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ORIGINAL ARTICLE

Do aphids actively search for ant partners?

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Abstract The aphid–ant mutualistic relationships are not necessarily obligate for neither partners but evidence is that such interactions provide them strong advantages in terms of global fitness. While it is largely assumed that ants actively search for their mutualistic partners namely using volatile cues; whether winged aphids (i.e., aphids' most mobile form) are able to select ant-frequented areas had not been investigated so far. Ant-frequented sites would indeed offer several advantages for these aphids including a lower predation pressure through ant presence and enhanced chances of establishing mutuaslistic interactions with neighbor ant colonies. In the field, aphid colonies are often observed in higher densities around ant nests, which is probably linked to a better survival ensured by ants' services. Nevertheless, this could also result from a preferential establishment of winged aphids in ant-frequented areas. We tested this last hypothesis through different ethological assays and show that the facultative myrmecophilous black bean aphid, *Aphis fabae* L., does not orientate its search for a host plant preferentially toward ant-frequented plants. However, our results suggest that ants reduce the number of winged aphids leaving the newly colonized plant. Thus, ants involved in facultative myrmecophilous interactions with aphids appear to contribute to structure aphid populations in the field by ensuring a better establishment and survival of newly established colonies rather than by inducing a deliberate plant selection by aphid partners based on the proximity of ant colonies.

Key words ant; aphid; *Aphis fabae*; *Lasius niger*; mutualism; partner research

Introduction

The ant–aphid mutualism is one of the most studied animal relationships (Verheggen *et al.*, 2012). While aphids provide ants with a stable and reliable food source (i.e., sugar-rich honeydew), ants improve the overall hygiene of the tended aphid colony and protect it against various natural enemies (Way, 1963; Stadler & Dixon, 2005). This relationship, which can be obligate or facultative depending on the aphid species considered, provides both partners with strong advantages in terms of global fitness. Therefore, ants and aphids would benefit from behavior favoring the establishment of a mutualism (Verheggen *et al.*, 2009b). Mechanisms enhancing encounters between ants and aphids are thus key elements in the success of such partnerships.

Ants are known to actively search for aphid partners namely by using volatiles cues like the aphid alarm pheromone (E-β-farnesene, constantly released at low levels by aphid colonies) to distantly orientate their search (Verheggen *et al.*, 2012). Once a potential aphid partner is detected, its suitability for a partnership is then assessed based on multiple criteria including aphid behavior, aphid morphological adaptation for ant-tending and overall honeydew quality and quantity (Way, 1963; Fischer *et al.*,

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2001; Stadler & Dixon, 2005; Detrain *et al.*, 2010; Lang & Menzel, 2011).

Small-sized aphid colonies such as newly established ones, experience proportionally the highest level of ant tending (Breton & Addicott, 1992). While presenting higher costs in terms of honeydew production for the aphids, this also increases the protection offered by ants, which would become particularly profitable for aphids under high predation pressure (Breton & Addicott, 1992; Yao *et al.*, 2000; Yoo & Holway, 2011). When the aphid colony becomes too large, ants invest less in tending behavior whereas they start preying on aphids, thereby reducing aphid benefits per capita (Breton & Addicott, 1992; Sakata, 1995; Sakata, 1999).

Ants are also known to structure aphid colonies in the field so that myrmecophilous aphid species are frequently observed at higher densities in the surroundings of ant colonies (Seibert, 1992; Hopkins & Thacker, 1999; Bishop & Bristow, 2003; Stewart-Jones *et al.*, 2008; Minarro *et al.*, 2010). Since ants provide myrmecophilous aphids with protected areas where both enemies and interspecific competition pressure are reduced (nonmutualistic species being considered as preys) (Fischer *et al.*, 2001; Bishop & Bristow, 2003; Stewart-Jones *et al.*, 2008; Minarro *et al.*, 2010); one can assume that winged aphids, the dispersal morph of most aphids, would find benefits in selecting host plants in such ant-frequented areas. However, whether such an active search for ants' proximity occurs in aphids has not been considered so far.

In this paper, we investigate the impact of ant occurrence on the host plant selection by winged aphids as well as on the behavior of aphids newly arrived on a host plant.

Materials and methods

Study model

We selected the facultative myrmecophilous black bean aphid, *Aphis fabae* (Scopoli) and the black garden ant *Lasius niger* L. as biological models, these insects being common mutualistic partners. For our bioassays, both colonies of ants and aphids were collected in the surroundings of Gembloux (Belgium).

