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Dispersal potential of native and exotic predatory ladybirds as measured by a computer-monitored flight mill

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Abstract The performance of three species of predatory ladybirds was compared in a flight mill and the effect of diet on their flight parameters was tested. The invasive ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) outperformed *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) in terms of flight distance, duration and velocity. *Harmonia axyridis* flew at least two times further, needed three times less breaks and flew two times faster than *C. montrouzieri* and *A. bipunctata* fed the same diet. Ladybirds reared on eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) performed better than their counterparts reared on natural prey (aphids for *H. axyridis* and *A. bipunctata*, mealybugs for *C. montrouzieri*). The findings of this study indicate that comparative flight studies can be useful to identify candidate biocontrol agents with pronounced dispersal abilities and thus can yield significant evidence to be used in an environmental risk assessment. However, it also demonstrates that variability related to mass rearing conditions should not be ignored when standardizing a risk assessment procedure for candidate biocontrol agents.

Keywords Biological control · Environmental risk assessment · Dispersal capacity · Flight mill · Coleoptera · Coccinellidae

Introduction

In commercial biological control both indigenous and non-indigenous beneficial organisms are used to suppress populations of harmful organisms below economic levels. However, the release of non-native natural enemies is not always considered to be environmentally safe. For instance, exotic biological control agents may cause undesired side-effects on non-target organisms and therefore threaten local biodiversity (van Lenteren et al. 2003; Loomans and van Lenteren 2005; De Clercq et al. 2011). An appropriate regulation concerning the import and use of natural enemies, based on a scientific risk assessment, could prevent these undesired side-effects. General guidelines for a risk assessment methodology have been elaborated, integrating information on a

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biocontrol agent's potential to establish (overwinter), its abilities to disperse, its host range, and its direct and indirect effects on non-target organisms (van Lenteren et al. 2003; van Lenteren and Loomans 2006; Ehlers 2011). Despite above-mentioned efforts, this general framework for risk assessment has hardly been translated into concrete experimental methods for the study of a candidate biocontrol agent's host range spectrum and its capacity to disperse (Babendreier et al. 2005; Mills et al. 2006; Brown et al. 2011). More work has been done, however, to develop a protocol to assess its establishment potential (Hatherly et al. 2005; Boivin et al. 2006; Bale 2011).

The lack of a standard protocol to predict the dispersal capacity of natural enemies is mainly due to its complex nature (i.e. the combination of long- and short-distance dispersal, the role of external factors such as wind or transportation by man) and the practical difficulties to monitor and quantify dispersal. In the context of environmental impacts of commercial biological control, dispersal can be defined as the movement of natural enemies away from the release site and into the surrounding landscape. Mills et al. (2006) recommended mark–release–recapture (MRR) experiments as the best suited approach to assess the dispersal potential of biocontrol agents, but the strong influence of landscape matrix and climatic conditions on the outcome of the experiments were noted as important drawbacks of this strategy. Moreover, open field tests can only be done in the native range of the candidate biological control agent, as quarantine considerations prevent the test from being done into the intended area of introduction (van Driesche and Murray 2004). Last, as most dispersal kernels are leptokurtic (Kot et al. 1996), with many propagules moving long distance, very large releases and trap numbers are necessary for accurate measurements at the tail of the insects' distribution. The use of laboratory techniques such as computer-monitored flight mills to assess the dispersal capacity of an exotic biocontrol agent could overcome this obstacle. Up until now, the tethered flight mill apparatus has mostly been used as a convenient and relatively inexpensive way to assess the migratory performance of insects (Riley et al. 1997; Nedved et al. 2001), to understand the ecological consequences of flight (Bruzzone et al. 2009) and to investigate the effect of flight performance on an insect's physiological state (Luo et al. 2002; Amat et al. 2012).

In the present study, the usefulness of the flight mill apparatus as a tool in a risk assessment procedure for predatory ladybirds as candidate biological control agents was investigated. We compared the flight performance of three species of predatory ladybirds. The first species selected, the two-spotted ladybird *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), is native to Europe. The second and third species selected are not indigenous to Europe: the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and the Australian ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae). Whereas there are no reports of invasiveness for the latter species, *H. axyridis* is known for its invasion of different continents (Adriaens et al. 2003; Koch et al. 2006; Brown et al. 2011). In addition, the effect of diet on the flight potential of these ladybirds was assessed. Diets used in commercial insectaries may have a strong impact on the physiological responses of the natural enemies produced. Unnatural (factitious) foods and/or artificial diets may change the fitness of a natural enemy in many ways (Grenier and De Clercq 2003) and may thus also influence their flight capacity. Therefore, we compared the flight potential of ladybirds fed on their natural prey with that of their counterparts reared on a factitious food source [frozen eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)]. Last, the influence of gender on the flight performance of the studied ladybirds was evaluated. Male insects tend to disperse farther than females, but the dispersal potential of females will ultimately determine the distribution of the progeny and is therefore of major importance (Hughes and Dorn 2002; Mills et al. 2006). The outcome of the flight mill experiments was linked to the ecological background of the tested species and the implications of our findings for conducting an environmental risk assessment at the use of ladybirds for biological control purposes are discussed.

