two nylon screens at the upwind end created a near laminar flow. An additional nylon screen closed the downwind end of the tunnel. Air was pulled through the tunnel at 0.1 m/sec with the use of a tubular duct fan (RR 125 C, Melios Ventilatoren AG. Urdorf, Switzerland) and was exhausted outside the room. The temperature in the room housing the windtunnel was between 22 and 23°C and the humidity around 55%.

Experimental procedure. The assay was designed as a dual-choice test for walking beetles. Two differently treated, potted plants were placed at the upwind end of the windtunnel 0.3 m apart. A glass plate (1.5 x 0.5 m) was placed horizontally in the windtunnel at a height adjusted such that air from the volatile emitting, upper plant parts would pass over it.

For each plant combination, 4 replicates with 20 beetles each (10 females and 10 males) were made. Replicates were done at an interval of at least one week with different plants and different beetles. The insects were denied food for at least 24 h before any experiment to increase the anemotactive response (Visser & Nielsen, 1977; Miller & Strickler, 1984). Glass jars with beetles were taken out of the incubator half an hour before an experiment and left near the wind tunnel to let the beetles acclimatise to the experimental conditions.

Five beetles at a time were released from a petri dish on the glass plate at the downwind end of the windtunnel, after 15 min another 5 beetles were released from the same spot. The distance between petri dish and the end of the glass plate in front of the plants was 1 m. As soon as a beetle had reached the end of the glass plate in front of a plant the choice was recorded and the beetle removed from the windtunnel. After 45 min all beetles were removed and the position of the plant pots was changed to avoid any directional bias. The glass plate was cleaned with 70% ethanol and five minutes later another 10 beetles were placed in the windtunnel and observed for 45 min. Beetles that started flying in the windtunnel were put back at the starting point (petri dish) as they bumped into the windtunnel wall and were incapable of directing their flight towards one of the plants. Beetles that reached the end of the glass plate in front of a plant were considered to have made a choice for that plant. Beetles that arrived at the upwind end of the glass plate after having followed the edge of the plate were never counted as having made a choice. The sexes were used equally often in the experiments.

Vision. To test if *O. cacaliae* use visual cues to locate their host plants we used a green leaf model made from a colour photocopy of a real leaf of *A. alliariae*. The stem of the model was strengthened with wire, which was covered with green paper. This leaf model, with exactly the same size and shape as a real leaf, was offered next to a real *A. alliariae* leaf, and the experiment was performed as described above.

Alkaloid extraction. Powdered air dried floral parts of *P. paradoxus*, calyx, petals and stems of the flowerheads (0.03 g each), were extracted in 200 µl 1N H_2SO_4 and stirred for 10 s (Vortex). The extract was left for some time for full extraction and after centrifugation (5 min, 8000 t/sec) the supernatant was mixed with Zn dust in excess and shaken for 2 h. The extract was basified with 200 µl NH_4OH (32%) and transferred on an Extroelut^{TM} column (Merck). After 20 min the alkaloids were recovered with 2 x 6 ml of dichloromethane.

After evaporation of the dichloromethane solvent the alkaloid residues were redissolved in MeOH for GC analysis.

Alkaloid analysis. Pyrrolizidine alkaloids (PAs) were separated by capillary GC on fused silica capillary columns (15 m x 0.25 mm; SPB1, Supelco) using a Perkin-Elmer 8500 apparatus. Conditions: injector, 250°C; temperature program: 150-300°C, 6°C/min; injection volume 1µl; carrier gas: He, 0.75 bar. Detection: flame ionisation detector. The PA retrosine (Sigma R-0382) was used as internal standard.

Statistical analysis. Results from the windtunnel choice experiments were analysed with a binomial test, using the total number of beetles that made a choice for a particular odour ($\alpha = 0.05$). If there was no effect of test odour, the mean number of beetles attracted to each side of the windtunnel would be equal.

RESULTS

Field results

Overwintering behaviour. O. cacaliae beetles were previously thought to overwinter in the soil close to their host plant *A. alliariae*. Three overwintering sites of *O. cacaliae* were found in the Val Ferret more than 200 m away from the nearest *A. alliariae* patch. Two overwintering sites are in the soil beneath a cliff (R1 and R2) raising up more than 2000 m, and one is a slope of open soil next to a newbuilt road (S) (Fig. 1, Fig. 3). Beetles were observed at these sites as early as March 17th in 1997, March 18th in 1998 and March 17th in 1999, they were covered with pieces of soil, and the vegetation was still hidden under a thick layer of snow.

Take-off direction. Beetles that overwintered away from their host plants engage in flight in spring. *O. cacaliae* were observed crawling up the cliff at the overwintering sites R1 and R2, wandering around exposed to the sun for several minutes, before taking-off. Beetles quickly gained altitude and where lost from sight soon after. The take-off direction of *O. cacaliae* was observed at wind speeds between 0.5 and 3,6 km/h. A constant wind speed of 6 km/h with gusts up to 12 km/h was too strong for the beetles to take off. In 57 cases of observed spontaneous take-off all these beetles took off within 20° against the prevailing wind direction (Fig. 1).

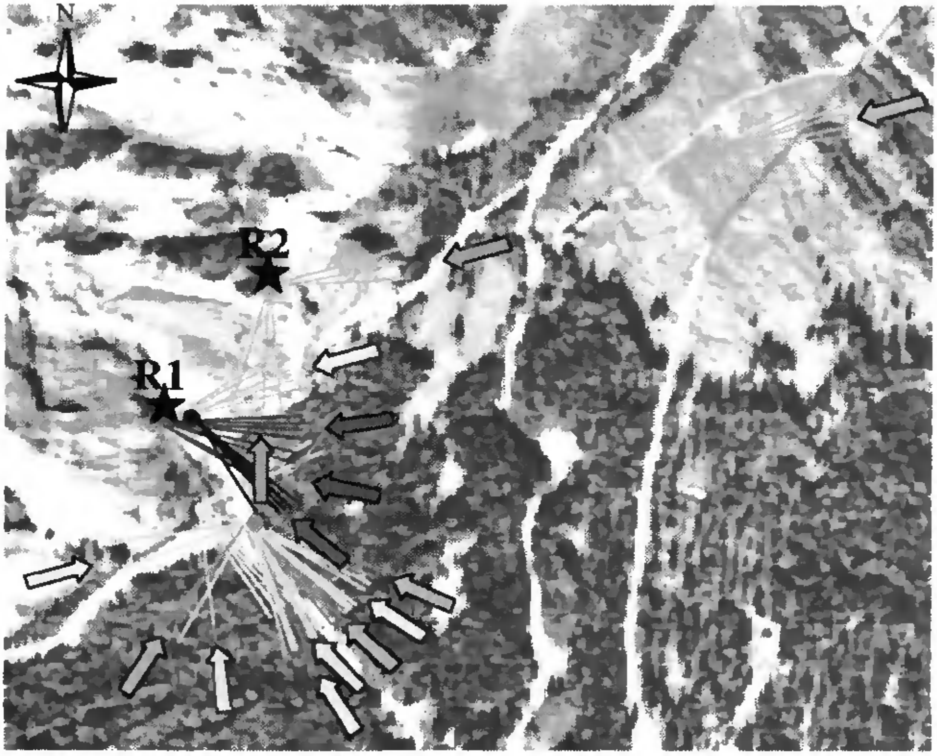


FIG. 1. Take-off directions of *O. cacaliae* near the overwintering places R1 and R2. The take-off direction (line) of single beetles were recorded. The arrows show the prevailing wind direction. Different shades represent different observation dates. The length of the lines is not representative of the flight distance.

There was no preferred take-off direction in this valley due to the distinct wind directions. The prevailing wind direction at the time of take-off determined the flight direction of the beetles. *O. cacaliae* was observed flying exclusively on sunny days preferably between 12.30. and 14.30, during the hottest period of the day.

Beetles' dispersal. Early in spring, on a sunny day, beetles were monitored along a walking path shown in Fig. 2. A high cliff, raising up more than 2000 m, bordered the area in the north. The vegetation was hidden under a thick layer of snow except for three places, where the snow had melted, and *P. paradoxus* flowers had already emerged. A total of 74 beetles were found, walking on the snow, at a considerable distance from *P. paradoxus*. Only 12 beetles were found within a distance of 20 m from the next *P. paradoxus* plant. There was no obvious aggregation of beetles near the spring host plant.

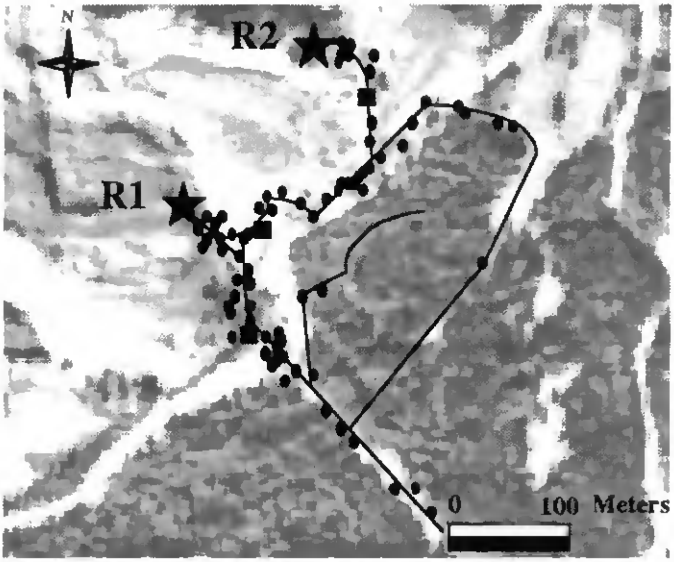


FIG. 2. Dispersal of *O. cacaliae* near the overwintering places R1 and R2 (stars) on a sunny day early in spring. The beetles (circles) were monitored along a walking path (-) where *P. paradoxus* had emerged at three places (squares). The rest of the vegetation was covered with snow.

Flight behaviour. A part of the population was observed engaging in flight in autumn and spring. These flyers left the *A. alliariae* patches in autumn and emerged up to two months earlier than the non-flyers that overwintered in the soil close to *A. alliariae,* in spring. The flyers faced the problem of emerging earlier than their main host plant *A. alliariae.* They overcame this problem by feeding on flowers of the early emerging *P. paradoxus.* The beetles left *P. paradoxus* and fly to *A. alliariae* as soon as this main host plant emerges (around the 20th of May). The flight distance and direction of recaptured beetles are represented in Fig. 3. The closest *A. alliariae* patch is 110 m away from a *P. paradoxus* patch. Of the 610 beetles marked at the overwintering sites, 20 were found in *P. paradoxus* patches between two and six weeks after marking, while 5 beetles were found in an *A. alliariae* patch between 7 and 12 weeks after marking (Table 1 for full data, Table 2 for summary). One of the 1008 beetles marked on *P. paradoxus* changed to another *P. paradoxus* patch and 5 beetles maked on *P. paradoxus* were found on *A. alliariae* later in the season. The longest distance travelled between overwintering place and site of recapture was 1.12 km. No beetle out of 390 marked 3 km further up the valley was found elsewhere. Once *O. cacaliae* found an *A. alliariae* patch it stayed there for the whole season. The beetles readily moved from one individual plant to others but they did not leave the *A. alliariae* patch by flight to find another patch.

Flight behaviour of *O. cacaliae* in autumn was observed in 1996 (26th and 30th of September) when beetles were observed to fly down the valley and were later found on walls of houses in the village of La Fouly, where they hid under the roofs to overwinter. We found some beetles on these walls the next spring but not in the consecutive years. In autumn 1998 (25th of August, 9th of September and 23th of September), we found 43 beetles wandering around at the overwintering places, at a distance of more than 250 m from the nearest host plant patch. During summer no beetles were found at those overwintering sites. One beetle marked at an overwintering place was refound at the same place two weeks later.

FIG. 3. Map with flight distances of marked beetles coming from overwintering places (stars, R1, R2, S) recaptured on the spring host *P. paradoxus* (squares) or marked on patches of the spring host and recaptured on *A. alliariae* patches (circles).

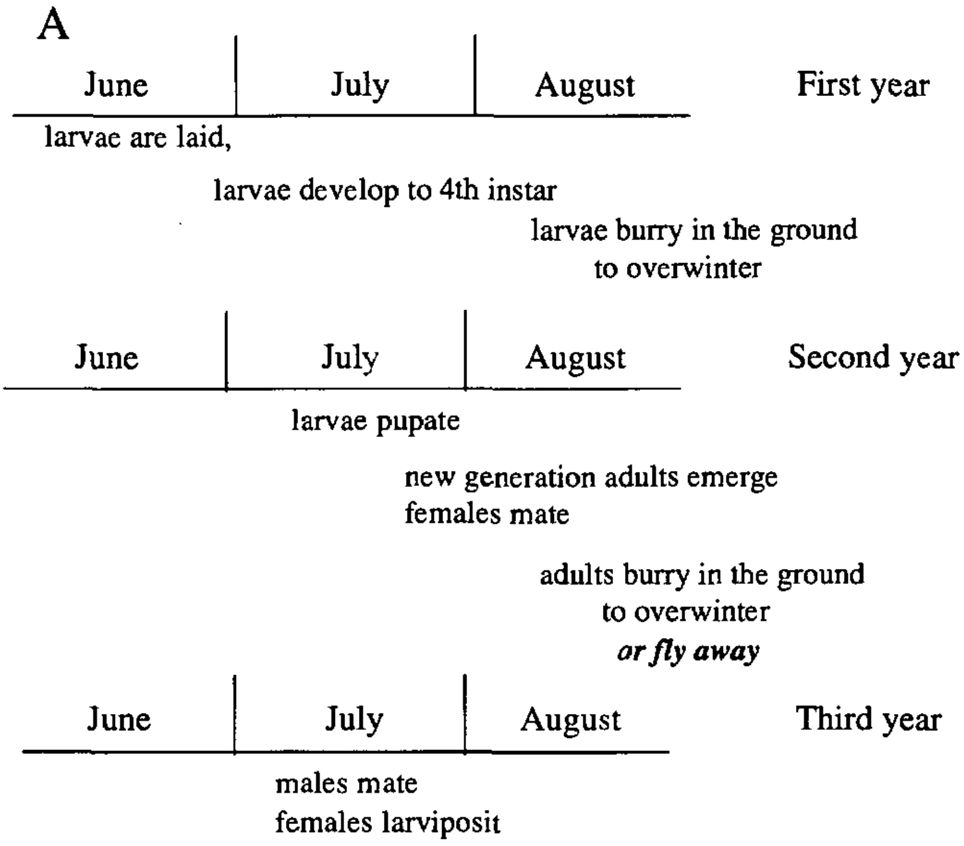
Table 1. Recaptured *O. cacaliae* marked either at the different overwintering places rock 1 (R1), rock 2 (R2) or soil (S) or in host plant patches of *P. paradoxus* (P) or *A. alliariae* (A). The date of marking (± days) and the place of marking is given, together with the information about the host plant where the beetles were found, the distance they travelled (in meters), the time they spent to arrive (days), as well as the sex of the beetle if determined (nd = not determined).

Number of beetles	Date of marking	Place of marking	Found on	Distance travelled (meters)	Travel time (days)	sex
13	27.3.97	R1	P	72±5	12±2	all nd
2	27.3.97±6	R1	P	455±115	41±7	nd, m
2	27.3.97	R2	P	205±15	15±2	nd, nd
1	27.3.97	R1	A	215	88	nd
2	27.3.97±6	R2	A	300±100	55±0.5	nd, nd
1	1.4.98	R1	A	235	50	nd
1	2.4.97	S	P	82	28	m
3	2.4.97±6	R1/2	P	120±10	30±17	nd, nd, m
1	2.4.97	S	A	1125	51	nd
2	11.4.98±5	R1/2	P	90±20	29±7	nd, m
1	29.4.98	P	P	75	7	nd
2	20.5.98	P	A	110	13±8	f, m
3	12.5.98±7	P	A	930±50	74±12	f, f, m
1	11.8.97	A	P	210	292	m

Table 2. Summary of Table 1 with the same abbreviations.

Number of beetles	Date of marking	Place of marking	Found on	Distance flown (meters)	Travel time (days)	sex
19	~1.4.	S, R1/2	P	86±30	17±9	most nd, m, m
3	~1.4.	R1/2	P	380±157	29±16	nd, m
1	12.5.98	P	P	75	7	nd
5	~1.4.	R1/2	A	435±394	58±15	nd
2	20.5.98	P	A	110	13±8	f, m
3	12.5.98±7	P	A	930±50	74±12	f, f, m
1	11.8.97	A	P	210	292	m

New life cycle. The results obtained with the mark-recapture experiments lead to additional information about the life cycle of *O. cacaliae*. It has been assumed so far, that all the beetles overwinter in the soil next to their host plant *A. alliariae*. But a part of the population left these host plant patches in autumn and flew to overwintering places at a distance of at least 200m from the next *A. alliariae* patch. Those flyers emerged earlier than the beetles that overwintered next to *A. alliariae* patches in spring and flew to the spring host *P. paradoxus* where they fed until the emergence of *A. alliariae*. As soon as *A. alliariae* had emerged the beetles left *P. paradoxus* by flight and flew to patches of the main host *A. alliariae*. In Figure 4 the new, complete life cycle of *O. cacaliae* is shown.



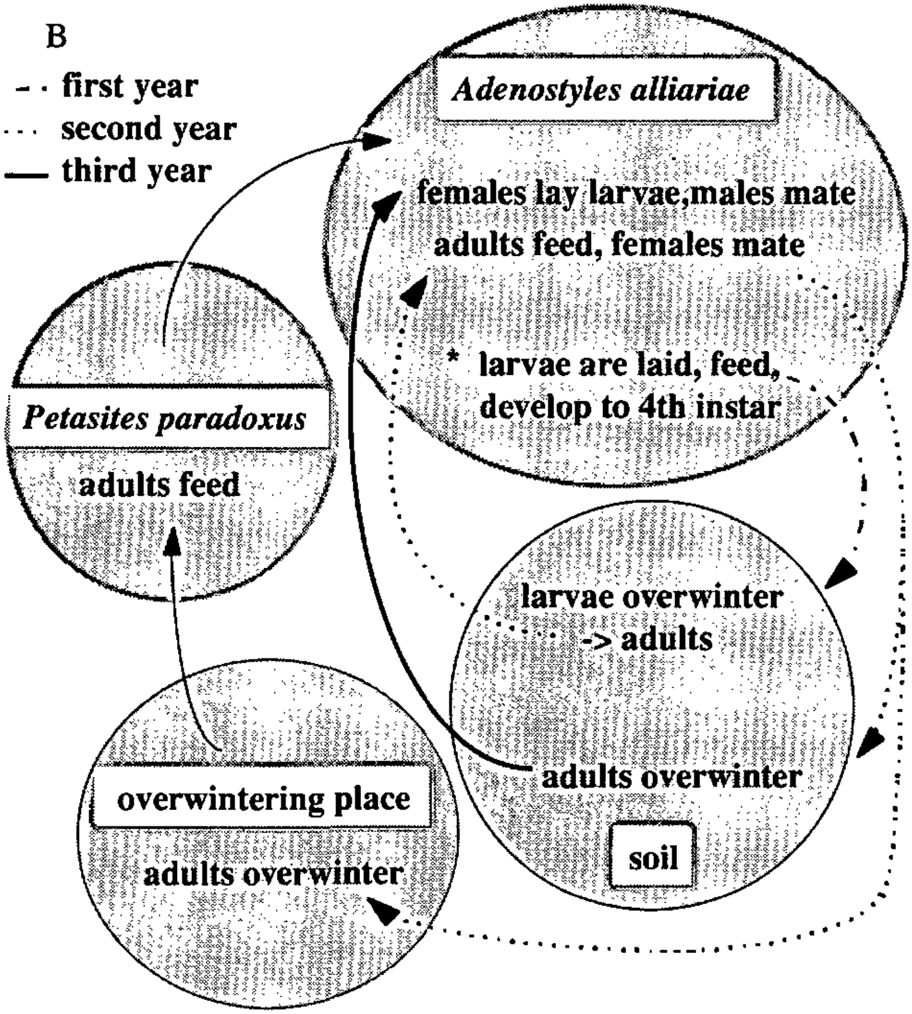


FIG. 4. New, complete life cycle of *Oreina cacaliae* with special emphasis on the dates (A) and locations (B). New is the part where the beetles take off by flight in autumn and fly to overwintering sites away from *A. alliariae*, from where they emerge earlier in spring and start feeding on *P. paradoxus* until the emergence of *A. alliariae*.