The 2 species were reared in a climate-controlled room (16 h light : 8 h dark photoperiod; 20 ± 2 °C). Aphid colonies were fed on broad beans (*Vicia faba* L.) cultivated in a mix of perlite and vermiculite (1/1) for several generations. Ant nests collected in the field were placed in plastic containers coated with polytetrafluoroethylene (Fluon®,Whitford, UK) to avoid escape. Six nests were used during the assays. Laboratory rearing nests consisted in test tubes covered with a red transparent foil and hosted a queen, brood and a minimum of 500 individuals. Dead insects were given weekly (flies, mealworms) in quantities consumed within 2 d. Both water and aqueous brown sugar solution (342 g/L) were provided *ad libitum* using dispenser made with a glass tube filled with water or sugar solution and closed with a cotton wool plug.

Ethological assays

Since winged individuals are the dispersal form of aphids and the most prone to establish new colonies away from the initial one, only this morph has been studied in the following bioassays. Two sets of assays have been developed: Y olfactometry and plant choice in wind tunnel.

Two-ways olfactometry

The attractiveness of ant-visited plants for winged *A. fabae* has been assessed using a Y shaped glass olfactometer (internal diameter: 0.5 cm; 7 cm long branches). All assays have been conducted at 22 ± 2 °C in a dark-walled chamber presenting no visual cues that might influence aphid choices. Samples and controls were placed in 4 L glass jars. Air purified on charcoal filters was pushed into those jars at 300 mL/min and then in the olfactometer's branches. The sample consisted in a pot of 9 *V. faba* plants and 1 nest tube containing 100 ants placed with the plants for 2 d. The control contained only "blank" plants, that is, without ants. Sixty winged aphids were collected on heavily infested plants using a soft brush and placed one by one in the olfactometer. Each test ended when aphids, tested individualy, reached the end of a branch. For each aphid, the chosen branch and the time needed to complete the assay were recorded.

Wind tunnel

A 2nd choice test has been performed in a wind tunnel made of Plexiglas (2.4 × 0.8 × 0.6 m). At one side of the wind tunnel, 2 plastic containers (0.56 × 0.36 × 0.11 m) wall-coated with Fluon® (Whitford Plastics Ltd., UK) were placed side-by-side (Fig. 1). A pot of 9 *V. faba* was placed at the center of each plate and 1 ant colony was added in one of the plates 2 d prior to the beginning of the assay. The Fluon coating prevented ant escape and ensured that aphids could reach the plants only by flight. At the other side of the tunnel (at 180 cm from the aforesaid plants), 250 winged aphids were introduced on a plant heavily infested by apterous aphids in order to favor their

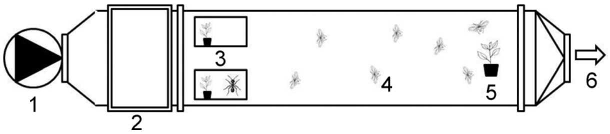

Fig. 1 Wind tunnel used to investigate *Lasius niger* impact on *Aphis fabae* alate choice of host plant. Experimental setup of wind tunnel used to investigate the impact of ant presence on the choice of host plant by aphids. (1) fan; (2) filters; (3) plastic plates containing *V. faba* frequented by *L. niger* and control *V. faba*; (4) winged *A. fabae*; (5) winged *A. fabae* release point (highly infested plant); (6) wind direction.

dispersal flight. The experimental conditions were as follows: velocity of 0.4 m/sec (laminar flow); T = 20 ± 2 °C; RH = 60%–70%; illumination = 2.300 lux. After 24 h, newly arrived aphids were counted on each healthy plant. Preliminary experiments have shown that this delay period was a good compromise to optimize the number of aphids reaching the plant and to limit its colonization by the offspring of newly arrived aphids. This assay (aphids counting) has been run in 2 replicates.

Retention effect of ants

The effect of ant presence on the retention of newly arrived winged *A. fabae* on a plant was investigated in a 3rd laboratory assay. Pots of 9 *V. faba* were infested by groups of 5 winged aphids. These infested plants were placed either in presence of an ant colony, so ants could freely access the plants, or absence of ants. The aphids remaining on the plant were counted after 1, 2, 3, 4, and 6 h. The tests were run at 22 ± 2 °C in 3.2 × 3 m greenhouse chambers. Four pots were placed per chambers at the corner of a 1.5 × 1.5 m square at the center of the room. The 2 conditions were tested in separate chambers. Twelve pots were analyzed (aphids counting after 1, 2, 3, 4, and 6 h) for each experimental condition.

