Dispersion of flightless adults of the Asian lady beetle, *Harmonia axyridis*, in greenhouses containing cucumbers infested with the aphid *Aphis gossypii*: effect of the presence of conspecific larvae

Lionel Gil¹, André Ferran²*, Jacques Gambier², Sandrine Pichat², Roger Boll² & Michelle Salles²

¹Institut du-Non-Linéaire de Nice, 1361 route des Lucioles, 06560 Valbonne, France; ²INRA, Equipe d’entomologie et de lutte biologique, 37 boulevard du Cap, 06600 Antibes, France

Accepted: 24 March 2004

**Key words:** *Harmonia axyridis*, Coleoptera, Coccinellidae, *Aphis gossypii*, Homoptera, Aphididae, cucumber greenhouse, adult dispersal, oviposition deterring larvae, biological control

**Abstract**
Most females of the Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), stop laying eggs if conspecific larvae are present. We studied the effect of this inhibition on the dispersion of this insect in a greenhouse containing cucumbers uniformly infested with the aphid *Aphis gossypii* Glover (Homoptera: Aphididae). In the absence of conspecific larvae, the adults moved around at random, sinuously, and independently. They spent most of their time walking on the ground and only a little time on the aphid-infested plants. When the cucumber plants in one half of the greenhouse had conspecific larvae on them, the whole adult population migrated to the larva-free half of the greenhouse. Consequently, most eggs were laid in that part of the greenhouse which was devoid of larvae. The consequences of this spatio-temporal interaction between larvae and adults for the biological control of aphids is discussed.

**Introduction**
Females of aphid-eating ladybird beetles are highly mobile (Ferran et al., 1991). This mobility appears to compensate for the limited visual and olfactory abilities of these insects when searching for prey and oviposition sites (Nakamuta, 1984; Obata, 1986; Jourdan et al., 1995; Lambin et al., 1995). However, an appreciation of geometric forms (Said et al., 1985), the presence of honeydew, aphid exuviae and aphids (Carter & Dixon, 1984), and their negative geotropism and positive phototropism (Dixon, 1959), lead these insects to colonise plants and parts of plants which are likely to be infested with aphids. Females move along the veins and borders of leaves, which brings them close to aphid colonies (Ferran & Deconchat, 1992). Once close enough to detect their potential prey visually and/or by smell, female beetles orient towards and attempt to capture an aphid. The ingestion of prey leads to a change in searching behaviour. The females adopt an intensive search mode, moving slowly and following a sinuous course, which increases the possibility of encountering other individuals in the aphid colony (Ferran & Dixon, 1993). The females resume an extensive search mode, consisting of rapid, rectilinear movements, and leave the aphid colony if they are unsuccessful in capturing further aphids, especially if they are sated with prey (Carter & Dixon, 1984). With a few exceptions (Ferran et al., 1989), female beetles lay eggs close to aphid colonies where the frequency of prey captures per unit time exceeds a certain critical minimum threshold (Dixon, 1997). Although they tend to cluster around dense aphid colonies, the females tend to lay a few eggs close to young aphid colonies (Dixon, 1997). This behaviour maximises the fitness of the species, ensuring that the larvae have sufficient food resources for their growth, and reduces the risk of cannibalism (Hemptinne et al., 1990). Females use the presence of larvae of their own species as an indication of the quality of an aphid colony for the development of their offspring (Hemptinne et al., 1992a). This mechanism enables females to quickly assess the quality of the aphid colonies...
and increases the probability of their discovering suitable sites for oviposition (Dombia et al., 1998). In the presence of larvae, females cease laying eggs and become more active, making it more likely that they will leave the area (Hemptinne et al., 1992a). Although females may detect the larvae by physical contact or visually, the principal signal inhibiting reproduction is a pheromone produced by the larvae (Dombia et al., 1998). This oviposition deterring pheromone (ODP) consists of a mixture of alkanes (Hemptinne et al., 1992b). Inhibitory effects of this type seem to exist in many predatory insects, including Chrysoperla oculata Say (Neuroptera: Chrysopidae; Ruzicka, 1996) and Cryptolaemus montrouzieri Muls (Coleoptera: Coccinellidae; Merlin et al., 1996).