Laboratory results

Beetle behaviour in the windtunnel. Beetles that were released from a glass Petri dish at the start of an experiment crawled up the edges of the dish and moved their antennae when they came in contact with the air that carried the plant odours. Several beetles were observed circling the edge while waving their antennae before leaving the dish towards one of the plants. During their walk between point of release and end of the glass plate in front of the plants (1m), most beetles frequently waved their antennae. Close to the odour source beetles often seemed to loose track, but abruptly changed their walking direction and continued their course in the original direction. This appeared to be a counterturning behaviour which turned the beetles back into an odour plume when they arrived at the plume edge, and has been described by Kennedy (1983). This counterturning behaviour of almost every *O. cacaliae* beetle was only observed at close distance (15 cm) from the plants, where the plume is well-defined, structured and narrow (David *et al.*, 1983). The beetles that arrived in front of a plant presented in the windtunnel (the ones who's behaviour was considered a choice) showed odour conditioned anemotaxis over the distance of 1 m.

Responses to plant volatiles. Significantly more *O. cacaliae* were attracted to the flowering spring host *P. paradoxus*, when it was presented next to flowering *T. farfara* in the windtunnel (Fig. 5A). Previous field experience with the plants did not influence this choice, since beetles used in two of the four replicates were collected on *P. paradoxus* and beetles used for the other two replicates were the only *O. cacaliae* beetles found on *T. farfara* in the field. Flowering *P. paradoxus* was significantly more attractive than its vegetative state (Fig. 5B). When comparing vegetative stages, the summer host *A. alliariae* was significantly more attractive than the spring host *P. paradoxus* (Fig. 5C). It was not possible to compare flowering *P. paradoxus* with flowering *A. alliariae*, because the generative stages of the plants occur at

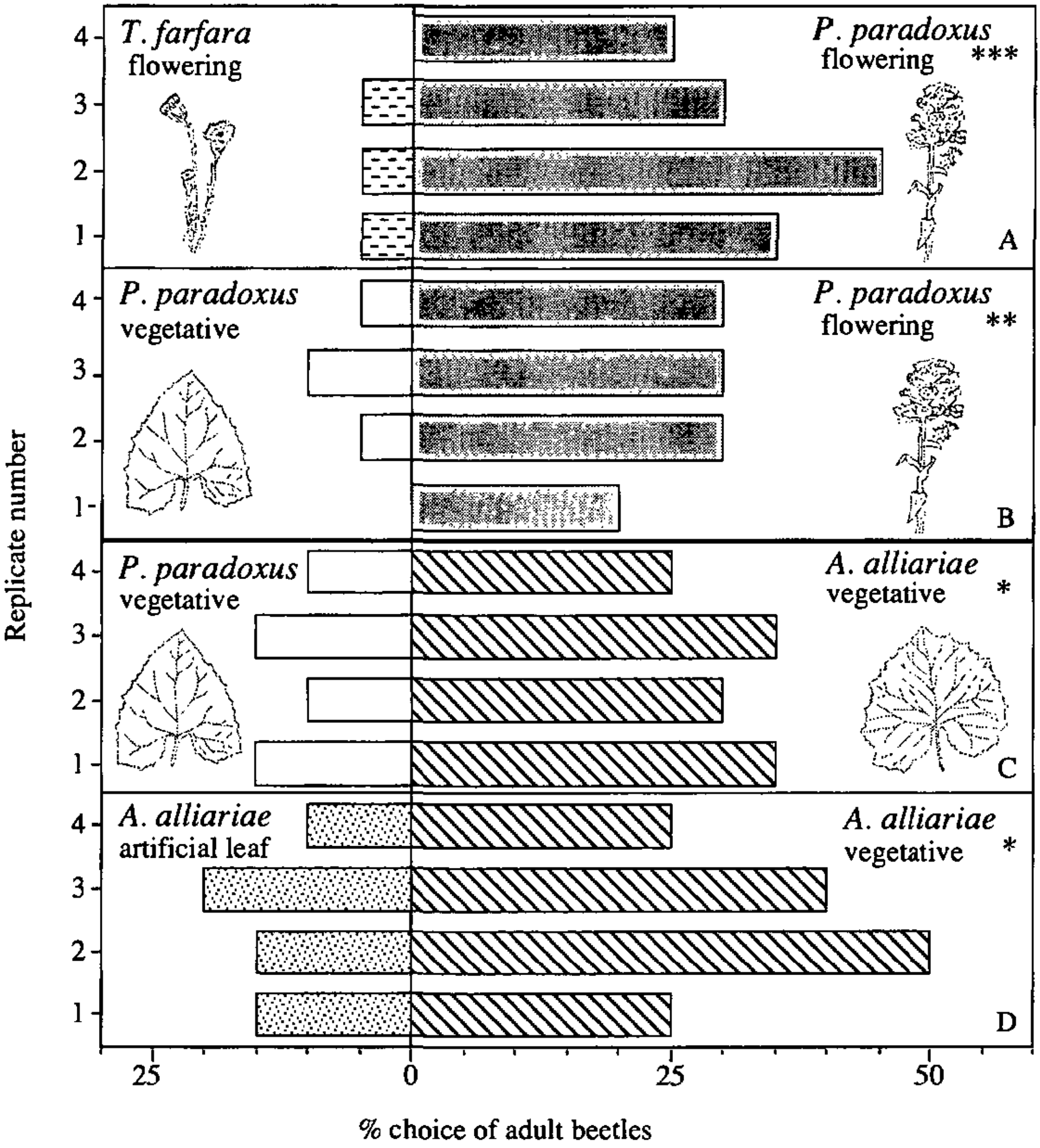


FIG. 5. Response in a wind tunnel of *Oreina cacaliae* towards odours emitted by uninfested potted host (*P. paradoxus* and *A. alliariae)* and non-host plants (*T. farfara.*) *P. paradoxus* was tested against *T. farfara.* (A); the flowering state of the spring host *P. paradoxus* against its vegetative state (B); *A. alliariae* against *P. paradoxus* in vegetative state (C) and an artificial leaf against a real, smelling leaf of *A. alliariae* (D). For each experiment four replicates were performed. Significance levels are indicated as $*P < 0.05$; $** P < 0.01$; $***P < 0.001$ (binomial test, n = 20 per replicate).

different times of the year. To test whether the leaf shape and colour might be part of the search image we offered an artificial *A. alliariae* leaf against a real, odorous leaf. The beetles directed their movement significantly more often to the real leaf (Fig. 4D). In all experiments both sexes were equally attracted to plant odours. Therefore, results for both sexes were combined. No attraction between beetles of different sex was ever observed during the experiments in the windtunnel (see Chapter 3 for details).

PA analysis of flowers of P. paradoxus. Flowering *P. paradoxus* contains PAs in its flowers. In the calyx, the petals and in the stems of the flowerheads the PA seneciphylline was detected.

DISCUSSION

It was shown in this study that *O. cacaliae* beetles are capable of flying away from host plant patches to overwintering sites in autumn. In spring beetles flew from overwintering sites to the spring host *P. paradoxus* to feed on. A male beetle that was marked in autumn on *A. alliariae* was found on *P. paradoxus* the next spring. Several beetles marked at distinct overwintering places were found later feeding on *P. paradoxus*. A *P. paradoxus* patch close to an overwintering place, was completely destroyed by the beetles. Flying *O. cacaliae* must have crossed a river and several streets before arriving at the host plant patches. We can therefore be sure that the beetles overcame these barriers by flight. Those mark-recapture studies served to colmplete the life cycle of *O. cacaliae*. Other mark-recapture studies with marked Chrysomelids found travel distances around 500 m (Follett *et al.*, 1996; Herzig, 1995), distances comparable with the ones presented here for *O. cacaliae*. One beetle marked in a *P. paradoxus* patch was found in another *P. paradoxus* patch later on, indicating that beetles may switch to other *P. paradoxus* patches when food sources are exhausted. After take-off beetles were observed landing on vegetation covered with snow from where taking-off was impossible. Beetles were found almost evenly distributed in the snow along a walking path, on a sunny day early in the season, when only at three places *P. paradoxus* had already emerged and the rest of the vegetation was still hidden beyond a thick layer of snow. We therefore suggest that *O. cacaliae* is not able to choose a specific host-plant patch during flight, but that the beetles first are driven by the prevailing wind direction, land on vegetation (maybe still covered with snow) and then either find the spring host by walking or take-off again after having found a grip. Migratory flight in insects is not necessarily a single flight but often consists of several consecutive flights, sometimes over an extended period of time (Solbreck, 1985).

Several beetles marked on the spring host *P. paradoxus* were found back on the main host *A. alliariae*. The time the beetles leave the spring host coincides with the emergence of their main host *A. alliariae*. Beetles marked at the distinct overwintering places away from the *A. alliariae* patches, and refound in an *A. alliariae* patch took a longer time (9 weeks, on average) to get there, than beetles refound on *P. paradoxus* (2 - 6 weeks). This is not surprising as *A. alliariae* emerges about 4 weeks after *P. paradoxus* and is further away from the overwintering sites than the spring host. Four beetles (3 males and 1 female), marked the previous year on *A. alliariae*, were refound in the same patch the following year indicating that some beetles overwinter next to the *A. alliariae* patches and walk to the same host plant patch the following year. The fact that no beetle switched between *A. alliariae* patches showed that once the beetles arrived in a patch of their main host plant, they stayed there for the whole season. An observation that confirms the findings of Knoll (1997).

No directional bias could be observed in the Val Ferret, 10 beetles headed down the valley and 9 up the valley but most (41) across the valley, in spring. Take-off directions were strongly influenced by the prevailing wind directions, with beetles always taking-off against the wind. Distances up to one kilometre were flown by four beetles arriving at an *A. alliariae* patch (two female, one male, one sex not determined). There seems to be no difference in the distance the two sexes cover. According to Caldwell and Rankin (1974) it would be a good strategy for a colonising species to mate prior to dispersal so that females arriving in a suitable habitat would not need to locate a mate in order to found a colony. Females of *Oreina cacaliae* do not need to remate in spring, females that mated only the year before, larviposited as successfully as females that remated in spring (Dobler and Rowell-Rahier, 1996), thus single females are theoretically very able to found a new colony on *A. alliariae*. Of 73 beetles marked early (the 22nd of April 1999), 84% were males indicating that males emerge some days earlier than females. Male *O. cacaliae* beetles are too soft for mating in year they develop into an adult from the pupal stage (personal communication M. Rahier). Males emerging from overwintering as an adult, able to copulate, may benefit from emerging early to mate with the females that are present that early in the season. As they copulate up to several days, they can profit from an early emergence by 'occupying' the females. If winter mortality is significant, females may increase the fitness of their offspring by remating after dormancy with males that survived the winter (Stevens & McCauley, 1989).

Flying beetles that overwinter at distinct overwintering places away from *A. alliariae* emerge earlier in spring, than their non-flying conspecifics that overwinter in the soil next to the main host *A. alliariae*. Overwintering places of flying *O. cacaliae* were sun exposed with a microclimatic temperature higher than in the surrounding area. Under rotten leaves of *P. paradoxus* from the year before, where hundreds of beetles were found, the temperature was 2.1°C higher than in the surrounding air. Walker (1966) also recorded increasing temperature at the soil surface beneath leaf litter. This microclimatic high temperature might be the reason why flying *O. cacaliae* emerge earlier than non-flyers overwintering next to shady *A. alliariae* patches. Temperature was found to be the most important extrinsic factor controlling diapause termination in the Colorado potato beetle (Kort, 1990). The microclimate of the overwintering place at the south exposed cliff, where

O. cacaliae first occur in spring, is likely to influence beetles' spring emergence date. For bollweevils it was shown that moisture combined with warm temperatures is an important factor in inducing spring emergence (Davis *et al.*, 1967). Comparing precipitation and temperature during winter 1996/97 and 1997/98 we found that the mean temperature from October to March was 1,1°C higher in 1997/98 and the mean precipitation was 12,5 mm higher in 1996/97. These differences are larger than the ones between 1997/98 and 1998/99 when the difference in temperature was 0.9°C and the one in precipitation only 1,5 mm. It looks as if for *O. cacaliae* the temperature and precipitation during the winter month does not determine their date of spring emergence as we found the first beetles March 18th in 1998 and March 17th in 1997 and 1999. The 12th of March 1998, no beetles were found at the field site. We suggest that the local temperature at the overwintering places determines the date of first spring emergence. As soon as the sun had melted away a crevice between rock and the snow layer next to that rock, the beetles crawled up the rock, wandered around and finally took off. The need for high temperature and particularly solar radiation for *O. cacaliae* to initiate flight, has also been found with the Colorado potato beetle (Le Berre, 1962; Caprio and Grafius, 1990; Zehnder and Speese 1987; May and Ahmad, 1983). Microclimatic factors like temperature and wind measured in the close surrounding of the beetles were more important than data obtained from the meteorological station nearest to the study area.

Olfaction turned out to be important for the host plant selection in *O. cacaliae.* Our visual cue tested, an artificial leaf with the same size and shape as a real leaf, attracted less beetles than the natural leaf. They very clearly preferred host plants odours over non-hosts offered in the windtunnel. Non-host plants may mask the attractive odour of a host plant. The Colorado potato beetle, for example, is not attracted to its host plant, potato, when a non-host plant is placed behind it (Thiery and Visser, 1987). But here when a host and a non-host plant are presented at the same time in a windtunnel, at a distance of 0.3 m between the two plants, *O. cacaliae* was able to discriminate the odour emitted by its host plant from the odour emitted by the non-host plant and showed an odour conditioned positive anemotaxis towards its host plant from a distance of 1m. The flowering spring host (*P. paradoxus*) was more attractive for *O. cacaliae* than the flowering non-host (*T. farfara*) even though the plants belong to the same subtribe of the family Asteraceae. Even the rare beetles that were collected from *T. farfara* in the field showed this clear preference. We interpret the very rare occurrence of *O. cacaliae* on *T. farfara* in the field as a choice forced by lack of alternative hosts in their near surrounding. Interestingly, the subspecies *Oreina cacaliae* var. *tussilaginis* (Suffrian) which occurs in the Pyrenees, regularly feeds on *T. farfara* in addition to species of the genera *Petasites* and *Adenostyles* (Bourdonné *et al.*, 1981).

O. cacaliae preferred the flowering stage of *P. paradoxus* over its vegetative stage, which occurs later in the season. *O. cacaliae* is (almost) exclusively found on *P. paradoxus* flowers early in the season before the emergence of *A. alliariae*. We consider flowering *P. paradoxus* to be the spring host *af O. cacaliae*. The time of emergence of *A. alliariae* coincided with the fanning of *P. paradoxus* flowers and the emergence of *P. paradoxus* leaves. It is at that time of the season that most *O. cacaliae* left *P. paradoxus* and switched to their main host *A. alliariae*. Almost never were *O. cacaliae* larvae and adults found on leaves of *P. paradoxus* during the summer in Val Ferret. Discrimination among plants that are closely related have been shown for another Chrysomelid. The goldenrod leaf beetle *Trirhabda canadensis* prefers the odour of its *Solidago* host plant over the odour of non-host plants belonging to different subtribes of the host family Asteraceae (Puttick *et al.*, 1988). *O. cacaliae* can discriminate plants that belong to the same subtribe of the family Asteraceae. Yet *T. canadensis* did not discriminate between their *Solidago* host and another *Solidago* species even though the latter, a non-host species, contains a potent antifeedant diterpene that may not be effective over distance

(Cooper-Driver *et al.*, 1986). Different species of the host genus were not tested with *O. cacaliae*, as they did not occur at the field site.

The leaves of *A. alliariae* were more attractive than *P. paradoxus* in its vegetative state. *A. alliariae* contains PAs in the leaves that are sequestered by the beetles for their own defence. The analysis of the floral parts of detected the PA seneciphylline in the calyx, the petals and the stems of the flowerheads. Seneciphylline is also the main PA of *A. alliariae*, where it represents up to 95% of the total amount of detected PAs (Pasteels *et al.*, 1995; Hägele and Rowell-Rahier, 1999). leaves on the other hand contain no PAs. Thus by preferring flowering over its vegetative state and vegetative *A. alliariae* over vegetative *P. paradoxus*, the beetles seem to avoid food without PAs.

Flight enables overwintered *O. cacaliae* beetles to disperse and to locate new host plant patches. The beetle is a weak flyer and cannot direct its flight towards a host plant. *O. cacaliae* always took-off against the prevailing wind direction and landed somewhere on the vegetation. After landing, the beetles orient to the host plants while walking. Olfaction appears to be very important in this last stage of the host-plant location process of *O. cacaliae*.

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REFERENCES

Berré, J. R. Le, 1962. Les bases scientifiques des advertissements agricoles -in- Dix anées de collaboration franco-britannique pour la defense des iles anglo-normandes contre les invasions de doryphore. Phytoma 142: 11-23.

Bourdonné, J. C. & R. Vincent, 1981. Matériaux pour un catalogue des Coléoptères des Pyrénés (2^e partie). L' Entom., Paris 37 (4-5): 184-190.

Caldwell, R. L. & M. A. Rankin, 1974. Separation of migratory from feeding and reproductive behavior in *Oncopeltus fasciatus*. Journal of Comparative Physiology 88: 383-394.

Caprio, M. A. & E. J. Grafius, 1990. Effects of light, temperature, and feeding status on flight initiation in postdiapause Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology 19: 281-285.

Cooper-Driver, G. LeQuesne, P. W., and Villani, M. 1986. Biologically active diterpenes from *Solidago* species: Plant-insect interactions. Stud. Org. Chem. 26: 271-282.

David, C. T., J. S Kennedy,. & A. R. Ludow, 1983. Finding a sex hormone source by gypsy moths released in the field. Nature 303: 804-806.

Davis, J. W., C. B. Cowan Jr. & C. R. Rarencia, 1967. Emergence of overwintered boll weevils from hibernation sites near Waco, Texas. Journal of Economic Entomology 60: 1102-1104.

Dobler, S. & M. Rowell-Rahier, 1996. Reproductive biology of viviparous and oviparous species of the leaf beetle genus *Oreina*. Entomologia Experimentalis et Applicata 80: 375-388.

Ehmke, A., M. Rowell-Rahier, Jacques M. Pasteels & Thomas Hartmann,1991. Sequestration of ingested (^{14} C)Senecionine N-oxide in the exocrine defensive secretions of chrysomelid beetles. Journal of Chemical Ecology 17: 2367-2379.

Ehmkc, A., M. Rahier, J. M. Pasteels, C. Theuring & T. Hartmann, 1999. Sequestration, maintenance, and tissue distribution of pyrrolizidine alkaloid-*N*-oxides in larvae of two *Oreina* species. Journal of Chemical Ecology 25: 2385-2395.

Follett, P. A., W. W. Cantelo & G. K. Roderick, 1996. Local dispersal of overwintered Colorado Potato Beetle (Chrysomelidae: Coleoptera) determined by mark and recapture. Environmental Entomology 25: 1304-1311.

Herzig, A. L., 1995. Effects of population density on long-distance dispersal in the goldenrod beetle *Trirhabda virgata*. Ecology 76: 2044-2054.

Hägele, B. F. & M. Rowell-Rahier, 1999. Genetic and environmental-based variability in secondary metabolite leaf content of *Adenostyles alliariae* and *A. alpina* (Asteraceae). Test of the resource availability hypothesis. Oikos 85: 234-246.