Statistical analyses

We performed a binomial test to detect significant differences in proportions for the Y-olfactometer assay. The average linear speeds recorded for the 2 branches of the Y-olfactometer were compared using Student's *t*-tests (normality and homoscedasticity assumptions met, tested with Ryan–Joiner and *F*-test, respectively). For the aphid retention assay, the significance of the effect of time and ant presence was assessed using a repeated measures analysis of variance (ANOVA) with time (1, 2, 3, 4, and 6 h) as within-subject factor, and treatment (ant presence vs. ant absence) as between-subject factor. Variables were assessed for distributional properties and degrees of freedom, and were adjusted with Greenhouse–Geisser correction when sphericity assumption was violated (i.e., Mauchly's test with P value less than 0.05). Repeated measures ANOVA was performed with R 3.0.2 (R Core Team, 2013); other analyses were computed with Minitab 15.1 (State College, Pennsylvania, USA). Significance was set at $P < 0.05$.

Results

Two-ways olfactometry

Among 60 aphids tested in 2-ways olfactometer, 29 individuals chose the ant-visited plant against 31 for the blank plant. Every tested aphid completed the assay and no choice changes were observed during the assays. The attractiveness of the sample and the control are not significantly different (binomial tests, $P = 0.483$). Average times spent in the olfactometer while going on the ant-visited plant branch or the control branch are 387 ± 212 and 340 ± 220 sec, respectively and do not significantly differ (*t*-test, $P = 0.403$).

Wind tunnel

In a wind tunnel, when facing the choice between ant-frequented plants and control plants, winged *A. fabae* did not seem to mark any preference. Among the 500 winged aphids released in the wind tunnel, one fifth flew to 1 of the 2 tested plants placed on the other side of the wind tunnel, with 49 and 51 aphids landing on the ant-frequented plants and the control plants, respectively.

Retention effect of ants

As expected, there is a main effect of ant presence as it results in a greater aphids retention (repeated measures ANOVA, $P = 0.037$), with a nonsignificant time × treatment interaction (repeated measures ANOVA with Greenhouse–Geisser correction, $P = 0.583$) (Fig. 2). The time elapsed since aphids release does not significantly influence the number of aphids remaining on plants (repeated measures ANOVA with Greenhouse–Geisser correction, $P = 0.537$) (Fig. 2). The greatest number of aphids leaves the plant within the 1st hour following their release. Then, the number of aphids remaining on plants remains quite stable and higher on ant-frequented plants

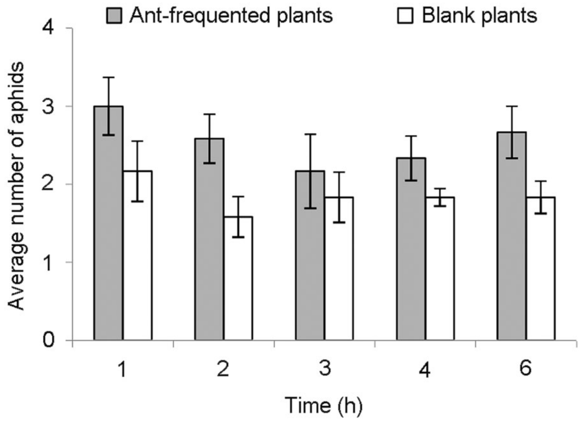

Fig. 2 Average number of aphids staying on plants depending on time and ant presence. Average numbers of aphids left on a plant at different times after the release of 5 winged aphids on the plant (mean ± SEM). $N = 12$.

(Fig. 2). Ants thus seem to limit aphid dispersal far from the infested plant and to stabilize their partner in their initial location.

Discussion

In the field, higher densities of myrmecophilous aphids colonies are observed in the surroundings of ant nests (Seibert, 1992; Hopkins & Thacker, 1999). Ants' presence enhance aphid survival, especially under a strong predation pressure, offering them diverse services including cleaning and protection, which can explain these observations. Whether this phenomenon is reinforced by the preferential establishment of winged aphids in ant-frequented areas had not yet been studied. The selection of such favorable sites, by an active orientation toward the area or by an enhanced retention on such ant-frequented plants, could indeed constitute an advantage for winged aphids, the dispersal morph of aphids, and for their offspring.