Young third-instar larvae of the flightless strain of the Asian lady beetle, Harmonia axyridis Pallas (Coleoptera: Coccinellidae), are marketed in France by BIOTOP (Tournaire et al., 2000). This beetle is reluctant to lay eggs in the presence of conspecific larvae (Yasuda et al., 2000). This behaviour, and the greater mobility of females in the presence of larvae could limit the efficacy of this biological control agent. In the laboratory, the strength of this deterrent effect probably depends on the age of the larvae. Therefore, two experiments were carried out in cucumber greenhouses uniformly infested with the aphid Aphis gossypii Glover (Homoptera: Aphididae), to estimate the capacity of adults to disperse in the presence or absence of larvae of their own species. The first experiment, without larvae (control), was carried out to check that there were no abiotic factors (light, wind, etc.) affecting the direction of the movement of the adult beetles. The second experiment, with larvae, aimed to quantify the effect of the presence of larvae on the movements of the adults and on the localization of the oviposition sites.

**Materials and methods**

**Deterrent effect of larvae on the reproductive potential of adults**

We first checked that the presence of larvae had a deterrent effect on the reproductive potential of adults. We placed young females of the flightless strain, the fecundity and fertility of which had been previously assessed over 3 consecutive days, into transparent plastic boxes covered with a grill (11 cm diameter × 3 cm high), one per box, along with a large number of Ephemia kuehniella Zeller (Lepidoptera: Pyralidae) eggs. Six batches of 20 females distributed into two replicates were used. In each batch, there were either no larvae (control), or five individuals at a particular preimaginal stage (eggs or one of the four larval stages). The females were left with the various preimaginal stages for 5 days.

Thirty to 40 conspecific eggs were added to each box. These eggs were stuck to a piece of black paper to make them easy to distinguish from eggs laid by the females. The larvae were reared so that there was a continuous supply of 1-day-old larvae in each of the various stages to replace those that moulted or died in the boxes with the females. The boxes were kept at a temperature of 24 ± 1 °C, 70–85% r.h., and a photoperiod of L16:D8.

The inhibitory effect of the presence of preimaginal stages on the reproductive activity of females was estimated by counting the number of females that laid eggs each day over the 5-day period. Egg-cluster predation appeared in boxes with second to fourth stage larvae. This conspecific predation mainly affected some eggs in egg-clusters. When eggs are predated, their presence is ascertained by their remains stuck to the box wall. The daily number of egg-laying females was compared over time (5 days) and larval stage (six cases), by means of a variance analysis for repeated measures (GLM procedure in SAS system). The test also allowed a comparison between the different numbers of egg-laying females obtained every day according to larval stage.

**Analysis of the dispersion of adults in the absence of larvae**

This experiment was carried out in May 2002, in a 60 m² glasshouse (4 × 15 m) containing 60 cucumber plants arranged in four rows, 1.2 m apart and in 15 lines, 0.70 m apart. The lines numbered from 1 to 15 were located by their distance x from line 8 where the adults were released [xᵢ = (i-8) × 0.7 m; values of i between 1 and 15]. The cucumbers (var. Carmin) were cultivated in nutrient solution in black plastic pots. The pots were placed 0.7 m apart, such that the foliage of neighbouring plants did not overlap. The terminal bud was removed after the emergence of the 15th leaf. A black plastic sheet (0.7 × 0.7 m) was placed under each pot to collect the honeydew and exuviae produced by the aphids.

Each cucumber plant was artificially infested with the aphid A. gossypii by placing on each of them five cucumber leaf disks infested with 20 wingless virginalarpous females, 1 week before releasing the beetles (Salles, http://bpi.antibes.inra.fr). This resulted in an abundant and homogeneous infestation, and ensured that excess food was available throughout the experimental period.