Jolivet, P., E. Petitpierre & M. Daccordi, 1986. Les plantes-hôtes des Chrysomelidae. Quelques nouvelles précisions et additions (Coleoptera). Nouv. Revue Ent. T.3 (Fasc. 3): 342-357.

Kennedy, J. S., 1983. Some current issues in orientation to odour sources. In: Payne & Birch (eds), Mechanisms in Insect Olfaction, pp. 11-26.

Knoll, S. 1997. Spatial population structure of an alpine leaf beetle. Ph. D. thesis, University of Neuchâtel.

Kort, C. A. D. de, 1990. Thirty-five years of diapause research with the Colorado potato beetle. Entomologia Experimentalis et Applicata 56: 1-13.

Kühnelt, W., 1984. Monographie der Blattkäfergattung *Chrysochloa* (Coleoptera, Chrysomelidae). Anz. Oesterr. Akad. Wiss. Math-Naturwiss. Kl. 1984: 171-287.

Lohse, G. A. & W. H. Luche, 1994. Die Käfer Mitteleuropas. In: G. Evers (ed), 14. Krefeld. pp. 73.

May, M. L. & S. Ahmad, 1983. Host location in the Colorado Potato Beetle: Searching Mechanisms in Relation to Oligophagy. In: S. Ahmad (ed), Herbivorous Insects: Host-seeking behaviour and mechanisms, Academic Press, New York. pp. 173-200.

Miller, J. R. & K. L. Strickler, 1984. Finding and Accepting Host Plants. In: W. J. Bell & R. T. Cardé (eds), Chemical Ecology of Insects, Chapman and Hall Ltd., pp. 127-155.

Nordenstam, B., 1977. Senecioneae and Liabeae-systematic review. In: V. H. Heywood, J. B. Harborne & B. L. Turner (eds), The biology and chemistry of the Compositae, Academic Press, London. pp. 799-829.

Pasteels, J. M., S. Dobler, M. Rowell-Rahier, A. Ehmke & T. Hartmann, 1995. Distribution of autogenous and host-derived chemical defenses in *Oreina* leaf beetles (Coleoptera, Chrysomelidae). Journal of Chemical Ecology 21: 1163–1179.

Peng, C. & M. J. Weiss, 1992. Evidence of an aggregation pheromone in the flea beetle, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Journal of Chemical Ecology 18: 875-884.

Prystupa, B., C. R. Ellis & P. E. A. Teal, 1988. Attraction of adult *diabrotica* (Coleoptera: Chrysomelidae) to corn silks and analysis of the host-finding response. Journal of Chemical Ecology 14: 635-651.

Puttick, G. M., P. A. Morrow & P. W. Lequesne, 1988. *Trirhabda canadensis* (Coleoptera: Chrysomelidae) responses to plant odors. Journal of Chemical Ecology 14: 1671-1686.

Rankin, M. A. & J. C. A. Burchsted, 1992. The cost of migration in insects. Annual Review of Entomology 37: 533-559.

Rowell-Rahier, M., J. M. Pasteels, A. Alonso-Mejia & L. P. Brower, 1995. Relative unpalatability of leaf beetles with either biosynthesized or sequestered chemical defence. Animal Behaviour 49: 709-714.

Solbreck, C., 1985. Insect migration strategies and population dynamics. Proc. Symp. ''Migration: mechanisms and adaptive significance''. Port Aransas marine Biol. Lab.

Stevens L. & D. E. Mc Cauley. 1989. Mating prior to overwintering in the important willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). Ecological Entomology 14: 219-223.

Thiery, D. & J. H. Visser, 1986. Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. Entomologia experimentalis et applicata 41: 165-172.

Thiery, D. & J. H. Visser, 1987. Misleading the Colorado potato beetle with an odor blend. Journal of Chemical Ecology 13: 1139-1146.

Visser, J. H., 1976. The disign of a low-speed wind tunnel as an instrument for the study of olfactory orientation in the Colorado Potato Beetle. Entomologia experimentalis et Applicata 20: 275-88.

Visser, J. H. & J. K. Nielsen, 1977. Specificity in the olfactory orientation of the Colorado Potato Beetle. Entomologia experimentalis et Applicata 21: 14-22.

Walker, J. K. 1966. Overwintered boll weevil infestations in trap crop plantings. Tex. Agric. Exp. Stn. Prog. Rept. 2433: 1-4.

Wilde, J. D.De, K. Hille Ris Lambers-Suverkropp & A. van Tol, 1969. Responses to air flow and airborne plant odour in the Colorado Potato Beetle. Netherlands Journal of Plant Pathology 75: 53-57.

Zehnder, G. & G. J. Speese, 1987. Assessment of color response and flight activity of *Leptotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) using window flight traps. Environmental Entomology 16: 1199-1202.

II

REPRODUCTIVE COST OF FLIGHT IN THE ALPINE LEAF BEETLE *OREINA CACALIAE*

Key words: Coleoptera, Chrysomelidae, *Oreina cacaliae*, dispersal by flight, reproduction, survival, oogenesis-flight syndrome

ABSTRACT

Flight polymorphism in an alpine population of the leaf beetle *Oreina cacaliae* (Coleoptera: Chrysomelidae) was studied. Part of this population engages in flight in autumn and spring whereas the other part does not fly. The existence of this flight polymorphism suggests that the ability to fly adversely affects life history components like reproduction and survival. This hypothesis was tested by analysing the differences in life history parameters of the two morphs of *O. cacaliae.* We specifically tested whether there was a trade-off between (a) dispersal and reproduction and between (b) size and survival for the two groups. Flyers were smaller in size, measured as elytra length, than non-flyers and small females of the non-flyer group larviposited significantly fewer larvae than large females of the same group. Flight was correlated with reduced reproduction only when the beetles did not have access to food after their flights, which represents the situation in the field. No access to food after flight had also a negative impact on survival, which then was reduced in the flyer compared to the non-flyer group. Insect dispersal by flight may be an investment, by a portion of the population, in colonising and exploiting resources in new habitats, at a cost of reduced reproduction and survival.

INTRODUCTION

Herbivores are continually faced with the problem of how to respond to the patchy distribution and quality of their host-plant resources (Lawrence & Bach, 1989). High mobility in order to quickly find and exploit new habitats is an obvious solution to this problem. In several herbivorous insect species a flight polymorphism has been observed. The advantage of the flying class is that it allows for the escape of deteriorating patches of host plants and for the colonisation of more nutritious and less crowded stands that occur elsewhere (Denno *et al.*, 1989). Migratory behaviour, is often displayed by only a portion of a population in response to appropriate environmental cues such as photoperiod, temperature, food quality, population density, and moisture (Rankin & Rankin, 1980). Migrants often inhabit temporary or early successional habitats, with very high reproductive potential and great capacity for rapid exploitation of a newly invaded habitat (Johnson, 1969; Dingle, 1972). But there may also be costs associated with dispersal such as increased predation risk or the failure to find a suitable habitat (Roff, 1984) as well as costs in terms of life history traits such as reproduction and survival due to energy investment.

The idea that migration involves reproductive costs seems to be supported by insects where interactions between flight and reproduction have been observed (Rankin & Burchsted, 1992). Insects often migrate during the prereproductive period of their life cycle (Kennedy, 1961). Johnson (1969) used the term oogenesis-flight syndrome to describe that the development of the ovaries causes a cessation of migratory behaviour through developmental effects on the flight apparatus, including biochemical aspects of wing functioning and flight fuel use and storage. Chrysomelids like the Colorado potato beetle (*Leptinotarsa decemlineata*) and the heather beetle (*Lochmea suturalis*) begin reproduction after they finish their dispersal period by flight (Voss & Ferro, 1990; Zillesen & Brunsting, 1983).

The alpine leaf beetle *Oreina cacaliae* Chevrolat (Coleoptera: Chrysomelidae) has a three-year life cycle. Larvae are laid on *Adenostyles alliariae* (Asteraceae) in June, develop to 4th instars until August on that plant, then bury in the ground to overwinter and pupate next summer. Adults of the new generation emerge in mid-July. Only females of the new generation mate in this second year (Rahier, unpublished results) and both sexes either overwinter in the soil beneath their host plant or fly away to overwintering places hundreds of meters away from *A. alliariae* patches. The next spring, beetles that overwintered away from *A. alliariae* patches emerge earlier than their non-flying conspecifics at the host plant patches. They then fly to patches of the spring host *P. paradoxus* (Chapter 1). As soon as their main host *A. alliariae* emerges, they leave their spring host and fly to *A. alliariae* patches. In their second summer as adults, both males and females mate and females larviposit. Occasional flight observations in the field during summer indicate that *O. cacaliae,* unlike the Colorado potato beetle (Ferro *et al.*, 1991), does not absorb its flight muscles after the dispersal period. In *O. cacaliae,* we observed two classes of beetles: a) flyers, that leave their host plant patch in autumn to overwinter away from their host plants, and that emerge early in spring to fly to the spring host *Petasites paradoxus*, where they feed until the main host *A. alliariae* emerges, and b) non-flyers, that overwinter in the ground at the host plant patches of *A. alliariae* and emerge in spring at the same time as their host plant and hardly ever fly (Chapter 1). Some *O. cacaliae* beetles remained in the original host plant patches for several years, as recaptured marked beetles showed (Chapter 1). *O. cacaliae* females do not need to mate in spring of their second year as an adult (Dobler, 1996), but are able to fly to new host plant patches and found a new colony. Nevertheless, mating in this species has been observed in spring thus, if selection on males acts during the winter season, females may increase their fitness by remating with these males (Stevens & Cauley, 1989). In addition, sperm which became inviable during the winter may be replaced by remating in spring.

The widespread occurrence of flight polymorphisms suggests that there are fitness costs associated with the ability to fly like increased development time, increased mortality, decreased fecundity or decreased mating success (Roff, 1984). In the cricket *Pteronemobius taprobanensis*, oviposition is delayed and fecundity is reduced in the winged morph (Tanaka, 1976). Long-winged morphs of the grasshopper *Chorthippus parallelus* take longer to reach sexual maturity after adult emergence and have reduced overall fecundity compared to short-winged morphs (Ritchie *et al.*, 1987). In the crickets *Allonemobius fasciatus* and *Gryllus firmus*, there were no differences in development time or adult survival, but egg production was delayed in the long-winged females, and the cumulative fecundity of the short-winged morph was only greater in *G. firmus* compared to the long-winged morph (Roff, 1984). In beetles, no evidence of a trade-off between flight and reproduction has been found. The red milkweed beetle, *Tetraopes teraophthalmus*: (Cerambicidae) (Davis, 1984) and Colorado potato beetles that flew longer distances did not lay significantly fewer eggs than beetles that flew less (Ferro *et al.*, 1991).

Young (1984) found that although wingless morphs of *Sigara scotti* and *S. dorsalis* (Heteroptera: Corixidae) produced more eggs than winged forms when starved in laboratory experiments, there was no difference in fecundity between different morphs in the field. This study mentions a problem with most studies of flight polymorphisms. Because the difference in morphology between morphs is obvious without any tedious analysis of flight behaviour, wing polymorphism has provided an easy way to assess costs of flight. However, the costs assessed in such studies are due to the possession of flight muscles and wings rather than to actual flight since winged individuals do not always migrate or even fly (Rankin & Burchsted, 1992). In addition, flight activity is usually tested under artificial circumstances, the insect being fixed to a pin or tethered to a mill (Solbreck, 1974). In our system, we can distinguish flyers easily from non-flyers due to the fact that flying *O. cacaliae* emerge earlier in spring and use the spring host *P. paradoxus*. while non-flyers emerge later on *A. alliariae* and we can be sure that the beetles of the flyer group have flown before they arrived on a *P. paradoxus* patch, where we collected them. The possibility that *O. cacaliae* overwintered in the soil or the leaf litter next to *P. paradoxus* could be excluded, as no beetles were ever observed there in autumn. In addition and the infestation of *P. paradoxus* patches seemed to be related to the distance from the next overwintering place with patches close to the overwintering place infested several weeks before the ones further away.

In the present study, we tested whether there are possible reproductive costs associated with dispersal by comparing female fecundity and survival between two groups, flyers and non-flyers, of *O. cacaliae* beetles originating from the same population. We were especially interested to test whether there is a trade-off between (a) dispersal and reproduction and between (b) size and survival.

MATERIAL AND METHODS

Beetles and collection sites. All beetles were collected in the Swiss Alps near La Fouly in the Val Ferret (Valais) at 1500 m above sea level (45.56.10 latitude, 7.05.95 longitude). The flyer group was collected on the spring host *P. paradoxus* and the non-flyer group was collected on *A. alliariae* at the time of emergence of this main host plant. The flyers were collected the 16th of May (1997), the 1st and 22th of April (1998) and the 14th of May (1999). Non-flyers were collected 23th May (1997), the 13th and 20th of May (1998) and the 1st of June (1999). Beetles were sexed using sexual polymorphism of the tarsi (Lohse & Luche, 1994).

Female size. In 1998, the length of the elytra were measured with a calliper after collection in the field to determine whether body size was correlated with fecundity and flight parameters.

Female fecundity. The fecundity of flyers and non-flyers was determined in the laboratory during the whole season for the three consecutive years. Females were kept individually in plastic containers lined with a thin layer of moist plaster and a filter paper to ensure constant humidity. They were maintained in an incubator at a day temperature of 16°C and a night temperature of 12°C and a 16L: 8D regime. This ensured a temperature rhythm concordant with natural conditions in the field at the beginning of June. Every week, the number of offspring was counted, the occurrence of dead females was noted, food foliage and filter paper were replaced and the plaster bottom in the pots was moistened. The number of offspring was summed over the season, starting with the first day that a female larviposited. Observations were continued for at least three weeks after the last larva was found. In 1997, the females of the flyer group were fed with flowering *P. paradoxus* until the emergence of *A. alliariae*. As soon as *A. alliariae* from the field was available, both groups were fed with this main host plant, in all years. In 1998, the flyers were not fed in the four weeks period between collecting the flyer beetles and the emergence of *A. alliariae* in the field. In 1999, three groups were maintained: flyers not fed, flyers fed with *P. paradoxus* until the emergence of *A. alliariae* and non-flyers which were fed with *A. alliariae* from the beginning.

Statistical analysis. Data on size of male and female beetles in the flyer and non-flyer group, respectively, were analysed with a two-factor ANOVA. Differences between the two dispersal groups in number of days living were analysed with a t-test in 1997 and 1998 and an ANOVA followed by a Bonferroni-Dunn post host test in 1999. Number of larvae produced versus size of females in the non-flyer group was subjected to a regression analysis like the relationship between number of days living and number of larvae produced. Reproduction over the season was analysed with a repeated measure ANOVA for each year studied, because successive weekly larvae counts are not independent.

RESULTS

O. cacaliae beetles found on *P. paradoxus* close to their overwintering places were regarded as flyers, whereas beetles collected in a patch of *A. alliariae* at a time when this plant was about to emerge were determined as non-flyers. Since we found four beetles that had been marked the previous year in the same *A. alliariae* patch as they were marked, we can therefore be sure that some beetles stayed in the same host plant patch for two consecutive years.

Size of flyers and non-flyers. The size of the beetles (length of elytra) was measured in 1998. Male beetles, flyers as well as non-flyers, were smaller than female beetles of the same group (Fig. 1). Female as well as male flyers were smaller in size than non-flyers in both sexes (two-factor ANOVA: MS=0.27, F=5.394, $P \leq 0.0211$, n=54 female flyer, n=51 female non-flyer, n=44 male flyer, n=94 male non-flyer).

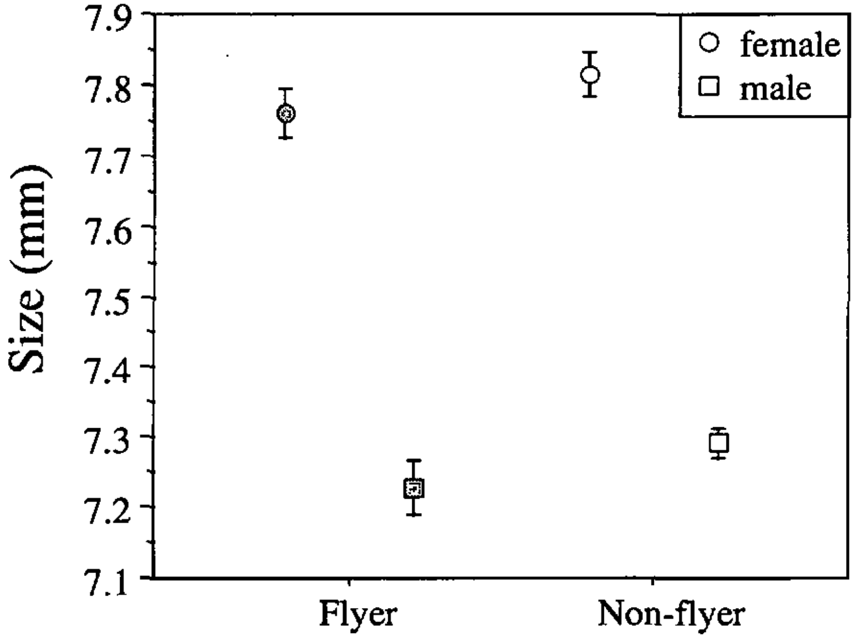

Fig. 1. Comparison of size (elytra length) between male and female flyers and non-flyers.

Size and fecundity. Small females laid significantly fewer larvae than large females as determined by regression analysis ($y= 34.83x - 223.49$, $R^2=0.144$, $F = 7.74$, $P \leq 0.0078$, $n = 48$) (Fig. 2). From a total of 50 individuals, two beetles were excluded because they did not reproduce at all over the entire season and their size was close to the mean. The analysis was done with data from the non-flyer group of 1998 because the size of the beetles was only measured in 1998 and the flyer group of that year produced only few larvae (see below).

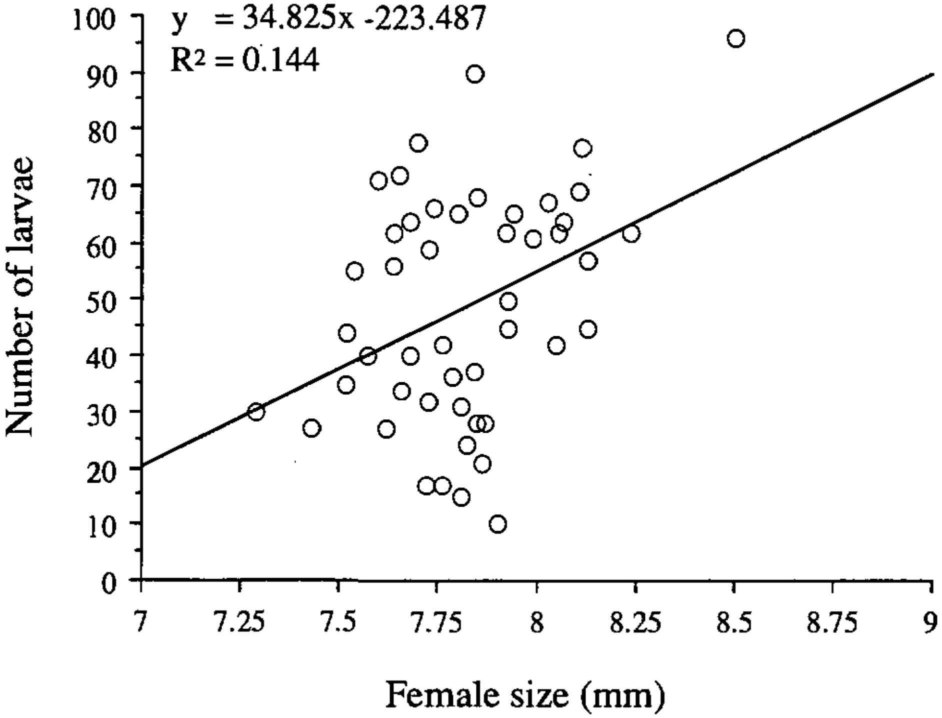

Fig. 2. Regression analysis of the size of females (mm) and the number of larvae laid in the non-flyer group of 1998 ($F = 7.744$, $P \leq 0.0078$, $n = 48$).