Our results show no significant impact of ant presence on the distant orientation of winged *A. fabae* toward host plants. Indeed, both 2-ways olfactometry and wind tunnel assays showed no choice preference for an ant-frequented plant. Moreover, the attraction level of aphids expressed by their average linear speed in olfactometer was similar in the 2 branches of the system. In another assay in greenhouse under seminatural conditions undertaken to support the results, only 1% of the 1000 winged aphids (500 aphids, replicated twice) released in the center of the room reached the plants placed either side of the chamber (C.Y. Fischer, unpublished data). In contrast with previous studies (Pickett *et al.*, 1992), this suggests that *A. fabae* attraction toward host plants is not very efficient in case of isolated plants under these conditions. For other aphid species, like *Cryptomyzus korschelti* (Börner), host plant volatiles induce a positive upwind anemotaxis (Pickett *et al.*, 1992). Thus, the absence of wind in the greenhouse assay might partially explain the low number of *A. fabae* aphids reaching plants. Nevertheless, in the wind tunnel assay, the proportion of aphids reaching their host plant was also relatively low, most of them making short erratic flights around their release point and struggling against the wind. These two assays confirm bad flight abilities of the black bean aphid (Dixon & Howard, 1986; Goldansaz & McNeil, 2006). Being bad flyers, winged aphids would then benefit from clinging to a favorable site when they reach it.

Ant presence can have an impact on aphid distribution in the field by providing them with enemy- and competition-reduced spaces (Fischer *et al.*, 2001; Bishop & Bristow, 2003; Stewart-Jones *et al.*, 2008; Minarro *et al.*, 2010). The extent of that protection depends on the ant species, its aggressiveness ant territoriality, its affinity for the aphid partner, the intensity of the tending and the enemy considered (El-Ziady & Kennedy, 1956; Way, 1963; Stadler & Dixon, 2005; Guénard, 2007; Verheggen *et al.*, 2009a; Novgorodova & Gavrilyuk, 2012). For example, *Harmonia axyridis* (Pallas) adults and larvae are quickly attacked and removed from the plant (Herbert & Horn, 2008), while other enemies, like the parasitoid *Lysiphlebus cardui* (Marshall) or the cecidomyid predator *Aphidoletes aphidimyza* (Rondani), are able to prey freely on aphids without being disturbed by ants (Liepert & Dettner, 1996; Guénard, 2007). Moreover, ants play a role in structuring aphid populations, favoring their preferred species and preying on the others (Fischer *et al.*, 2001; Bishop & Bristow, 2003; Stewart-Jones *et al.*, 2008; Minarro *et al.*, 2010). They are also known to limit aphid dispersal in well-established tended colonies by reducing winged aphids production and affecting their flight abilities (Kleinjan & Mittler, 1975; Oliver *et al.*, 2007; Yao, 2012). Our results bring one more possible structuring effect of ants on aphid population. Indeed, while winged *A. fabae* are not attracted toward ant-frequented areas, they tend to stay more on ant-frequented plants, and are thus more likely to start new colonies on these plants. Once detected by ants, newly arrived aphids and their 1st born are thus likely to experience high levels of attendance and the related hygienic and protection benefits. However, *A. fabae* is a facultative myrmecophile and is thus able to survive without ant tending. It might be interesting to investigate whether obligate myrmecophilous species show the same behavior.

Within ant–aphid mutualism, ant seems thus to be the only 1 actively searching for partnership. Our results show no attraction of winged *A. fabae* by ant presence, while ants are known to orientate their scouting based on aphid-linked volatile organic compounds (Verheggen *et al.*, 2012). Nevertheless, winged aphids stay significantly more on ant-frequented plants. Thus, the higher prevalence of aphid colonies in the surroundings of ant nests observed in the field, primarily linked to a better aphid survival through protection and reduction of competition, does not seem related to a deliberated flight orientation of winged aphids toward the ant partner, but could be influenced by a better retention of winged aphids on ant-frequented plants.

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Disclosure

The authors declare that there is no conflict of interests.

References

Bishop, D.B. and Bristow, C.M. (2003) Effects of the presence of the Allegheny mound ant (Hymenoptera: Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. *Annals of the Entomological Society of America*, 96, 202–210.

Breton, L.M. and Addicott, J.F. (1992) Density-dependent mutualism in an aphid-ant interaction. *Ecology*, 73, 2175–2180.

Detrain, C., Verheggen, F.J., Diez, L., Wathelet, B. and Haubruge, E. (2010) Aphid–ant mutualism: how honeydew sugars influence the behaviour of ant scouts. *Physiological Entomology*, 35, 168–174.

Dixon, A.F.G. and Howard, M.T. (1986) Dispersal in aphids, a problem in resource allocation. *Insect Flight* (ed. W. Danthanarayanas), pp. 145–151. Springer, Berlin Heidelberg.

El-Ziady, S. and Kennedy, J.S. (1956) Beneficial effects of the common garden ant, *Lasius niger* L., on the black bean aphid, *Aphis fabae* Scopoli. *Proceedings of the Royal Entomological Society of London*, 31, 61–65.

Fischer, M.K., Hoffmann, K.H. and Völkl, W. (2001) Competition for mutualists in an ant–homopteran interaction mediated by hierarchies of ant attendance. *Oikos*, 92, 531–541.