The population of A. gossypii on each cucumber plant was quantified using the method of Lapchin et al. (1997) before the adult lady beetles were released. The number of aphids present on the leaves of each cucumber plant was rapidly estimated by counting. The leaves were then classified in abundance classes according to the number of aphids. The total number of aphids present on each cucumber plant was then calculated using the number of leaves in each abundance class.
The adult flightless beetles came from a laboratory strain reared on E. kuehniella eggs. Young adults (250 pairs), the fertility of which had been verified, were placed on the soil early in the morning along line 8. On the first day, adults were counted 10 times from 08.30 hours onwards, at 45-min intervals, on each of the 60 experimental plants, pots, and surrounding black plastic sheets. Over the following 9 days, the adults were only counted once per day, first thing each morning. The egg-clusters were destroyed in case they affected the mobility of the adults.

The distance the adults moved from the release line was measured by determining the mean position (\(X_m\)) and variance (\(\rho^2\)) of the presence probability distribution. A detailed description of this method can be found in Okubo (1980). \(X_m\) corresponds to the centre of gravity of the adult population. In the absence of biotic and abiotic factors affecting the direction of adult movement in the greenhouse, \(X_m\) fluctuated around zero in the control. The variance (\(\rho^2\)) corresponds to the area over which this population is spread. It varied between a very low value at the time of release to a quarter of the area of the greenhouse, depending on the capacity of the species to disperse. The probability \(p_i\) of the presence of a beetle at line \(x_i\) is defined by the ratio between the number of adults present at line \(i\) (\(n_i\)) and the total number of beetles in the greenhouse at the time of the assessment (\(N\)):

\[
n_i = \text{total number of adults found on plants in the four plants in line } x_i,
\]

\[
N = \sum n_i \quad \text{(total number of adults observed during the assessment)}, \quad p_i = n_i/N.
\]

Mean position and variance were defined as follows:

\[
X_m = \sum p_i x_i \quad \text{(in m)}
\]

\[
\rho^2 = \sum p_i (x_i)^2 - (X_m)^2 \quad \text{(in m}^2)\]

**Analysis of the dispersion of adults in the presence of larvae**

The experimental protocol used was identical to that for the experiment without larvae, except that each cucumber plant was infested with a larger number of aphids (four discs of 40 apterous virginiparous females), to encourage the larvae to stay on these plants. Line 8 served as the boundary between the half of the greenhouse with larvae and that without larvae. The zone with larvae and without larvae, respectively, included 32 cucumber plants in lines 8 (\(x_i = 0\)) to 15 (\(x_i = +4.9 \text{ m}\)) and 28 cucumber plants in lines 1 (\(x_i = -4.90 \text{ m}\)) to 7 (\(x_i = -0.70 \text{ m}\)). One hundred young second stage larvae were placed on each of these plants the day before the adults were released (24 May 2002). The few larvae found in the ‘without larvae’ half of the greenhouse were collected and released in the other half of the greenhouse. The number of egg clusters and total number of eggs were counted at each assessment, and then destroyed.

**Results**

**The effect of preimago stages on the reproductive behaviour of female beetles**

Whether or not the females laid eggs depended on time (\(F = 4.10; \ P = 0.0113\)) and age of the preimago stage (\(F = 25.67, \ P = 0.0006; \) Figure 1). Whatever the day, the presence of eggs had no effect on the number of females that laid eggs relative to the control. In the presence of first-stage larvae, the number of females that laid eggs declined from the fourth day onwards. For older larvae, the effect was apparent on the first day, and tended to increase in magnitude with age of the larvae.

**Dispersion of Harmonia axyridis adults in the absence of larvae**

The mean number of aphids on the cucumber plants at the time of release of the adult ladybirds was 1619 aphids per plant, with a 95% confidence interval of \(\pm 47\) aphids, indicating that the cucumber plants were homogeneously infested. The total number of adults in the greenhouse (\(N\)) decreased from one assessment to the next. The population had decreased by about 20% at the end of the first day and by about 85% at the end of the tenth day.