Larvae production. The production of larvae of both flyers and non-flyers is shown as total numbers in Fig. 3 and as mean number during three seasons in Fig. 4. Unfed flyers produced significantly fewer larvae than non-flyers determined with an Anova for all year studied (MS=20682.7, F=30.56, $P \leq 0.0001$, n=265). Interestingly, when flyers were fed, the total number of larvae they produced was similar to the beetles of the non-flyer group. The time course of larviposition per week showed different patterns of production between the groups studied. Surprisingly, the production was not continuous but seemed to occur in more than one phase (Fig. 4). In 1997, the mean number of larvae produced at the first day of larviposition was 24 and 23 larvae in the flyer and non-flyer group, respectively. Then there was a sharp decline in larvae production of both flyers and non-flyers (down to 0 and 5 larvae per female respectively), after which the larvae production increased again (Fig. 4). This increase in larvae production after the first drop was observed in the flyer and non-flyer group of 1997, in the flyer group of 1998 and the flyer fed group in 1999. In the other groups larvae production peaked in the second week then decreased and never rose again. The last larva was laid on day 74. In 1997 and 1999 the flyers started reproducing one week earlier than the non-flyers. The time needed to produce half the number of larvae is shown in Table 1. Although flyers started reproducing earlier than the non-flyers they took significantly more time to produce half of the total number of larvae (t=9.992, P=0.0001 in 1998; F=34.189, P=0.0001 in 1999), whereas fed flyers did not differ from the non-flyers in the time they needed to produce half of their offspring. In 1997, the time spent to produce half the number of larvae was not significantly different between the flyer and the non-flyer group.

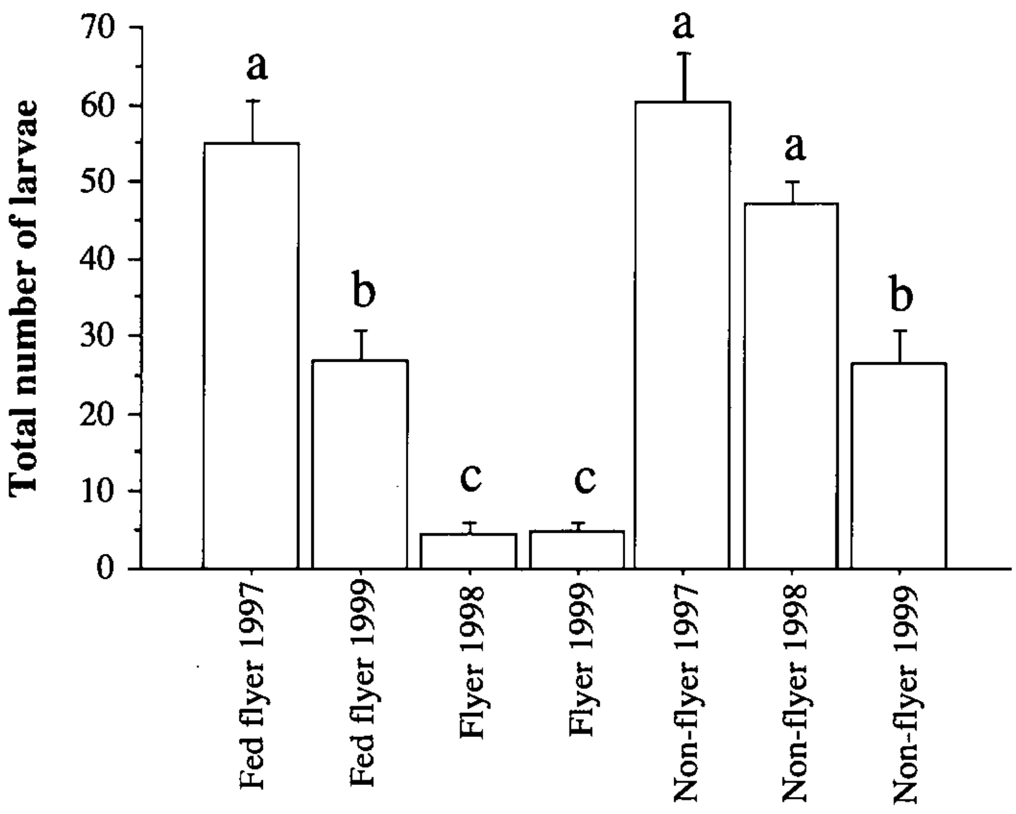

Fig. 3. Total number of larvae produced over a whole season in the different dispersal groups (mean and SE). n=39 flyer 1997, n=30 fed flyer 1999, n=50 flyer 1998, n=30 flyer 1999, n=39 non-flyer 1997, n=50 non-flyer 1998, n=30 non-flyer 1999. Different letter show significant differences determined by an Anova.

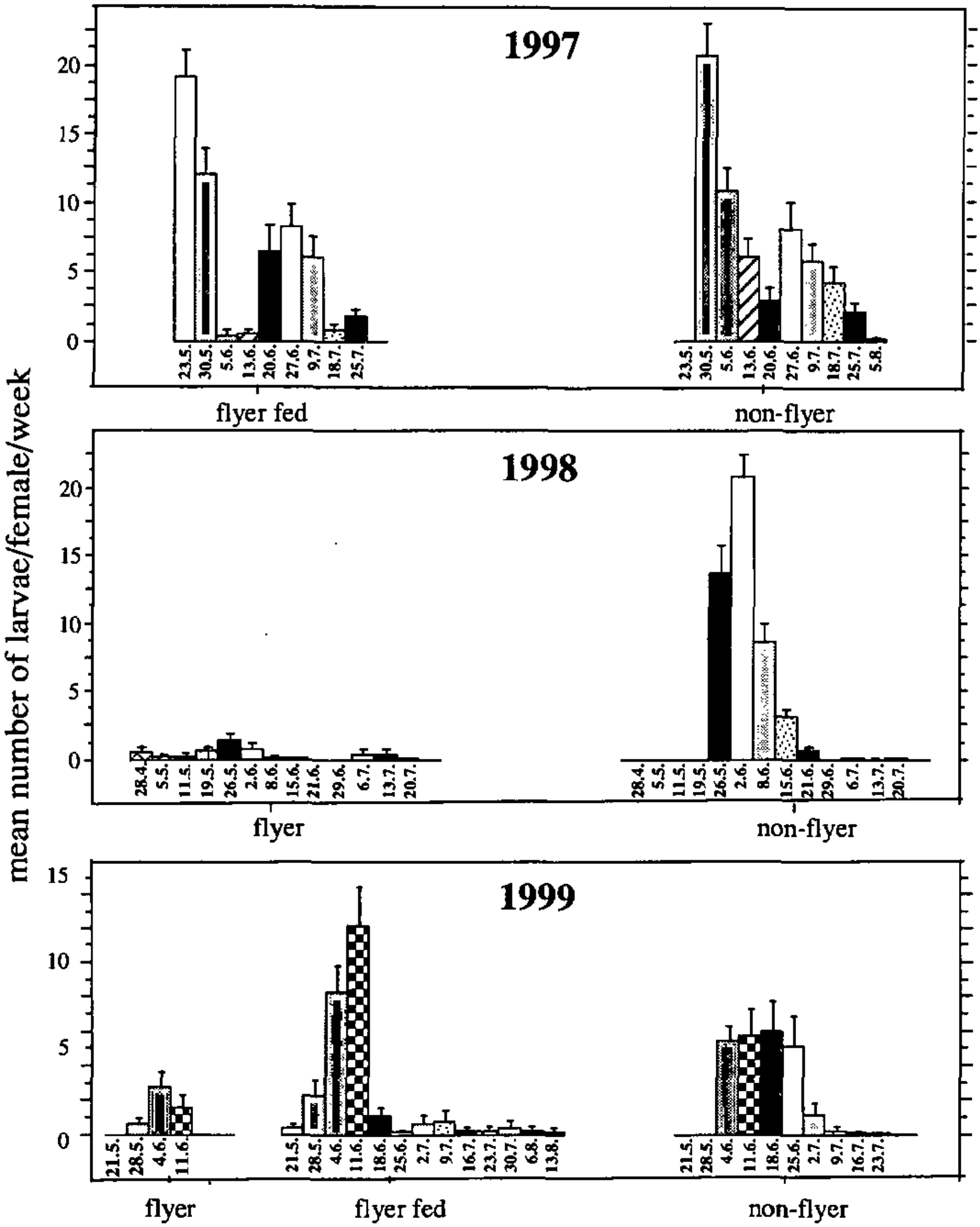

Fig. 4. Time course of larviposition by flyers, fed flyers and non-flyers throughout the seasons of three successive years. Flyers start reproducing earlier than non-flyers.

Survival. The time course of female survival showed that only 10% of the flyers survived the first 28 days after larviposition had started, whereas approximately 80% of both, fed flyers and non-flyers lived longer than 28 days and had the same reduction in survival after 84 days (Fig. 5). Survival in the fed flyer and non-flyer groups was regularly declining for two months after the first larviposition. At that time reproduction had almost finished. The lifespan of the three groups are shown in Table 2.

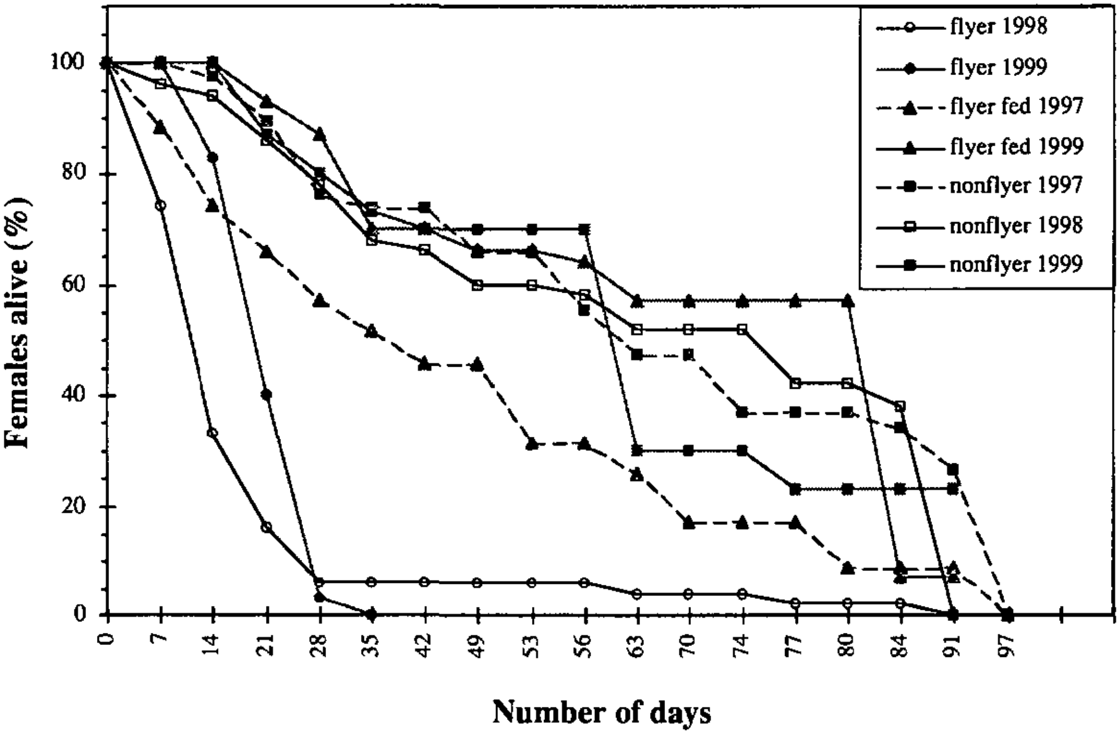

Fig. 5. Survival curves of adult *O. cacaliae* females of the flyer (circles), fed flyers (triangles) and non-flyer (squares) groups of three years. Day zero is the day the beetles were collected in the field.

Regression between larva production and life span. There was a positive correlation between female lifespan and the total number of larvae produced (Fig. 6) ($y = 0.91x + 11.34$, $R^2=0.41$, $F = 53.21$, $P \leq 0.0001$, $n = 78$). The analysis was done with data from the flyer and non-flyer group of 1997, because in the flyer group of 1998 too few larvae were laid.

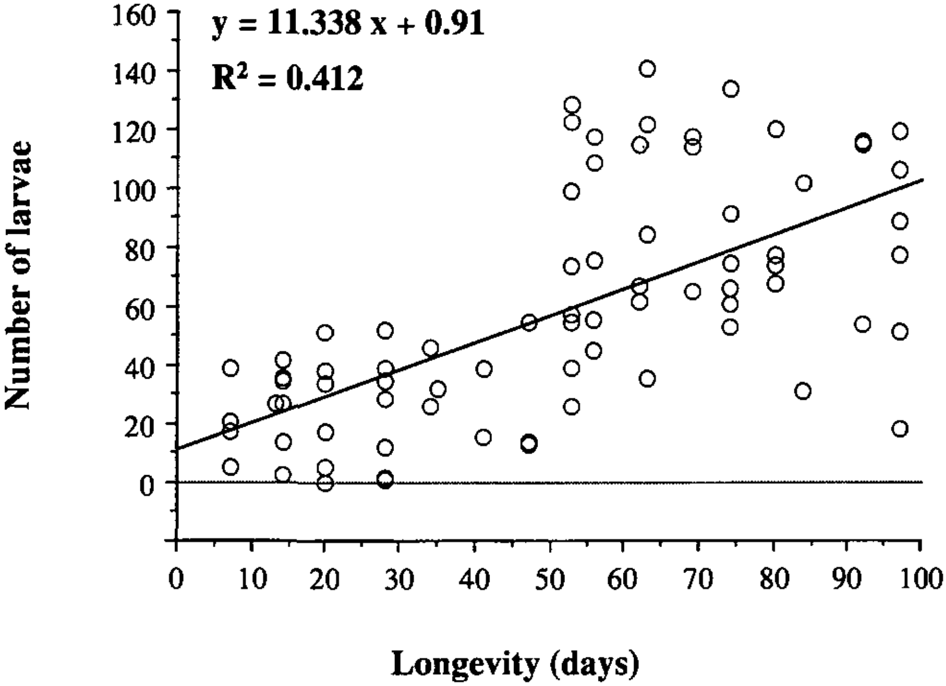

Fig. 6. Regression analysis of the number of larvae laid per individual female over the season 1997 and the according number of days the females lived ($F = 53.21$, $P \leq 0.0001$, n = 78).

TABLE 1. Comparison of the time needed for flyers and non-flyers to produce half of the total number of larvae, during different seasons.

Flying state and year	*n*	mean time ± SE (days)	-*n*
Flyers (fed) 1997	38	15.9 7±1.95	0
Non-flyers 1997	39	14.90 ±1.26	1
Flyers 1998	22	23.23 ±1.48	28
Non-flyers 1998	48	10.35 ±0.55	2
Flyer 1999	16	21.00 ±1.11	14
Flyer (fed) 1999	29	23.41 ±1.27	1
Non-flyer 1999	25	10.92 ±1.0	5

n = number of females , -n = number of females not reproducing all over the season.

TABLE 2. Life span of flyers and non-flyers in three consecutive years. Mean number of days ± standard error are given.

Flying state and year	*n*	mean time ± SE (days)
Flyers (fed) 1997	38	42.0 ± 64.2
Non-flyers 1997	39	58.4 ± 4.3
Flyers 1998	22	23.5 ± 1.8
Non-flyers 1998	48	64.1 ± 4.6
Flyer 1999	16	22.8 ± 1.0
Flyer (fed) 1999	29	65.0 ± 6.4
Non-flyer 1999	25	57.4 ± 4.5

DISCUSSION

The distinct flight behaviour of *O. cacaliae* observed in Val Ferret allowed to distinguish flyers from non-flyers. While non-flyers overwintered close to the main host *A. alliariae*, flyers overwintered away from those plants and could be found on *P. paradoxus*, in spring. Both groups were compared for size, reproduction and survival. Like in many other Coleoptera male *O. cacaliae* were smaller in size than females. The flyer beetles, males as well as females, were significantly smaller in size than the non-flyers. Roff (1991) suggested that bioenergetic constraints mandate that migrants should be as large as they can be to maximise the distance they can travel without refuelling, and thus predicts that migrants will be larger, on average, than nonmigrants. This prediction was not confirmed in *O. cacaliae*, but seems to be true for small *L. decemlineata* females which engage in reproduction rather than dispersal (Weber & Ferro, 1996) and for the red milkweed beetle (*Tetraopes tetraophthalmus*, Cerambicidae) where large individuals were more agile than small ones in tethered flight tests (Davis, 1984). We found a positive relationship between the amount of larvae produced and the size of *O. cacaliae* females. Large females laid more larvae than small individuals. This was in contrast to Colorado potato beetle females where no significant relationship between the size of the beetles (width of elytra) and the number of eggs was observed (Ferro *et al.*, 1991).

In 1997, the larviposition increased again after a first peak both in the (fed) flyer and the non-flyer group. In the other years no second phase of increasing reproduction was observed. Favourable temperature conditions in winter 1996/7 might be the reason for the increased reproduction in 1997. In the Colorado potato, the production of eggs peaked at about 15 days after emergence and dropped off exponentially until day 86; most production occurred after day 15 (Peferoen *et al.*, 1981). The *O. cacaliae* flyers produced significantly fewer larvae than the non-flyers, if the flyers were not fed. Roff

(1986) summarised the data for 22 wing-dimorphic species (mainly Hemiptera and Orthoptera) and concluded that the non-flying morph is more fecund and reproduces earlier than the flying morph. Nevertheless, Desender (1989) found a higher egg production in long-winged females of *Pogonus chalceus* (Coleoptera: Carabidae) than in short-winged females. The same result was found in studies with Ptiliidae (Coleoptera) by Taylor (1978). Fed flyers produced as many larvae as non-flyers in *O. cacaliae*. Access to food before and after flight seems to be an important factor for the number of larvae produced. When the flyers were not fed in the lab they had access to food after spring emergence in the field, as they were collected on flowering *P. paradoxus*. In other insect orders, access to food after flight was shown to be important to reduce the cost of flight on reproduction, too. In the migrant bug, *Oncopeltus fasciatus*, flights of several hours over 6 days had no effect on life history characteristics of female bugs unless they were also starved over that period, what reduced fecundity and longevity (Slansky, 1980). For *Heliothis virescens* (Lepidoptera: Noctuidae) Willers *et al.* (1987) showed that enforced tethered flight depressed fecundity and reduced longevity, but both were largely restored by allowing the moths access to sucrose after flight. Prolonged flight by *Spodoptera exempta* (Lepidoptera: Noctuidae), depressed fecundity in females that were maintained on distilled water but had no significant effect if they were allowed access to sucrose solution after flight (Gunn *et al.*, 1988). A period of starvation for 10 days after eclosion was lethal to virtually all females of the summer generation of Colorado potato beetles, regardless of subsequent availability of food (Weber & Ferro, 1996).

O. cacaliae flyers started reproducing earlier in the lab than non-flyers. They were ready to larviposit as soon as they had access to their main host plant *A. alliariae*. Non-flyers that were collected between one (1997 and 1999) and three (1998) weeks later in the field started reproducing later. In the field the flyers took some time, after having left *P. paradoxus*, to find emerging *A. alliariae* and it is very unlikely that they start reproducing on *A. alliariae* before non-flying *O. cacaliae* that overwinter in the vicinity of that main host plant and emerge at the same time as *A. alliariae*. We are convinced that the flyers need more than a week, after having left *P. paradoxus*, to find *A. alliariae* to feed on, at distances between 110 m and 1km, and they risk not to be able to feed during that time. Therefore we consider the situation presented in 1998, where the flyers were not fed in the lab, more realistic than the one of 1997 were the flyers were fed between collection on *P. paradoxus* in the field and emergence of *A. alliariae*. Although the flyers started reproducing earlier than the non-flyers they took significantly more time to produce half of their total number of larvae. The later onset of reproduction in the flyer groups has been found to be the overwhelming conclusion from studies on wing polymorphism (Rankin and Burchstedt, 1992).