Goldansaz, S.H. and Mcneil, J.N. (2006) Effect of wind speed on the pheromone-mediated behavior of sexual morphs of the potato aphid, *Macrosiphum euphorbiae* (Thomas) under laboratory and field conditions. *Journal of Chemical Ecology*, 32, 1719–1729.

Guénard, B. (2007) *Mutualisme Fourmis Pucerons et Guilde Aphidiphage Associée: le Cas de la Prédation Furtive*. p. 121. Université du Québec à Montréal, Montréal.

Herbert, J.J. and Horn, D.J. (2008) Effect of ant attendance by *Monomorium minimum* (Buckley) (Hymenoptera: Formicidae) on predation and parasitism of the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). *Environmental Entomology*, 37, 1258–1263.

Hopkins, G.W. and Thacker, J.I. (1999) Ants and habitat specificity in aphids. *Journal of Insect Conservation*, 3, 25–31.

Kleinjan, J.E. and Mittler, T.E. (1975) A chemical influence of ants on wing development in aphids. *Entomologia Experimentalis et Applicata*, 18, 384–388.

Lang, C. and Menzel, F. (2011) *Lasius niger* ants discriminate aphids based on their cuticular hydrocarbons. *Animal Behaviour*, 82, 1245–1254.

Liepert, C. and Dettner, K. (1996) Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. *Journal of Chemical Ecology*, 22, 695–707.

Minarro, M., Fernandez-Mata, G. and Medina, P. (2010) Role of ants in structuring the aphid community on apple. *Ecological Entomology*, 35, 206–215.

Novgorodova, T.A. and Gavrilyuk, A.V. (2012) The degree of protection different ants (Hymenoptera: Formicidae) provide aphids (Hemiptera: Aphididae) against aphidophages. *European Journal of Entomology*, 109, 187–196.

Oliver, T.H., Mashanova, A., Leather, S.R., Cook, J.M. and Jansen, V.A. (2007) Ant semiochemicals limit apterous aphid dispersal. *Proceedings of the Royal Society–Biological Sciences*, 274, 3127–3131.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M. and Hardie, J. (1992) The chemical ecology of aphids. *Annual Review of Entomology*, 37, 67–90.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Sakata, H. (1995) Density-dependent predation of the ant *Lasius niger* (Hymenoptera: Formicidae) on two attended aphids *Lachnus tropicalis* and *Myzocallis kuricola* (Homoptera: Aphididae). *Researches on Population Ecology*, 37, 159–164.

Sakata, H. (1999) Indirect interactions between two aphid species in relation to ant attendance. *Ecological Research*, 14, 329–340.

Seibert, T.F. (1992) Mutualistic interactions of the aphid *Lachnus allegheniensis* (Homoptera: Aphididae) and its tending ant *Formica obscuripes* (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, 85, 173–178.

Stadler, B. and Dixon, A.F.G. (2005) Ecology and evolution of aphid–ant interactions. *Annual Review of Ecology Evolution and Systematics*, 36, 345–372.

Stewart-Jones, A., Pope, T.W., Fitzgerald, J.D. and Poppy, G.M. (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. *Agricultural and Forest Entomology*, 10, 37–43.

Verheggen, F., Diez, L., Detrain, C. and Haubruge, E. (2009a) Aphid–ant mutualism: an outdoor study of the benefits for *Aphis fabae*. *Biotechnologie, Agronomie, Societe et Environnement*, 13, 235–242.

Verheggen, F., Diez, L., Detrain, C. and Haubruge, É. (2009b) Mutualisme pucerons–fourmis: étude des bénéfices retirés par les colonies d'*Aphis fabae* en milieu extérieur. *Biotechnology, Agronomy, Society and Environment*, 13, 235–242.

Verheggen, F.J., Diez, L., Sablon, L., Fischer, C., Bartram, S., Haubruge, E. and Detrain, C. (2012) Aphid alarm pheromone as a cue for ants to locate aphid partners. *PLoS ONE*, 7, e41841.

Way, M.J. (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology*, 8, 307–344.

Yao, I. (2012) Ant attendance reduces flight muscle and wing size in the aphid *Tuberculatus quercicola*. *Biology Letters*, 8, 624–627.

Yao, I., Shibao, H. and Akimato, S. (2000) Costs and benefits of ant attendance to the drepanosiphid aphid *Tuberculatus quercicola*. *Oikos*, 89, 3–10.

Yoo, H.J.S. and Holway, D.A. (2011) Context-dependence in an ant–aphid mutualism: direct effects of tending intensity on aphid performance. *Ecological Entomology*, 36, 450–458.

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