Starting from the release line (\(x_i = 0\)), the adults dispersed throughout the greenhouse by walking on the ground (this strain is flightless). They explored the polythene sheeting on the ground and the pots, which were attractive in terms of their colour and the presence of honeydew and exuviae.
At the end of the first day, the adults were present in approximately equal numbers on the polythene sheets, the pots, and the plants.

Figure 2 shows the mean position ($X_m$) of the adults during the course of the experiment (circles). The position varied around zero, indicating an absence of factors directing movements. The adults dispersed homogeneously in these experimental conditions. The amplitude of fluctuations in the mean position around zero increased markedly with time, due to statistical errors associated with the decrease in the size of the population of adults.

The changes in variance ($\rho^2$) over time are shown in Figure 3 (circles). This variation was linear over the first few hours: $\rho^2 = 2D_t + b$ [$D = 7 \text{ m}^2/\text{day}, b$ (area occupied by the population at the time of release) = 1.2 m$^2$, error = 0.12].

The fact that the observed variation was linear with time is consistent with the random and independent movement of each beetle, i.e., with diffusion (Okubo, 1980). The slope $D$ (coefficient of diffusion) indicates the speed of colonisation of the greenhouse by the adults. The value we obtained (7 m$^2$/day) indicates that 1 day is sufficient for the colonisation of the whole greenhouse, after which the variance showed no further change and was close to that corresponding to the uniform distribution ($\rho_{\text{uniform}}^2 = 9.1 \text{ m}^2$; Figure 3).

**Dispersion of Harmonia axyridis adults in the presence of larvae**

The mean number of aphids per plant changed from 3089 ± 524 (confidence interval at $P = 0.05$) at the time of release of the adult ladybirds to 21283 ± 1814 at the end of the experiment. This resource was not limiting oviposition. The adult *H. axyridis* population decreased over time in a similar manner in both the absence and presence of larvae. The larvae and the resulting pupae mainly remained in the zone in which they were released. No more than 10 individuals were collected each day in the half of the greenhouse that initially contained no larvae. The spatial distribution of the larval stages therefore remained uniform.

Whereas $X_m$ fluctuated around zero in the absence of larvae, the presence of larvae immediately displaced the population towards lines with a negative $x_i$, i.e., towards the half of the greenhouse without larvae (Figure 2; plusses). After 1 day, this stabilised at about $-1.8 \text{ m}$. The variance of the distribution of adults rapidly increased during the first day and then stabilised at $\rho^2 = 9.0 \text{ m}^2$ (Figure 3; plusses), indicating that the adults occupied a zone extending between approximately $X_m - \rho = -4.8 \text{ m}$ and $X_m + \rho = +1.2 \text{ m}$. Thus, the adults were mostly located in the half of the greenhouse lacking larvae and in the two lines with larvae closest to the boundary between the two halves of the greenhouse.

The total number of eggs collected in the half greenhouse without larvae was about threefold higher than in the half with larvae (Figure 4). This difference cannot be explained by the predation of egg-clutches by larvae, because egg-clutch remains were not observed.

**Discussion**

The reproductive potential and spatio-temporal behaviour of the Asian lady beetle, *H. axyridis*, are sensitive to the presence of conspecific larvae. Female reproductive activity was inhibited by the presence of conspecific active larvae, but not eggs. Females laid several egg clusters in the proximity of a single aphid colony. In the presence of first-stage larvae, the reproductive activity of females was gradually inhibited over time. This is possibly because
the small amount of oviposition-deterring pheromone secreted by larvae of this age probably needs to accumulate on the substrate before it can be perceived by the females. Later larval stages had a more immediate effect on the reproductive potential of the females, and the effect increased with the age of the larvae. In the two-spotted ladybird, *Adalia bipunctata* L., the presence of conspecific larvae also deters egg laying (Dombia et al., 1998).