Similar to the results for number of larvae produced are the results for longevity. As for reproduction, feeding seems to be an important factor for longevity. In 1997 and 1998 there was a large difference in longevity between the females from different flying states due to the fact, that in 1997 the beetles were fed *P. paradoxus* before the emergence of *A. alliariae*. The 1999 study confirmed that feeding is the factor leading to the difference in survival as well as in reproduction. Fed flyer beetles survived significantly better than unfed flyers. Survival was influencing reproduction since the females larviposited during three months. Another strategy might have been to lay all larvae during the first weeks and die soon after.

O. cacaliae live in a relatively stable environment in the Swiss Alps. *A. alliariae* patches provide everything beetles need to survive and reproduce. Those patches can persist for several years. Nevertheless catastrophic events like flooding (as observed at a site in 1997) can completely destroy a host plant patch within hours. In the absence of reliable environmental cues, it may be advantageous to spread the risk (den Boer, 1968). Selection might have favoured individuals that produce heterogeneous offspring, some of which stay in the patch (non-flyers), and some of which disperse to other patches (flyers) (Davis, 1984). Our results indicate that possible costs exist in survival and reproduction when the flying beetles have no access to food after flight. Since there is a high degree of patchiness in plant communities and herbivores continually try to find high quality host plants, investing energy in dispersal may be justified.

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REFERENCES

Boer, P. J. den, 1968. Spreading the risk and stabilization of animal numbers. Acta biotheoretica 18: 165-194.

Davis, M. A., 1984. The flight and migration ecology of the red milkweed beetle (*Tetraopes teraophthalmus*). Ecology 65: 230-234.

Denno, R., K. L. Olmstead & E. S. McCloud, 1989. Reproductive cost of flight capability: a comparison of life history traits in wing dimorphic planthoppers. Ecological Entomology 14: 31-44.

Desender K. (1989). Heritability of wing development and body size in a carabid beetle, *Pogonus chalceus* Marsham, and its evolutionary significance. Oecologia 78: 513-520.

Dingle, H. 1972. Migration strategies in insects. Science, 175: 1327-1335.

Dobler, S. & M. Rowell-Rahier, 1996. Reproductive biology of viviparous and oviparous species of the leaf beetle genus *Oreina*. Entomologia experimentalis et applicata 80: 375-388.

Ferro, D. N., A. F. Tuttle & D. C. Weber, 1991. Ovipositional and flight behavior of overwintered Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology 20: 1309-1314.

Gunn, A., A. G. Gatehouse & K. P. Woodrow, 1988. Trade-off between flight and reproduction in the African armyworm moth *Spodoptera exempta*. Physiological Entomology 14: 419-427.

Johnson, C. G., 1969. Migration and dispersal of insects by flight, Methuen, London.

Kennedy, J. S. 1961. A turning point in the study of insect migration. Nature 198: 674-677.

Lawrence, W. S. & C. E. Bach, 1989. Chrysomelid beetle movement in relation to host-plant size and surrounding non-host vegetation. Ecology 70: 1679-1690.

Lohse, G. A. & W. H. Luche, 1994. Die Käfer Mitteleuropas. In: G. Evers (ed), 14. Krefeld. pp. 73.

Peferoen, M., R. Huybrechts & A. D. Loof, 1981. Longevity and fecundity in the Colorado potato beetle, *Leptinotarsa decemlineata*. Entomologia Experimentalis et Applicata 29: 321-329.

Rankin, M. A. & J. C. A. Burchsted, 1992. The cost of migration in insects. Annual Review of Entomology 37: 533-559.

Rankin, M. A. & S. M. Rankin, 1980. Some factors affecting presumed migratory flight activity of the convergent ladybeetle, *Hippodamia convergens* (Coccinellidae: Coleoptera). Biological Bulletin 158: 356-369.

Ritchie, M. G., Butlin, R. K., Hewitt, G. M. 1987. Causation, fitness effects and morphology of macropterism in *Chorthippus parallelus* (Orthoptera: Acrididae). Ecological Entomology 12: 209-218.

Roff, D. A., 1984. The cost of being able to fly: a study of wing polymorphism in two species of crickets. Oecologia 63: 30-37.

Roff, D. A., 1986. The evolution of wind dimorphism in insects. Evolution, 40: 1009-1020.

Roff, D. A. 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. Am. Zool. 31: 205-215.

Slansky, F., 1980. Food consumption and reproduction as affected by tethered flight in female milkweed bugs (*Oncopeltus fosciatus*). Entomologia Experimentalis et Applicata 28: 277-286.

Solbreck, C. 1974. Maturation of post-hibernation flight behaviour in coccinellid *Coleomegillo maculata* (de Geer). Oecologia 17: 265-275.

Stevens, L. & D. E. M. Cauley, 1989. Mating prior to overwintering in the important willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). Ecological Entomology 14: 219-223.

Tanaka, S. 1976. Wing polymorphism, egg production and adult longevity in *Pteronemobius taprobanensis* Walker (Orthoptera: Gryllidae). Kontyu 44: 327-33.

Taylor V. A. 1978. A winged élite in a subcortical beetle as a model for a protermite. Nature 276: 73-75.

Voss, R. H. & D. N. Ferro, 1990. Ecology of migrating Colorado Potato Beetles (Coleoptera: Chrysomelidae) in Western Massachusetts. Environmental Entomology 19: 123-129.

Weber, D. C. & D. N. Ferro, 1996. Flight and fecundity of Colorado Potato Beetle (Coleoptera: Chrysomelidae) fed on different diets. Annuals of the Entomological Society of America 89: 297-306.

Willers, J. L., Schneider, J. C. Ramaswamy, S. B. (1987) Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. Journal of Insect Physiology 33: 803-808.

Young, E. C. 1984. Flight muscle polymorphism in British Corixidae: ecological observations. Journal of Animal Ecology 34: 353-90.

Zillesen, P. G. v. S. & A. M. H. Brunsting, 1983. Capacity for flight and egg production in *Lochmaea saturalis* (Col., Chrysomelidae). Netherlands Journal of Zoology 33: 266-275.

III

ATTRACTION OF A LEAF BEETLE (*OREINA CACALIAE*) TO ALREADY INFESTED HOST PLANTS

Key words: Coleoptera, Chrysomelidae, *Oreina cacaliae*, olfaction, host plant location behaviour, windtunnel, plant volatiles

Abstract

Early in spring, just after the snow melts, the leaf beetle *Oreina cacaliae* feeds on *Petasites paradoxus* flowers. Later in the season they switch to their principle host plant *Adenostyles alliariae*. The potential role of olfaction in the localisation of these host plants was studied in a windtunnel. The spring host *P. paradoxus* was more attractive to the beetles after they had been damaged overnight by conspecifics. Increased attractiveness was also found after artificial damage the evening before, but not when the plants were similarly damaged half an hour before the windtunnel experiments. Contrary to *P. paradoxus*, the principle host plant, *A. alliariae* was more attractive shortly after an attack by conspecifics (half an hour before the experiment), but lost its increased attractiveness when infested overnight. In all treatments, no preference of plants infested by larvae or adults of *O. cacaliae* over plants artificially damaged was observed. Volatiles emitted by injured and healthy host plants were collected and gas chromatographic analyses of the collected odours showed qualitative and quantitative differences between infested and uninfested plants. Among the volatiles recorded, green leaf volatiles and mono- and sesquiterpenes dominated. Nonene, α-phelandrene, limonene and eremophylene, were the compounds induced by insect feeding in *P. paradoxus*. Linalool, α-humulene and germacrene D the ones induced in *A. alliariae*. Volatiles emitted by host plants attracted *O. cacaliae* and the enhanced attraction of infested plants was longer lasting in the spring host *P. paradoxus* than in the main host *A. alliariae*.

Introduction

Herbivory by beetles leads to plant injury and thus to an enhanced emission of plant volatiles (Loughrin et al., 1995; Bolter et al., 1997). In beetles such volatiles may increase the attractiveness of their host plants. The scarabid *Maladera matrida* is attracted to volatiles of an injured host plant (Harari, 1994). Host-derived volatiles can also interact with aggregation pheromones to produce synergised attraction like it has been described in bark beetles (Jones, 1985). In female mountain pine beetles the host-produced monoterpene α-pinene is converted into trans-verbenol, which is an aggregation pheromone for this species (Borden, 1984). Bartelet *et al.*, (1990) reported an aggregation pheromone from nitidulids (Coleoptera), which interacts synergistically with some host volatiles.

The alpine *Oreina cacaliae* (Coleoptera: Chrysomelidae) were found to feed on two host plants at the field site in La Fouly (Val Ferret: Valais) in the Swiss Alps. In spring, just after the snow melts, the beetles feed on *Petasites paradoxus* (Asteraceae) in its flowering state, which is one of the first annual plants to occur at sun exposed sites. Wind tunnel experiments confirmed the attractiveness of *P. paradoxus* over a non-host (*Tussilago farfara*) in the lab (Chapter 1). Up to 80 individual *O. cacaliae* beetles can be found on a single flowering *P. paradoxus* plant in the field (Kalberer, personal observation). This observation led to the hypothesis that an aggregation pheromone might exist, which could explain the accumulation of adults on an individual host plant. Female *O. cacaliae* beetles copulate immediately after their emergence in summer, while male beetles first overwinter as an adult before they copulate the next spring and summer (Rahier, unpublished results). Intraspecific attraction of the opposite sex has never been observed in the genus *Oreina*.

Adenostyles alliariae (Asteraceae), the main host plant of *O. cacaliae*, emerges later in the season, 3-4 weeks after *P. paradoxus*. The leaves of *A. alliariae* contain pyrrolizidine alkaloids, that are sequestered by the *O. cacaliae* larvae and adults for their own defence (Ehmke *et al.*, 1991; Dobler & Rowell-Rahier, 1994). In the field the beetles are less aggregated on the *A. alliariae* leaves than on their spring host *P. paradoxus* (Kalberer, unpublished data). Quite often two or three beetles can be found on one single *A. alliariae* leaf, but their number rarely exceeds more than four (Kalberer, personal observation). This observation weakens the hypothesis about the existence of an aggregation pheromone in *O. cacaliae* except if this pheromone was released only on *P. paradoxus*.

We wanted to test a) if an aggregation pheromone exists, b) if plant injury changes the attractiveness of the host plants, and c) if *O. cacaliae* reacts behaviourally different to short and old injury to *P. paradoxus* and *A. alliariae* plants. Different responses to already *P. paradoxus* and *A. alliariae* may explain the observed differences in aggregation on these plants. Behavioural experiments with walking beetles were conducted in a windtunnel. In addition the odours emitted by healthy and infested host plants were collected, identified and compared.

Material and Methods

The insects. Adults of *O. cacaliae* were collected weekly from *P. paradoxus* in spring and from *A. alliariae* in summer, near La Fouly (Val Ferret: Valais) in the Swiss Alps. To prevent stress, beetles were transported to the laboratory in cooled containers. The beetles were maintained in glass jars in an incubator at a day temperature of 16°C and a night temperature of 12°C and a 16L: 8D light-regime. Beetles were used for the experiments within 3 days after collection. Beetles were sexed using sexual polymorphism of the tarsi (Lohse and Luche, 1994).

The plants. Plants used in the experiments were dug out weekly at, or near the sites were beetles were collected. They were immediately potted into ceramic pots of 15 cm diameter in the field and brought to the laboratory, where they were placed near a window and kept at room temperature until use. To obtain herbivore-infested plants, 10 adult beetles (5 males and 5 females) were placed on the plants either for half an hour before the experiment (short damage) or during the night before the experiment (old damage). For overnight attack the beetles were placed on the plants at 20h00, where they were feeding until darkness at 22h30, and the experiments were conducted during the next day. For larval short attack, five last instar *O. cacaliae* larvae were allowed to feed on a plant for half an hour before the experiment. Artificial injury on *P. paradoxus* was inflicted by a razor blade, with which two out of tens of flowerheads were removed together with the edge of a basal leaf surrounding the stem of the flower. In *A. alliariae* artificial injury was administered by cutting out two pieces (35 x 10 mm) from the edge of the leaf with a razor blade. Additionally a hole was punched in the middle of the leaf with a cork borer (14 mm diameter). Each individual plant was used once as a healthy plant and once after attack treatment.

Wind tunnel. The plexiglas windtunnel (0.60 x 0.60 x 1.80 m) was surrounded by a white tent to eliminate visual distraction of the beetles. Air was pulled through the tunnel at 0.1 m/sec with the use of a tubular duct fan (RR 125 C, Melios Ventilatoren AG, Urdorf, Switzerland) and was exhausted outside the room. A charcoal impregnated fabric cleaned the air at the tunnel entrance. The temperature in the room housing the windtunnel was between 22 and 23°C and the humidity around 55% (for details see Chapter 1).

Experimental procedure. The assay was designed as a dual-choice test for walking beetles. Two differently treated, potted plants were placed at the upwind end of the windtunnel 0.3 m apart. A glass plate (1.5 x 0.5 m) was placed horizontally in the windtunnel at a height adjusted such that air from the volatile emitting, upper plant parts would pass over it. The distance between point of release on the glass plate and the plants was 1 m. The glass plate was cleaned with ethanol (70%) after testing ten beetles. Each plant combination was replicated four times with 20 beetles each (10 females and 10 males). Replication took place at an intervals of at least one week with different plants and different beetles. The insects were denied food for at least 24 h before each experiment to increase their anemotactive response (Visser & Nielsen, 1977; Miller &Strickler, 1984). Glass jars with beetles were taken out of the incubator half an hour before an experiment and left near the wind tunnel to let the beetles acclimatise to the experimental conditions. Details on the behaviour of the beetles in the windtunnel choice situation are described in Chapter 1.

Windtunnel experiments excluding visual cues. For this experiment the plants were not put in the windtunnel but placed outside the white curtain surrounding the windtunnel. Compressed air, humidified and purified by a charcoalfilter, entered the sealed Nalophan® cooking bags (Kalle Nalo, Wiesbaden, Germany) containing the potted plant. Teflon tubes lead the air enriched with plant odours out of the cooking bag and into the windtunnel through two holes at the upwind end of the windtunnel. The end of the tubes were taped to the upwind edge of the glass plate.

An additional experiment tested the quality of the collected headspace volatiles. Samples (50µl each) containing the volatiles collected from two different treatments, were offered on a folded filter paper disc (diam: 90 mm, Schleicher & Schuell, Dassel, Germany) on the glass plate in the windtunnel. This experiment was also conducted to test if the volatiles that elicited a behavioural response were collected.

Pheromone test. To test for attractiveness of one sex to the other, we hid either 10 overwintered males or 10 freshly emerged females in a metallic cage with big meshes, where volatiles could readily pass through. Beetles of the opposite sex were used to test for pheromonal attraction. To establish a dual choice test we had two cages, one with beetles and one empty. The experiments were conducted with 20 beetles and replicated four times.

Collection of plant volatiles. Volatiles were collected from potted plants inside a climate chamber at 15°C, 50-60% RH, and 35'000 Lux. The plants were placed inside odourless PET Nalophan® cooking bags. One bag opening was sealed before use with a heat sealer (TEW electric heating equipment Co. Ltd, Type TISH-400), the other was tied around the plant stem with a plastic tie provided with the bags.

Humidified and charcoal filtered air, was pushed into the bag via a teflon tube at a rate of 1000 ml/min. The teflon tube was connected to the bags by a glass tube. The glass tube entered the PET bag and was introduced through a screw cap containing a teflon sealed O-ring of the same diameter (6 mm). For details see Turlings *et al.*, (1998).

A volatile collection trap consisting of a 10-cm-long, 5-mm-diameter glass tube containing 25 mg Super Q adsorbent (as described by Heath & Manukian, 1992) was attached to the PET bag in the same way as the inlet glass tube before. Air was pulled through the collection trap in the bag via Tygon tubing at a rate of 800 ml/min (Turlings *et al.*, 1998). Volatiles were collected for 4 h after which each trap was rinsed with 150 µl methylene chloride, and internal standards were added (400 ng *n*-octane and nonyl-acetate) before injection in a gas chromatograph (GC). Four replicated per treatment were collected.

Plant volatile analysis Volatiles were analysed by a Hewlett Packard 6890 GC equipped with an on-column injector and a flame ionisation detector. Aliquots of 3µl were injected into a 30 m x 0.25 mm non-polar column (EC1, film thickness 0.25 µm, Alltech Associates, Inc, USA) preceded by a 10m x 0.25 mm deactivated retention gap (Connex, USA) and a deactivated pre-column (30 cm, 0.53 mm, Connex, USA). Helium was used as carrier gas at 24 cm/s. The following temperature program was used: after a hold time of 3 min at 50° C, temperature was linearly increased at a rate of 8 °C/min to 230°C and held for 9.5 min. Data were collected and processed with ChemStation software (Hewlett-Packard).

Statistical analysis. Odour preferences in the windtunnel were tested with a two-tailed binomial test, using the total number of beetles that made a choice for a particular odour ($\alpha = 0.05$). To analyse the quantitative differences in the plant odours emitted by healthy and infested plants, we used an ANOVA followed by a Bonferroni-Dunn versus control (healthy plant) post hoc test. Collected odour data were log (X + 1) transformed before analysis, to stabilise variance.

Results

Choice experiments

Petasites paradoxus, *flowering state*

In choice experiments with flowering *P. paradoxus,* beetles showed a significant preference for flowers that had been infested overnight by feeding beetles (Fig. 1A, $P \leq 0.001$) and for plants that had been artificially damaged the previous evening (Fig. 1B; $P \leq 0.01$) over healthy flowers. There was no difference in attraction between overnight attack caused by adult beetles and artificial overnight attack ($P \leq 0.43$). However, beetles were not more attracted to *P. paradoxus* flowers when they had been infested only half an hour before the experiment than to uninfested flowers ($P \leq 0.43$).

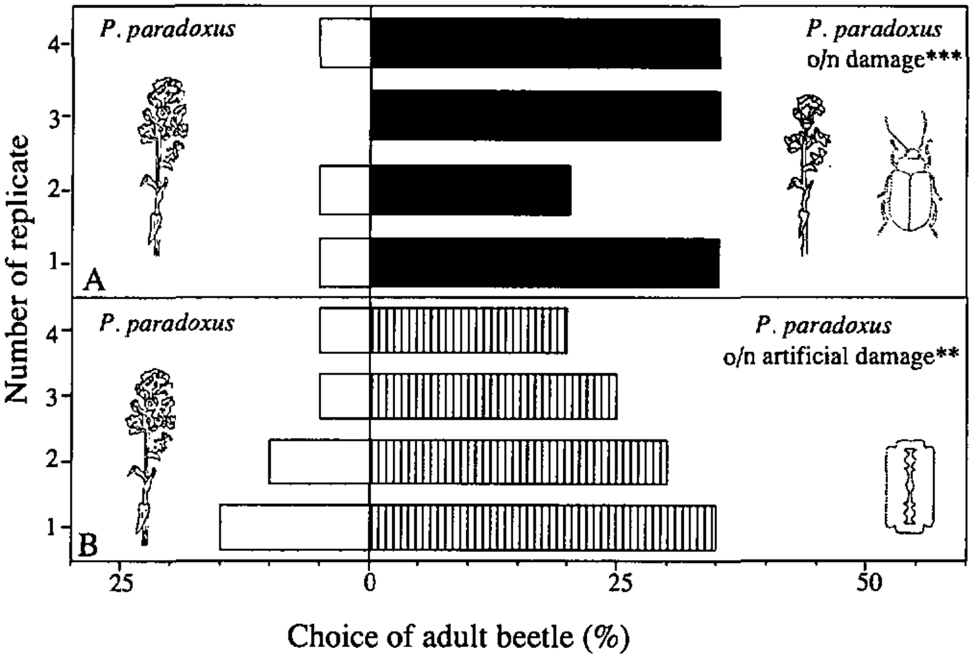

Fig. 1. Choices of *Oreina cacaliae* in windtunnel experiments with healthy and overnight (o/n) infested *Petasites paradoxus*. The bars represent the percentage of tested beetles that made a particular choice. The asterisks indicate a significant preference for the indicated treatment (two-tailed binomial test; **$P \leq 0.01$, ***$P \leq 0.001$).

Adenostyles alliariae, *vegetative state*

Leaves of *A. alliariae* infested half an hour before starting the wind tunnel experiments, turned out to be more attractive than healthy leaves, regardless if adult beetles or larvae had been feeding on the plant (Fig. 2A and 2B). Artificial fresh attack was as attractive as fresh damage caused by adult beetles feeding on the plant ($P \leq 0.42$). Plants infested over night by adult beetles were not more attractive than healthy plants ($P \leq 0.42$). In contrast to *P. paradoxus* flowers, freshly infested *A. alliariae* leaves were significantly more attractive than overnight infested leaves (Fig. 2C).

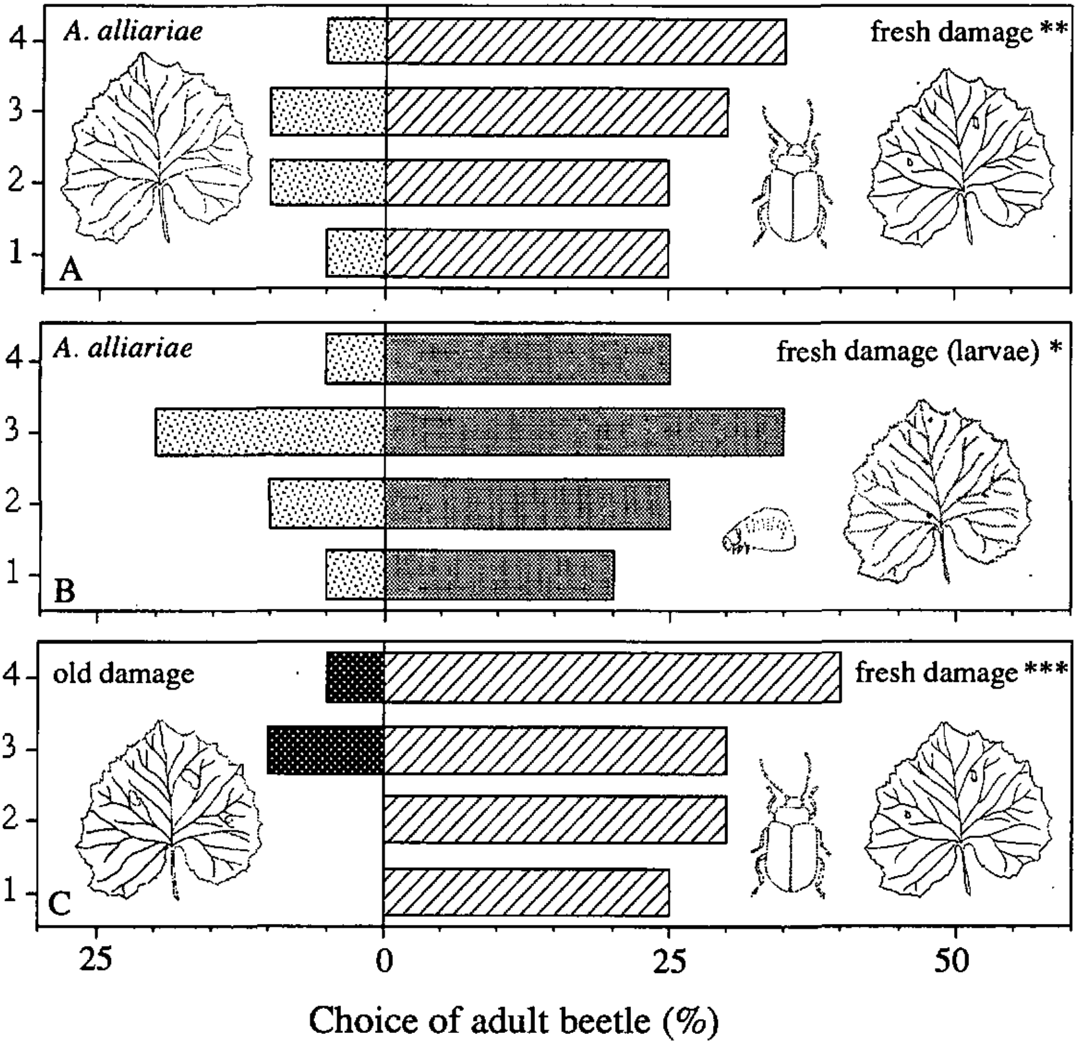

Fig. 2. Choices of *Oreina cacaliae* in the windtunnel experiments with *Adenostyles alliariae*. The bars represent the percentage of tested beetles that made a particular choice. The asterisks indicate a significant preferences for indicated treatment by a two-tailed binomial test ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$).

Host odour without visual cues.

The odour of a healthy *A. alliariae* plant coming from outside the windtunnel attracted significantly more beetles ($P \leq 0.05$) than clean air (Fig. 3A). Collected headspace volatiles of a freshly infested *A. alliariae* leaf attracted more beetles ($P \leq 0.01$) presented on a filter paper than collected headspace volatiles of a healthy leaf (Fig. 3B).

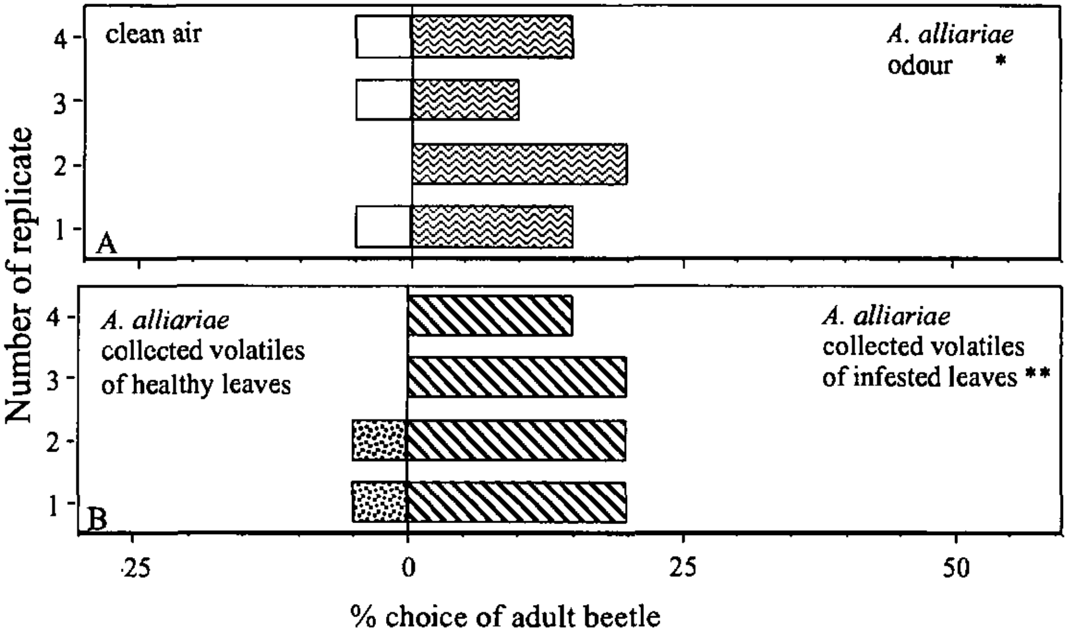

Fig. 3. (A) Choice experiments without visual cues, where the host odour entered the windtunnel from plants standing outside the windtunnel. Odour from *A. alliariae* plant attracted more beetles than pure air. The asterisks indicate a significant preferences for indicated treatment by a two-tailed binomial test. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. (B) Collected headspace volatiles from an infested *A. alliariae* leaf put on a filter paper, attracted more beetles than the collected odour of a healthy *A. alliariae* leaf.

Attractiveness of conspecifics.

O. cacaliae beetles of one sex were not attracted by their conspecifics of the other sex. Overwintered males, which were ready to copulate were not more attracted to 10 new generation females hidden in a metallic cage than to an empty cage. Vice versa new generation females were not able to find their overwintered male conspecifics in a cage ($P \leq 0.34$, both sexes). When we added two potted *A. alliariae* plants at the upwind end of the windtunnel, the beetles still would not discriminate between the cage with conspecifics and the empty cage ($P \leq 0.4$).

Analysis of headspace volatiles.

Petasites paradoxus *flowers.*

Representative gas chromatographic profiles of headspace volatiles collected from *P. paradoxus* flowers (Fig. 4.) illustrate the differences in volatile blends between healthy and infested flowers. Control collections with odours coming from empty PET bags showed that the method was clean since no background impurities were detected. The sesquiterpene α-phellandrene was emitted in large amounts by freshly infested *P. paradoxus* plants (Fig. 4). Overnight infested attractive flowers (adult and artificial attack) emitted significantly more limonene than both healthy and freshly damaged flowers. The other volatile compounds nonene, benzaldehyde 4-methoxy, eremophylene, and two unknown substances were released in significantly larger amounts by infested flowers (either fresh damage old damage) than by healthy flowers (Table 1). Chromolaenin was the only identified substance emitted from *O. cacaliae* beetles and was also present in plant odour collections from infested plants.

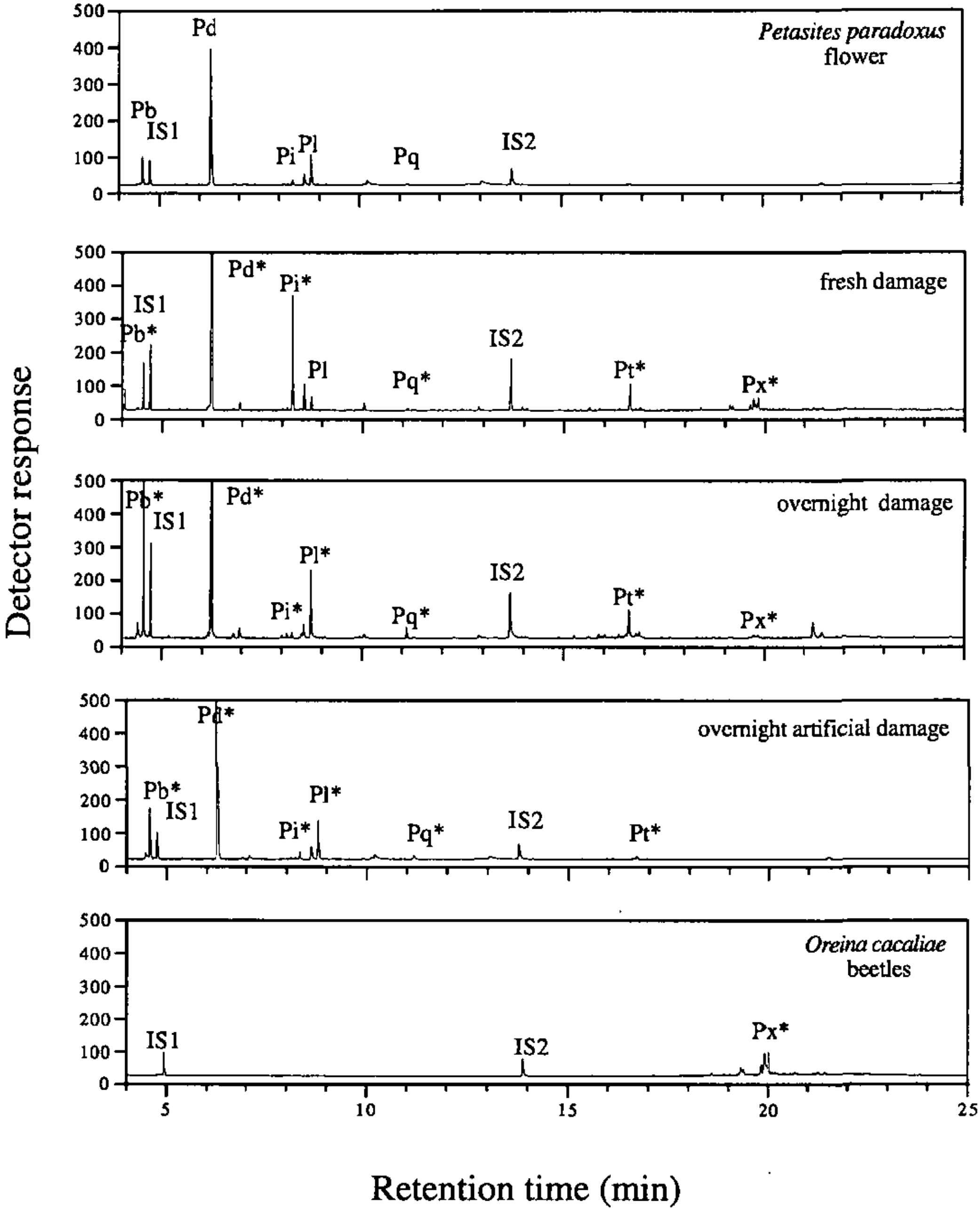

Fig. 4. Representative gas chromatographic profiles of headspace volatiles from healthy, freshly infested, overnight infested and artificially overnight infested *P. paradoxus* flowers. For comparison the odour from *Oreina cacaliae* beetles is shown, too. Peak identities: Pb, unknown; Pd, nonene; Pi, α-phelandrene; Pl, limonene; Pq, unknown; Pt, eremophylene; Px, chromolaenin. IS1 and IS2 represent the internal standards n-octane and nonyl acetate, respectively. Asterisks indicate significant differences in the amount of an odour compound emitted compared to a healthy *P. paradoxus* flower.

Adenostyles alliariae *leaves.*

Representative gas chromatograms of head-space volatiles collected from *A. alliariae* leaves following different treatments are shown in Fig. 5. Freshly infested leaves, damaged either by adult beetles, larvae, or artificially, contained significantly larger amounts of linalool, dodecanol, α-humulene and germacrene D than uninfested leaves (Table 1). When *A. alliariae* leaves were infested overnight by adults, they emitted additional volatiles like (Z)-3 hexenol, (*Z*)-3-hexenyl acetate, (*E,E*)-α-farnesene and an unknown substance. (*E,E*)-α-farnesene was emitted in a similar amounts by infested and healthy leaves (MS=0.25, F=2.42, $P \leq 0.075$), but *A. alliariae* infested overnight emitted significantly more of this common sesquiterpene than freshly infested leaves by larvae ($P \leq 0.005$).

TABLE 1. Data of the ANOVA test for collected plant odour compounds of infested plants compared with the healthy control plant. Significant differences between treatments are indicated with * in the Figures 4 and 5.

Compound	MS	*F*	*P*
Petasites paradoxus **flowers**			
α-phellandrene	5.81	11.7	0.0001
limonene	1.15	4.94	0.01
4-methoxy benzaldehyde	4.33	12.63	0.0001
eremophylene	4.29	23.59	0.0001
Adenostyles alliariae **leaves**			
linalool	0.94	7.38	0.0005
dodecanol	2.78	6.14	0.0014
α-humulene	0.87	7.51	0.0004
germacrene D	0.48	3.61	0.019
(Z)-3 hexenol	2.69	3.34	0.025
(Z)-3 hexenyl acetate	1.9	3.84	0.015

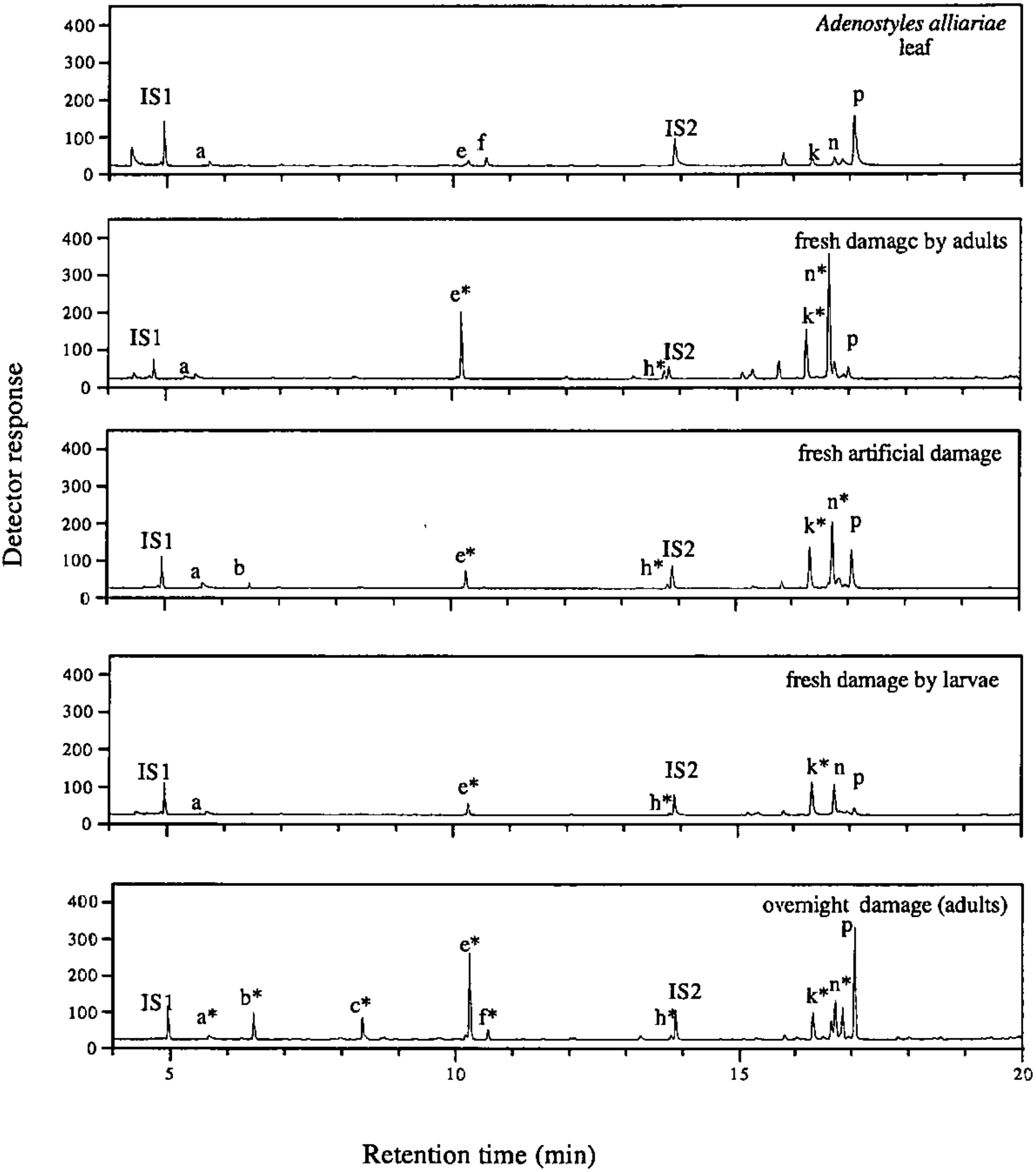

Fig. 5. Representative gas chromatographic profiles of *Adenostyles allliariae* headspace volatiles collected from leaves after various treatments. Treatments from top to bottom were: healthy *A. alliariae* leaf, a leaf freshly damage by adult *O. cacaliae*, a leaf artificially damaged with a razor blade, a leaf freshly damaged by *O. cacaliae* larvae and a leaf infested overnight by *O. cacaliae* adults. Peak identities: a, *Z*-3-hexenol; b, unknown; c, *Z*-3 hexenylacetate; e, linalool; h, dodecanol; k, α-humulene; n, germacrene D; p, (*E,E*)-α-farnesene. IS1 and IS2 represent the internal standards n-octane and nonyl acetate, respectively. Asterisks indicate significant differences in the amount of an odour compound emitted compared to a healthy *A. alliariae* leaf.