In the absence of larvae, the mean position of adults in the greenhouse fluctuated around where they were released, confirming the absence of abiotic or biotic factors affecting the movements of the adults. The population moved sufficiently fast to colonise the entire greenhouse within 1 day. The characteristics of the spatial dispersion of the adults (mean position and variance) were consistent with each beetle walking at random. Dependence of variance on time ($t^n$ for $n = 1$) indicates both that the time spent foraging on plants was limited (if this were not the case then variation in $\rho^2$ would be proportional to $t^n$ for $n < 1$), and that the beetles showed frequent changes of direction (if this were not the case then $\rho^2$ would be proportional to $t^n$ for $n > 1$).

Most egg clusters were laid in the part of the greenhouse without larvae, indicating that the presence of larvae either prevented egg-laying without affecting the diffusion of adults, or prevented egg-laying with migration of inhibited beetles into the part of the greenhouse with no larvae. If the deterrent effect was the sole reason for the pattern of oviposition, then the adults would have moved randomly in both halves of the greenhouse but only laid eggs in the half of the greenhouse lacking larvae. The mean position of the population over time would therefore remain close to zero, and the distribution of adults would be as uniform as in the control experiment. If the only reason for this pattern was that the beetles moved away from the oviposition-deterring pheromone produced by the larvae, the whole population would be located in the half of the greenhouse that lacked larvae. In this case, the mean position would be in the middle of the half greenhouse ($X_m = -2.45$ m) with a uniform distribution across the middle and a variance of $\rho^2 = 6.4$ m$^2$. The experimental results do not rule out one of these two assumptions. Indeed, the observed values ($X_m = -1.8$ m, $\rho^2 = 9.0$ m$^2$) indicate both directed and random displacements. In particular, the presence of egg clusters in the first two lines of plants in the half of the greenhouse with larvae indicates both kinds of movement.

Random movements, combined with the inhibition of oviposition, would result in a small change in mean position. Whilst laying eggs, the insects were immobile and mostly located in the half of the greenhouse without larvae. This fraction of the population therefore had a negative mean position. The rest of the population was moving randomly throughout the greenhouse, and therefore had a mean position which fluctuated around zero. Consequently, the mean position for the entire population (expressed as a weighted mean of the mean positions of the two subpopulations) was therefore markedly shifted towards the half of the greenhouse without larvae. This deviation would be weak, much weaker than that observed, because laying eggs immobilises females for much less than 1 day (computation by L. Gil).

Over 3 days, a single female can consume a quantity of aphids equivalent to that required by a larva over a period of 12 days (data not shown). It would therefore be tempting to use adult females for inundative biological control. Indeed, the results obtained indicate that the females were able to disperse their egg clusters throughout the greenhouse and to lay several clusters close to each colony of aphids. However, there are three major drawbacks associated with the use of adults: (1) Their rapid, random displacements may result in them leaving the crop. (2) The inhibition of oviposition in the presence of larvae of the same species limits the duration of the period of reproduction (called the ‘egg window’ by Dixon, 1997). This should lead to the development of successive cohorts of ladybirds.

In addition, larvae are not voracious until late in their development, as the first two larval stages have very low food requirements (about 15% of the total needs of larvae for growth, data not shown). However, not all females react to the presence of larvae (Hemptinne et al., 1992a). (3) Females seem to lay eggs on a range of prey densities limited by a critical minimum threshold (Mills, 1982b), and a maximum threshold, beyond which the females lay too few eggs for the larvae hatching from them to be effective (Dixon, 1997). The use of larvae is not subject to these drawbacks (Branquart et al., 1996), and larvae have been
widely used for the biological control of aphids in various crops (Ferran et al., 1996; Trouvé et al., 1997a, b; Weissenberger et al., 1997).

Acknowledgements

The authors thank Prof. A.E.G. Dixon (University of East Anglia, UK) for his comments and his invaluable help in translation.

References