Results of beetle odour collections

Oreina cacaliae

O. cacaliae beetles emitted a few volatiles that were distinctly different from the ones emitted by their host plants (Fig. 5). These volatiles were also detected in the profiles of infested *A. alliariae* leaves, but not in a healthy or artificially infested leaves. GC-MS analysis of *O. cacaliae* odour showed that chromolaenin could be a compound characteristic for the beetles.

Discussion

Wind tunnel experiments with *O. cacaliae* and the host plants *P. paradoxus* and *A. alliariae* provide evidence that this alpine leaf beetle is more attracted to infested than to healthy host plants. However, timing of increased attractiveness differed for the two plants. The spring host *P. paradoxus* was more attractive after an overnight infestation, whereas *A. alliariae* had an enhanced attractiveness when attacked half an hour before the experiment, but lost this enhanced attractiveness hours later. Visser *et al.,* (1979) showed that fresh attack results in the emission of green leaf volatiles from injured plant cells These volatiles are emitted by all plant species after damage as a result from the oxidative degradation of plant lipids (Visser & Avé, 1978). The compounds (Z)-3-hexenol and (Z)-3-hexenyl acetate that were released by briefly attack from infested *A. alliariae* leaves belong to this category, but they were also produced after an overnight attack. Several studies have shown that the production of other volatiles is induced in the plant following the attack by a herbivore (Turlings *et al.*, 1990, Takabayashi *et al.,* 1994), and is the result of active chemical processes in the plant (Pare & Tumlinson, 1997). Mono- and sesquiterpenes, built via the isoprenoid pathway, belong to this category (Pare & Tumlinson, 1997).

Other beetles have been found to be attracted by injured hosts (Loughrin *et al.*, 1995; Bolter *et al.*, 1997). Injured cucurbit leaves were extremely effective in arresting movement and eliciting feeding in diabroticini chrysomelids that sequester cucurbitacin (Metcalf *et al.*, 1980, 1982). Male or female green June beetles (Coleoptera: Scarabaeidae) feeding on ripe peach attracted significantly more conspecifics than ripe peach slices and male and female beetles without fruits (Domek and Johnson, 1988). *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) was attracted to conspecifics feeding on the host plant oilseed rape, but not to conspecifics feeding on the non-host crambe (Peng, 1992). This shows that the beetles are not attracted to a general odour of wounded plants, but that the injured host plant emits characteristic odours that beetles are able to distinguish. Specific attraction to the injured host plant was also found in *Altica carduorum* (Coleoptera: Chrysomelidae) which aggregate on damaged (artificially or by conspecifics) *Cirsium arvense* host plants, but other *Cirsium* species were not attractive (Wan, 1996). Japanese beetles feeding on crabapple react similarly to damaged host plants as *O. cacaliae* on *P. paradoxus*. Crabapple leaves damaged overnight attract more Japanese beetles than undamaged leaves and leaves with fresh damage, caused artificially or by conspecifics (Loughrin *et al.*, 1995). An enhanced attraction to infested host plants similar to the one that has been described for the scarabid beetle *Maladera matrida* (Harari, 1994) was found in *O. cacaliae*. In all these cases, it is not a beetle-emitted aggregation pheromone that attract the beetles, but feeding-induced odours emitted by host plants that elicited the behavioural response.

When we kept the plants in PET bags outside the windtunnel and introduced their odour via a teflon tube inside the tunnel, significantly more beetles approached the plant odour source (healthy *A. alliariae* plant) than the clean air source, which came from an empty PET bag. The fact that fewer beetles responded in experiments without visual cues, than with real plants in the windtunnel, might show that indicate that some visual cues help the beetles to find a plant. Samples of volatiles collected from infested *A. alliariae* leaves were more attractive to the beetles than samples from healthy *A. alliariae* leaves. Apparently our system was able to collect the compounds that trigger the behavioural response. The compounds emitted by the host plants of *O. cacaliae* were examined in order to determine how the composition of the odour blend changed as a result of herbivore attack. After an overnight infestation by beetles as well as after artificial damage the previous evening, *P. paradoxus* flowers released significantly more limonene than plants that received one of the other treatments. These plants with older damage were also found to be more attractive to the beetles. The profile of *A. alliariae* leaves infested overnight, which showed no increased attractiveness compared to uninfested leaves, did show an increase in emission of the sesquiterpenes α-humulene and (*E,E*)-α-farnesene. In freshly infested, attractive *A. alliariae* leaves the sesquiterpene germacrene D is emitted in higher amounts than in other *A. alliariae* treatments. It is tempting to speculate that the enhanced attractiveness of the freshly infested *A. alliariae* leaves may be caused by a increased amount of germacrene D, and the enhanced attractiveness of overnight infested *P. paradoxus* by a increased amount of limonene in the emitted plant volatiles. Nevertheless it is risky to speculate about single compounds to evoke a behavioural response in an insect, several authors have shown that the blend of odour components is crucial to provoke a certain behavioural response (Visser & Avé, 1978; Saxena & Goyal, 1978).

Most of the feeding induced compounds that we found for host plants of *O. cacaliae* have been found for infested host plants of other systems before. For instance (*E,E*)-α-farnesene was released in *Psylla*-infested pear trees (Scutareanu *et al.*, 1997). (*Z*)-3-hexenyl acetate was found to be associated with herbivory in cotton and maize (Turlings *et al.*, 1990; McCall *et al.*, 1994). The terpenes (*E,E*)-α-farnesene was one of the most abundant of induced volatiles in crabapple (Loughrin, 1995). The sesquiterpene (-)Germacrene D appeared to mask the attractiveness of healthy pine trees to *Monochamus alternatus* (Coleoptera: Cerambycidae) (Yamasaki *et al.*, 1997).

Plants that were damaged artificially do not always elicit the same behavioural response as an injury caused by insects. Artificially damaged cabbage leaves are less attractive to the parasitoid *Cotesia glomerata* than herbivore-damaged leaves (Mattiacci *et al.*, 1994). Nevertheless volatiles released by plants immediately at the beginning of feeding damage did not differ from volatiles released by plants artificially damaged with a razor blade in maize, but several hours after damage caterpillar-damaged maize release large amount of terpenoids that are rarely released by artificially damaged maize (Turlings *et al.*, 1990).

O. cacaliae beetles showed the same behavioural response to infested host plants no matter if the injury was caused by larvae or adult beetles feeding on the plants or artificially with a razor blade. The fact that old artificial damage elicits the same response as overnight feeding damage shows that the volatile blend emitted is solely a result of the plant injury and is not part of the odours emitted by the insect while feeding. Evidently, *O. cacaliae* introduce no elicitor to their host plants that could enhance the production of attractive odours by infested plants as it has been found in the regurgitant of caterpillars (Turlings et al., 2000).

Pasteels *et al.* (1994) speculated about the existence of a sexual pheromone in Chrysomelids, after differences in the composition of defensive secretion according to sex had been found in *O. gloriosa* (Eggenberger & Rowell-Rahier, 1991 and 1993). No evidence was found that possible sex differences in the defensive secretion of *O. cacaliae* result in an attraction of the opposite sex in the windtunnel. We could not show any attraction to the opposite sex. However, beetles were transported together in a pot from the field to the lab and they may have released their defensive secretion during this transport.

This could have affected their subsequent responses. Keeping the beetles separate may yield different results.

An aggregation pheromone was not found for *O. cacaliae*, but infested host plants were more attractive than healthy plants and this may lead to aggregation on leaves suitable for feeding and larval development. The time after attack was crucial for the behavioural response elicited in the beetles. On the spring host *P. paradoxus* tens of beetles were observed feeding on that plant in the field. Old attacked plants were more attractive than healthy plants and the attraction was long-lasting. Whereas the increased attractiveness of infested *A. alliariae* disappeared after prolonged attack and only few beetles were observed on the same leaf in the field. The long lasting attraction in flowering *P. paradoxus* early in the season might help *O. cacaliae* to mates. It might be especially rewarding for the females to remate in spring with a male who successfully overwintered, to increase their fitness of the offspring (Stevens & Cauley, 1989).

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References

Bartelet, R. J., P. F. Dowd, R. D. Plattner & D. Weisleder, 1990. Aggregation pheromone of driedfruit beetle, *Carpophilus hemipterus*. Wind-tunnel bioassay and identification of two novel tetraene hydrocarbons. Journal of Chemical Ecology 16: 1015-1039.

Bolter, C. J., M. Dicke, J. J. A. v. Loon, J. H. Visser & M. A. Posthumus, 1997. Attraction of CPB to herbivore-damaged plants during herbivory and after its termination. Journal of Chemical Ecology 23: 1003-1023.

Borden, J. H. 1984. Semiochemical-mediated aggregation and dispersion in the Coleoptera, pp. 123-149, in T. Lewis (ed.). Insect Communication. Academic Press, Orlando.

Dobler, S. & M. Rowell-Rahier, 1994. Production of cardenolides versus sequestration of pyrrolizidine alkaloids in larvae of *Oreina* species (Coleoptera, Chrysomelidae). Journal of Chemical Ecology 20: 555–568.

Domek, J. M. & D. T. Johnson, 1988. Demonstration of semiochemically induced aggregation in the green June beetle, *Cotinis nitida* (L.) (Coleoptera, Scarabaeidae). Environmental Entomology 17: 147-149.

Eggenberger, F. & M. Rowell-Rahier, 1991. Chemical Defence and Genetic Variation. Naturwissenschaften 78: 317-320.

Eggenberger, F. & M. Rowell-Rahier, 1993. Physiological sources of variation in chemical defense of *Oreina gloriosa* (Coleoptera: Chrysomelidae). Journal of Chemical Ecology 19: 395-410.

Ehmke, A., M. Rowell-Rahier, J. M. Pasteels & T. Hartmann, 1991. Sequestration of ingested (^{14} C)Senecionine N-oxide in the exocrine defensive secretions of chrysomelid beetles. Journal of Chemical Ecology 17: 2367-2379.

Harari, A. R., D. Ben-Yakir & D. Rosen, 1994. Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabidae). Journal of Chemical Ecology 20(2): 361-371.

Heath, R. R. & A. Manukian, 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. Journal of Chemical Ecology 18: 1209-1126.

Jones O. T.1985. Chemical mediation of insect behavior. pp. 311-373, in D. H. Hutson and T. R. Roberts (eds.). Progress in Pesticide Biochemistry and Toxicology, Vol. 5, Insecticides. Wiley, New York.

Lohse, G. A. & W. H. Luche, 1994. Die Käfer Mitteleuropas. In: G. Evers (ed), 14. Krefeld. pp. 73.

Loughrin, J. H., D. A. Potter & T. R. Hamilton-Kemp, 1995. Volatile Compounds induced by Herbivory act as Aggregation Kairomones for the Japanese beetle (*Popillia japonica* Newman). Journal of Chemical Ecology 21: 1457-1467.

Mattiacci, L., M. Dicke & M. A. Posthumus, 1994. Induction of parasitoid attracting synomone in brussel sprouts plants by feeding on *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. Journal of Chemical Ecology 20: 2229-2247.

Miller, J. R. & K. L. Strickler, 1984. Finding and Accepting Host Plants. In: W. J. Bell & R. T. Cardé (eds), Chemical Ecology of Insects, Chapman and Hall Ltd., pp. 127-155.

Pare, P. W. & J. H. Tumlinson, 1997. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiology 114: 1161-1167.

Pasteels, J. M., M. Rowell-Rahier, J.-C. Braekman & D. Daloze, 1994. Chemical defense of adult leaf beetles updated. In: P. Joliver, M. Cox & E. Petitpierre (eds), Novel aspects of the biology of chrysomelidae, Kluver, Dordrecht. pp. 298-301.

Peng, C. & M. J. Weiss, 1992. Evidence of an aggregation pheromone in the flea beetle, Phyllotreta cruciferae (Coleoptera: Chrysomelidae). Journal of chemical ecology 18(6): 875-884.

Saxena, K. N. S. Goyal, 1978. Host-plant relations of the citrus butterfly *Papilio demolus* L.: Orientation and ovipositional responses Entomologia experimentalis et applicata 24: 1-10.

Scutareanu, P., B. Drukker, J. Bruin, M. A. Posthumus & M. W. Sabelis, 1997. Volatiles from *Psylla*-infested pear trees and their possible involvement in attracting of anthocorid predators. Journal of Chemical Ecology 23: 2241-2260.

Stevens, L. & D. E. M. Cauley, 1989. Mating prior to overwintering in the important willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). Ecological Entomology 14: 219-223.

Takabayashi, J., M. Dicke & M. A. Posthumus, 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: Variation caused by biotic and abiotic factors. Journal of Chemical Ecology 20: 1329-1354.

Turlings, T. C. J., J. H. Tumlinson & W. J. Lewis, 1990. Exploitation of herbivore-induced plant odours by host-seeking parasitic wasps. Science 250: 1251-1253.

Turlings, T. C. J., M. Bernasconi, R. Bertossa, F. Bigler, G. Caloz & S. Dorn, 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. Biological Control 11: 122-129.

Turlings T. C. J., Alborn, H. T., Loughrin, J. H., Tumlinson, J. H., 2000. Volicitin, an elicitor of maize volatiles in oral secretion of *Spodoptera exigua:* Isolation and bioactivity. Journal of Chemical Ecology 26: 189-202.

Visser, J. H. & D. A. Avé, 1978. General green leaf volatiles in the olfactory orientation of the Colorado Beetle, *Leptinotarsa decemlineata*. Entomologia experimentalis et applicata 24: 538-549.

Visser, J. H. & J. K. Nielson, 1977. Specificity in the olfactory orientation of the CPB. Entomologia Experimentalis et Applicata 21: 14-22.

Visser, J. H., S. V. Straten & H. Maarse, 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. Journal of Chemical Ecology 5: 13-25.

Wan, F.-H. & P. Harris, 1996. Host finding and recognition by *Altica carduorum*, a defoliator of *Cirsium arvense*. Entomologia experimentalis et Applicata 80: 491-496.

Yamasaki, T., M. Sato & H. Sakoguchi, 1997. Germacrene D: masking substane of attractants for the cerambycid beetle, *Monochamus alternatus* (Hope). Applied Entomology and Zoology 32: 423.429.

IV

HOST PLANT FINDING IN THE ALPINE LEAF BEETLE *OREINA CACALIAE*

Key words: Coleoptera, Chrysomelidae, *Oreina cacaliae*, olfaction, behaviour, host plant finding, mark-release recapture.

Abstract

The host-plant finding behaviour in walking *Oreina cacaliae* was studied in the field, to test the importance of olfaction in this process. A total of 200 marked adult beetles were released under different wind directions on a selected site surrounded by their host plant *Adenostyles alliariae*. Locations of the beetles on the host plants were recorded at the day of release and once a week during three successive weeks. Analysis of the data by circular statistics revealed that the movement of the beetle was dependent on the wind direction: under northwind conditions the majority of the beetles was found northeast of the release point, in contrast beetles moved south-east when the wind blew from the south. Because 96% of 200 beetles released in an identical visual surrounding moved to host plants in the upwind direction with the wind blowing from opposite directions, we conclude that host plant finding by *O. cacaliae* relies on olfaction rather than on vision.

Introduction

In the Chrysomelidae, host plant finding is typically performed by adults, commonly by walking towards the host (Hsiao, 1988). The term host-plant finding has been used to describe the phenomenon of insects arriving near or on such a resource (Thorsteinson, 1960; Beck 1965). It involves searching from a distance after having perceived plant characteristics and is affected by the spatial distribution of host plants (Visser, 1988). The distribution of plants that insects use for refugia, mating sites, and food, is usually patchy, dictated by the patchiness of suitable soils and growing conditions. The resources offered by plants vary qualitatively as judged by relative contributions to the fitness of the insect (Miller & Strickler, 1984). *Oreina cacaliae* (Coleoptera: Chrysomelidae) need to locate their host plants either after overwintering in the soil next to their main host *Adenostyles alliariae*, or after flight from distinct overwintering places towards the spring host *Petasites paradoxus*, and after flight from there towards *A. alliariae* later during the season (Chapter 1). In windtunnel experiments walking *O. cacaliae* adults responded to host plant volatiles and showed a clear preference for the host plant when given a choice between a host and a non-host plant (Chapter 1). Attraction to specific host plant volatiles has been reported in a field trapping experiment for flea beetles, *Phyllotreta* spp., which are attracted to isothiocyanates characteristic of the Brassicaceae (Pivnick *et al.*, 1992). Odours seem to be the most important cues to locate plants for many species, but some phytophagous insect species also use visual cues to locate plants, like tephritid fruit flies (Prokopy & Owens, 1978) and cabbage root flies, which even distinguish among host plants by reflectance patterns of leaves (Prokopy *et al.*, 1983). The overwhelming majority of insects and even most of those relying upon visual cues, are assumed to switch on to olfactory guidance in the vicinity of the host (Sabelis & Schippers, 1984). A variety of studies demonstrate olfactory attraction of chrysomelids to plant chemicals in the laboratory (Visser 1976, Loughrin *et al.*, 1995, Bolter *et al.*, 1997). In contras, examples of distant olfactory attraction to host plants in nature is much rarer. Experiments with laboratory olfactometers can demonstrate upwind movements to an odour source, but do not always have relevance to the activity of the same odour in the field (Sabelis & Schippers, 1984). Steep odour gradients may exist in a lab olfactometer, thus permitting chemotactic orientation which operates usually only over very short distances in walking insects, however, their relevance to location of distant host in the field is questionable (Kennedy, 1977). Prokopy (1986) suggested that experiments on the host-finding behaviour of phytophagous insects require a thorough analysis of the movements and behaviour of individual insects under both non-stimulus and resource-stimulus conditions. The essential features of the host-finding process are: moving, contacting cues from the resource, and then responding to the cues by adjusting locomotion to increase proximity to the resource (Miller & Strickler, 1984). To date, only few studies have attempted to examine the host-finding behaviour of herbivorous insects under field conditions (Judd & Borden, 1988). Our study is among the first not to use field traps when looking for cues important in host plant finding. In field experiments we wanted to confirm the importance of olfaction observed in the windtunnel experiments (Chapter 1 and 3). If *O. cacaliae* find its host plants with the use of visual cues, released beetles can be expected to find individual plants independent of the prevailing wind condition.

Material and Methods

Data collection. Adult beetles were collected on *A. alliariae* at a site 0.5 km from the release point. They were marked in the morning of a sunny release day (at 10.00) with correction fluid (tipp-ex) dots on the elytra in a date specific patter, so that the code could be read without disturbing the insect. At 4 different dates (7th, 8th and 10th and 21st of July), 50 beetles (25 of each sex) were released from a glass jar in the middle of a path covered with small stones resulting from flooding during the previous year. The beetles were released at the same point in all experiments so that their visual surrounding was each time the same. All plant patches surrounding the release point were searched for marked beetles the day of release, and once a week during three successive weeks. The position of each recaptured beetle was recorded, the distance to the point of releasc was measured. We divided the search area in 6 sectors, 3 on the left side of the path and 3 on its right side. The 3 sectors on each side were devided in north, east and south. Each recaptured beetle was again marked according to the sector where we found it. This allowed us to check the dispersal within the *A. alliariae* patch during the three weeks following the release.

Wind speed measurement. Wind speed was measured with a portable anemometer (50518, Bioblock Scientific, Frenkendorf, Switzerland) 70 cm above ground and direction was noted with the aid of a compass.

Statistical analysis. Statistical analyses of circular distributions follow Batschelet (1981). The angle of the mean vector (ϕ) (ranging from 0 to 360), which represents the mean direction of movement of all recaptured beetles was calculated. In addition we determined the magnitude of the mean vector R (ranging from 0 to 1) which represents the directidness of the movements of recaptured beetles. R would be close to 0 if dispersal is random and if R is sufficiently large, the null hypothesis of random dispersal can be rejected in favour of a preferred dispersal direction using the Rayleigh test (Batschelet, 1981).

Results

The location of beetles that found a host plant on the day of release, when the wind was blowing from the north is shown in Fig. 1. The wind was always blowing from the north at an angle between 330° and 50°, with a very unsteady speed ranging from windstill up to short gusts at speeds of 2.83 m/s. The result of three releases, each with 50 beetles is shown in Fig. 1. Of the 150 beetles released, 47 found a host plant within 7 hours at the day of release. Of those 45 were localised on a leaf that was upwind from the release point. The fastest walking beetle (a female) reached the leaf of its host plant at a distance of 5.2 m after 33 min.

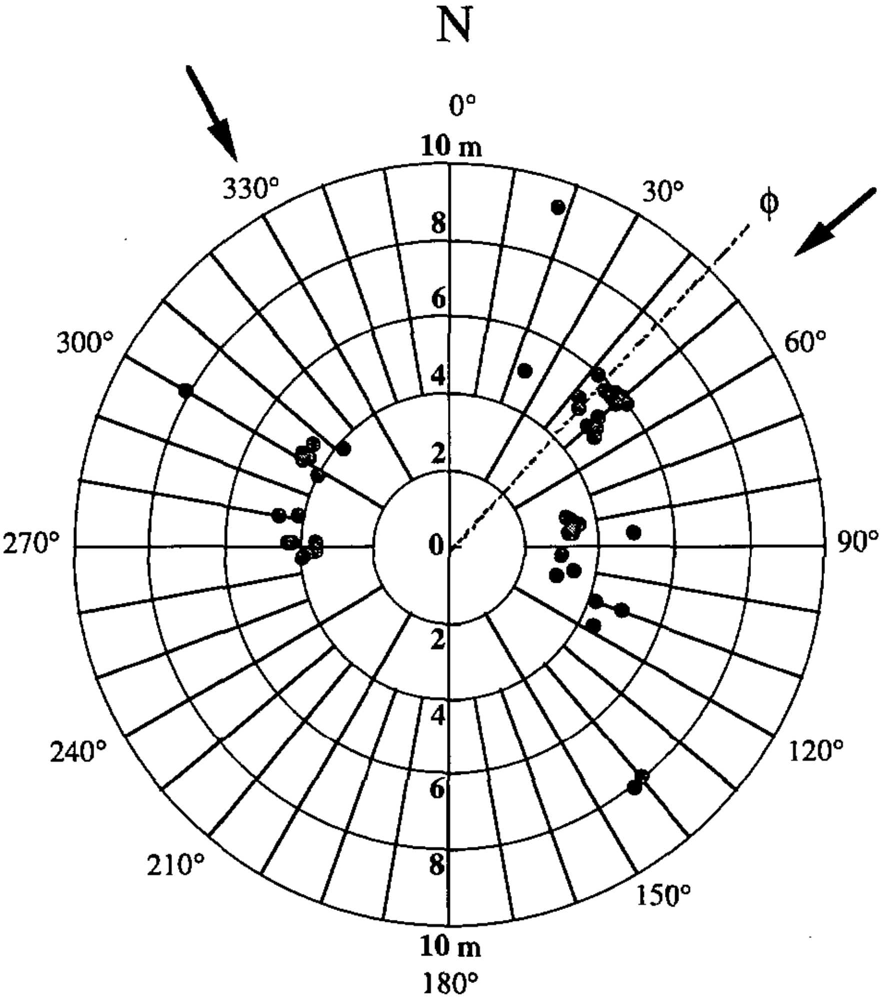

Fig. 1. Beetles recaptured on a host plant (•) at days of release when the wind was blowing from the north. The arrows indicate the extremes of the wind direction which was shifting between 330° and 50°. The mean angle of beetle movement (ϕ) was 43°. Data represent values of three releases at different dates.

Determination of the mean angle beetles moved after release, under north wind conditions resulted in a value of 42.7°, R (a measure of directidness) was 0.38 and $P= 0.002$ (Rayleigh test, Batschelet 1981).

The localisation of beetles released at the same place under south wind conditions is shown in Fig. 2.

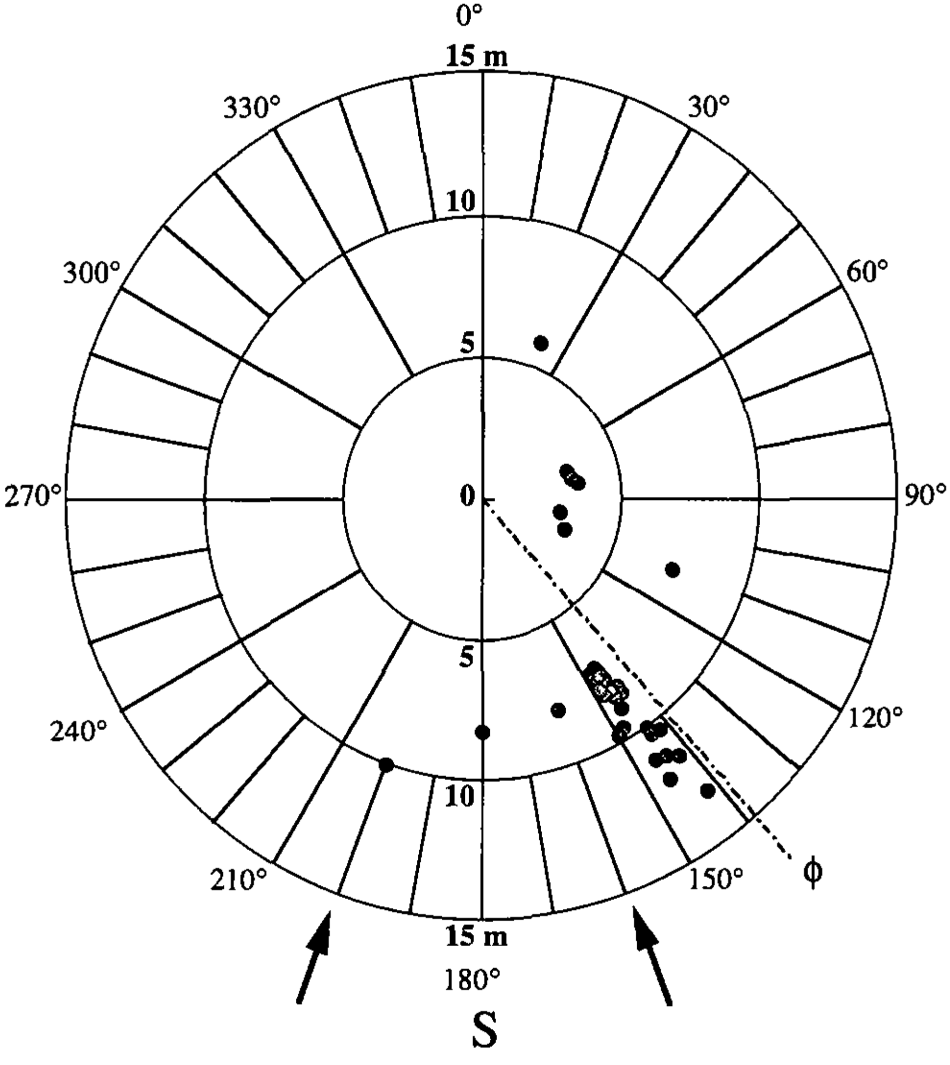

Fig. 2. Beetles recaptured on a host plant (•) at the release day when the wind was blowing from the south. The arrows indicate the extremes of the wind direction which was shifting between 160° and 200°. The mean angle of beetle movement (ϕ) was 139°.

The wind was blowing at an angle between 160° and 200° at a constant speed of 2.08 m/s. This meteorological situation, in which the wind blows from the south, is rare in the Val Ferret. Of 50 beetles marked, 34 were recaptured on host plant leaves located upwind the day of release, whereas only one was recaptured downwind. The mean angle of the beetles' movement was 138,5°; R= 0.87 and $P \leq 0.001$ when the wind was blowing from the south.

Movement between the host plant sectors.

Once a week, during three weeks we searched for beetles that had been remarked after recapture on a host in order to see if they had moved between the host plant sectors. We recaptured 12 of the 47 remarked beetles at north wind conditions, for a second time. Of these, 8 were recaptured in the same sector they had been marked the week before. Another 17 beetles that had not been found on a plant before were recaptured on *A. alliariae*. Eighteen beetles that had not been captured before, were recaptured the second week after release, and an additional 8 after three weeks. Of a total of 150 beetles marked and released with the wind blowing from the north, 78 were found within three weeks after release. (43 males and 30 females, the sex of the missing beetles had not been determined).

From the 35 beetles that were recaptured on a host plant the day of release when the wind was blowing from the south, 24 were recaptured on a host plant one week later. Just one of those had moved to another sector during the week. Eight new beetles that had not reached a plant the day of release were recaptured one week later, all of them east to the release point. Of a total of 50 beetles released when the wind was blowing from the south, 43 beetles (19 males and 23 females) were recaptured on *A. alliariae* either the day of release or one week later.

Discussion

The influence of prevailing wind directions on the host plant finding behaviour of *O. cacaliae.* was tested in the field. Marked beetles were released in the middle of a path, which was surrounded by host plants at a minimal distance of at least 3.2 m. Male and female beetles dispersed upwind from the point of release with a mean movement vector which were directional (Rayleigh test, $P<0.002$) when the wind was blowing from the north as well as when it blew from the south ($P<0.001$). Most of the beetles that had been recaptured were still in the same sector where they were marked but some readily moved towards another plant, even in another sector, within a week after being remarked. This finding confirms the results of Knoll (1997) who described the beetle movement within a host-plant patch. Compared with our mark-recapture studies during spring, where we found only 2.5 % of our 610 marked beetles several hundred meters away from the points of marking (Chapter 1), the 60.5% marked beetles recaptured on a host plant within several meters indicated that those beetles had already finished their dispersal period, usually occurring in autumn and spring. No take-off behaviour was observed during the host-plant finding experiments. The beetles find their host-plants while walking, not during flight because they appear not to be able to direct their flight towards a host plant (Chapter 1). Continuous field observations for several hours showed that the beetles moved in a straight line towards their host plants, while correcting their walking direction only slightly once they had started walking. Kennedy (1977) stated that the most important change in behaviour that signals an insect's orientation towards a distant odour source, is a change from non-directed movement to upwind movement. In our experiment beetles seemed to move only after they had perceived an odour.

Host-plant finding in the field must be mediated by vision, olfaction or both (May & Ahmad, 1983). Because of the limitations of an insect's visual system, long-range host finding behaviour is likely to be primarily dependent on olfactory information (Kennedy, 1977). Olfactory orientation may occur through chemotaxis, which depends on a steep odour gradient occurring only at short distances (within centimetres) from an odour source (May & Ahmad, 1983). For longer distances, insects are thought to orient by means of odour-conditioned positive anemotaxis (Kcnncdy, 1977). This anemotaxis comprises mechanical stimulation by wind, which primes the insect, when it then perceives an attractive odour, it turns upwind (Visser, 1986). An odour plume released from a host-plant is not continuous, but intermittent, and at a fixed point downwind of the odour source the plume will be perceived as a series of bursts of odour, which are variable in strength and duration, separated by odourless air pockets (Murlis & Jones, 1981). Walking towards an odour in wind is a relatively simple task for an insect because perceiving the wind direction only requires mechanoreceptive detection (Murlis et al., 1992).

Several authors have reported odour conditioned as a means by which beetles locate food plants. In complete darkness and without wind, *Geotrupes silvaticus* beetles move in circles, but as soon as the beetles perceive wind, they start moving upwind, in a straight line (Linsenmair, 1973). In Tenebrionidae a windspeed of 0.3 to 0.5 m/sec is required for them to orient upwind (Linsenmair, 1973). Of eight *Trirhabda canadensis* beetles released downwind of their host plant, seven moved upwind and six of these found the host (Puttick et al., 1988). The same beetles chose between monoculture and polyculture host plant plots on days with low windspeed, when they probably received olfactory information from a wide arc of vegetation, and colonised only upwind plots on windy days when information was likely to be available only from upwind plots (Morrow, 1989). In *O. cacaliae,* windspeed in addition to wind direction might have influenced the host-finding behaviour. More beetles found a host-plant during the day of release at a constant windspeed (the situation when the wind was blowing from the south) than when the wind was blowing unsteadily with strong gusts (when the wind was blowing from the north). The straightness of an airflow, as measured by the displacement of puffs of smoke, increases as the wind speed increases (Brady *et al.*, 1989).

The distribution of *O. cacaliae* beetles that had found their host plants was clearly not random as confirmed by a Rayleigh test. Considering that the visual surrounding was the same on the different release dates and that the beetles moved upwind towards host plants, we conclude that *O. cacaliae* relies rather on olfactory than on visual cues to locate their host plants.

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References

Batschelet, E. 1981. Circular statistics in Biology. Academic Press, New York.

Beck, S. D. 1965. Resistance of plants to insects. Annual Review of Entomology 10: 207-232.

Bolter, C. J., M. Dicke, J. J. A. v. Loon, J. H. Visser & M. A. Posthumus, 1997. Attraction of CPB to herbivore-damaged plants during herbivory and after its termination. Journal of Chemical Ecology 23: 1003-1023.

Brady, J. Gibson, G & M. J. Packer. (1989). Odour movement, wind directions, and the problem of host-finding by tsetse flies. Physiological Entomology, 14: 369-380.

Hsiao, T. H. 1988. Host specificity of certain chrysomelids for Solanaceae. In: D'Arcy, W. G. (ed.); Solanaceae: Biology and Systematics. Columbia University Press, New York. pp. 345-377.

Judd, G. J. R. & J. H. Borden, 1988. Long-range host-finding behaviour of the onion fly Delia antiqua (Diptera: Anthomyiidae): ecological and physiological constraints. Journal of Applied Ecology 25: 829-845.

Kennedy, J. S., 1977. Olfactory responses to distant plants and other odour sources. In: H. H. S. a. J. J. M. Jr (ed), Chemical Control of Insect Behaviour, Wiley-Interscience, New York. pp. 67-91.

Knoll, S. 1997. Spatial population structure of an alpine leaf beetle. Ph. D. thesis, University of Neuchâtel.

Linsenmair, K. E., 1973. Die Windorientierung laufender Insekten. Fortschr. Zool 21: 59-79.

Loughrin, J. H., D. A. Potter & T. R. Hamilton-Kemp, 1995. Volatile Compounds induced by Herbivory act as Aggregation Kairomones for the Japanese beetle (Popillia japonica Newman). Journal of chemical ecology 21: 1457-1467.

May, M. L. & S. Ahmad, 1983. Host location in the Colorado Potato Beetle: Searching Mechanisms in Relation to Oligophagy. In: S. Ahmad (ed), Herbivorous Insects: Host-seeking behaviour and mechanisms, Academic Press, New York. pp. 173-200.

Miller, J. R. & K. L. Strickler, 1984. Finding and Accepting Host Plants. In: W. J. Bell & R. T. Cardé (eds), Chemical Ecology of Insects, Chapman and Hall Ltd.,

Morrow, P. A., D. W. Tonkyn & R. J. Goldburg, 1989. Patch colonisation by Trirhabda canadensis (Coleoptera: Chrysomelidae): effects of plant species composition and wind. Oecologia 81: 43-50.

Murlis, J., J. Elkinton & R. T. Cardé, 1992. Odour plumes and how insects use them. Annual Review of Entomology 37: 505-532.

Murlis, J. & C. D. Jones, 1981. Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources. Physiological Entomology 6: 71-86.

Prokopy, R. J., 1986. Visual and olfactory stimulus interactions in resource finding by insects. In: T. L. Payne, M. C. Birch & C. E. J. Kennedy (eds), Mechanisms in Insect Olfaction, Clarendon Press, Oxford. pp. 81-89.

Prokopy R. J. & E. D. Owens, 1978. Visual generalist-visual specialist phytophagous insects: host selection behaviour and application to management. Entomologia experimentalis et applicata 24: 409-420.

Prokopy R. J., R. H. Collier & S. Finch, 1983. Leaf color used by cabbage root flies to distinguish among host plants. Science 221: 190-192.

Puttick, G. M., P. A. Morrow & P. W. Lequesne, 1988. Trirhabda canadensis (Coleoptera: Chrysomelidae) responses to plant odours. Journal of Chemical Ecology 14(8): 1671-1686.

Sabelis, M. W. & P. Schippers, 1984. Variable wind directions and anemotactic strategies of searching for an odour plume. Oecologia 63: 225-228.

Thorsteinson. A. J. 1960. Host selection in phytophagous insects. Annual Review of Entomology 5: 193-218.

Visser, J. H., 1976. The design of a low-speed wind tunnel as an instrument for the study of olfactory orientation in the CPB. Entomologia Experimentalis et Applicata 20: 275-88.

Visser, J. H., 1986.Host Odour Perception in Phytophagous Insects. Annual Review of Entomology 31: 121-144.

Visser, J. H. 1988 Host-plant finding by insects: orientation, sensory input and search patterns. Journal of Insect Physiology 34: 259-268.

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Summer 1988	Language course in Oxford (U.K.) (4 months)
Autumn1988-autumn 1990	Studies in Biochemistry, (4 Semesters) University of Basel
Autumn1990-spring1996	Studies in Biology University of Basel Main branch: Phytopathology (U. Gisi) Secondary branches: Neurobiology (H. Reichert) Ecology (C. Körner) Biology of Herbivores (M. Rahier)
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ATTENDED CONFERENCES

Oral presentation at the Second Congress of Alpine Ecology and Biogeography, La Thuile (Italy), summer 1997: An alpine leaf beetle *(Oreina cacaliae, Chrysomelidae)* attracted by the smell of its host plant(s).

Poster presentation at the International Symposium of Insect-Plant Interaction (SIP 10) in Oxford, summer 1998: The effect of plant volatiles in host discrimination by an alpine leaf beetle, *Oreina cacaliae* (Coleoptera: Chrysomelidae).

Poster presentation at the Satellite symposium of Zoologia et Botanica in Zürich spring 1999: The cost of dispersal by flight in an alpine leaf beetle.

ATTENDED COURSES

Course in handling the geographical information system (GIS) entitled: Introduction aux bases de données spatiales et systémes d'information géographique. Université de Neuchâtel, Dr. Mahmoud Bouzelboudjen, February, 1998.

Course in Statistics entitled: Elements of Statistics for research. Organised by Formation continue Université de Neuchâtel, Prof. Yadolah Dodge, October and November 1999.

ATTENDED 3IEMES CYLCLES ROMANDS

Herbivory and plant secondary metabolites, ecological and evolutionary issues. Organisation: Prof. M. Rahier and Dr. F. Saucy. Château d'Oex, March 1995.

Influence of insects and pathogens on plant competition. Organisation Dr. J. Frantzen and Prof. H. Müller, Fribourg. Délémont, February 1998.

The coevolutionary process. Organisation: Dr Betty Benrey, Prof. B. Betschart and Prof. M. Rahier, Neuchâtel. Le Peupéquignot, September 1998.

INVITED SPEAKER FOR SEMINARS

ARLDN Seminar in Tucson invited by Prof. J. Hildebrand, November 1998: Host-plant odour recognition in an alpine leaf beetle.

Ecology seminar at the University in Zürich invited by Dr. B. Hellriegel, June 1999: The cost of dispersal by flight in an alpine leaf beetle.

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Outlook

Based on the presented results new questions arose. The factors that drive a part of the *O. cacaliae* population to leave their host plant patches in autumn and to fly to distinct overwintering sites away from that host are not known. It is possible that the deteriorating quality of the *A. alliariae* patches or the age of the beetles are important in that decision.

We showed that plant odours play an important role in the host-plant recognition process of *O. cacaliae*. Even if some known volatiles could be identified, it is not known which compounds are detected by the insects receptor system in the antennae. To identify the substances that elicit the behavioural response in *O. cacaliae* electrophysiological studies are needed.

Our windtunnel experiments could not show intraspecific communication via pheromones in *O. cacaliae*. Nevertheless the existence of pheromones can not be completely excluded. The experiments should be repeated with virgin females from a newly emerging generation, either coming from a lab culture or being transported singly from the field, which would prevent the beetles from releasing their defensive secretion before the experiment.