Materials and methods

Insect populations

A laboratory colony of *H. axyridis* was initiated in 2011 by collecting individuals from an established wild population in a park in Ghent (Belgium). The ladybirds were reared on frozen eggs of *E. kuehniella* as described by De Clercq et al. (2005) and Berkvens et al. (2008a, b). A second *H. axyridis* colony was established by taking insects from the stock colony (generation 15) and feeding them on the pea aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) instead of *E. kuehniella* eggs. Both *H. axyridis* populations were maintained at 23 ± 1 °C, 65 ± 5 % relative humidity (RH) and a 16:8 h (L:D) photoperiod. Ladybirds reared for 17 generations on *E. kuehniella* eggs and for two generations on *A. pisum* were subjected to the experiments.

Two laboratory colonies of *C. montrouzieri* were established in 2010 with larvae acquired from Katz Biotech AG (Baruth, Germany) and maintained in a climatic chamber set at 25 ± 1 °C, 75 ± 5 % RH and a 16:8 (L:D) h photoperiod. The first colony was reared on frozen *E. kuehniella* eggs. Water was provided by way of a moist piece of cotton wadding fitted into a 1.5 cm plastic dish. A larger piece of dry cotton wadding (5 × 5 cm) was offered to adult beetles and served as an oviposition substrate for females (Maes et al. submitted). The second *C. montrouzieri* colony was reared on the citrus mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae). Mealybugs were cultured on potato sprouts and kept in a dark room where temperature and RH were not controlled. Potatoes infested with mealybugs and covered with ovisacs were transferred to the colony of *C. montrouzieri.* Experiments were done using individuals of the fifth generation of both populations.

Two populations of *A. bipunctata* were initiated from specimens supplied by CRA-W (Gembloux, Belgium) in 2012: one population was fed a mixture of frozen *E. kuehniella* eggs and bee pollen as described by De Clercq et al. (2005), while a second population was reared on *A. pisum* aphids. Both populations were kept in incubators set at 23 ± 1 °C, 65 ± 5 % RH and a 16:8 (L:D) h photoperiod. Insects of the second generation were used in the experiments.

Experimental setup

To investigate whether the flight activity of ladybirds was influenced by food source or gender, 120 newly emerged males and females of each species (*H. axyridis*, *C. montrouzieri* and *A. bipunctata*) and each population (naturally *vs.* artificially reared) were paired and caged in small Petri dishes. Adults of the different populations were allowed to feed on their respective diet for 7–10 days before being subjected to the experiments.

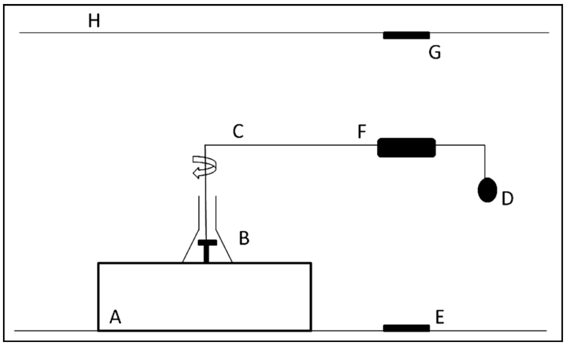

Fig. 1 Schematic overview of the flight mill used in the experiments (*A* flight mill base, *B* stator, *C* rotor, *D* ladybird, *E* photogate, *F* black flag, *G* signal receiver, *H* metal frame)

Morph type was determined for *H. axyridis* [f. *succinea* (referred to as non-melanic *H. axyridis* individuals), f. *conspicua* (melanic *H. axyridis* individuals)] and *A. bipunctata* [f. *typica* (non-melanic *A. bipunctata* individuals), f. *sublunata* (melanic *A. bipunctata* individuals)] (Majerus and Kearns 1989; Osawa and Nishida 1992). In order to check whether the flight parameters were correlated with body weight, insects were weighed using a semi-microbalance Sartorius Genius ME215P (Sartorius AG, Goettingen, Germany; ±0.01 mg) before being attached to the flight mills.

All flight mill trials were performed in the laboratory facilities at LUBIES (ULB, Brussels, Belgium) in an air conditioned room where temperature remained constant (23 °C) throughout the experiments. The ten flight mills were placed in Kewlox© cabinets. Each compartment (50 × 50 × 40 cm) was illuminated by a Fluorescent 10W tube fixed at the ceiling. To keep the RH in the cabinets around 60 % an electric air humidifier (Vicks©, V-5200) was installed in the testing room and a Petri dish covered with wet filter paper was placed in each compartment.

The base of each flight mill consisted of a crystal polystyrene box (Ø 4.8 cm, height 2 cm) filled with sand to increase stability (Fig. 1). A syringe needle (Terumo©) glued to the centre of the box acted as a stator. A steel arm (Ø 0.3 mm) bent at both ends and transversely inserted into the needle operated as the rotor (length 8 cm). Insects were secured to the rotor arm by a small amount of Pritt Poster Buddy (adhesive, synthetic rubber) fixed to their pronotum. An infrared beam emitted by a photogate was interrupted by a black opaque label attached to the rotor arm to record the time elapsed during each rotation. In each compartment, a signal transmitter was positioned on the wooden ground surface while the receiver, attached to a metal frame, was located 10 cm above the transmitter. The receptor cells were connected to a data acquisition board (National Instruments, NI USB 6501, 6.5 mA) and the program UlbDaqNiMoulin (Authors T. Ravet and A. Jannin) registered the transit time of the rotations.

Evaluation of flight potential

Because a single flight parameter might produce misleading results or fail to reveal important flight components (Dingle 1985; Luo et al. 2002), several flight parameters were analysed to compare the flight potential of *H. axyridis*, *C. montrouzieri* and *A. bipunctata*. So, for each trial, flight distance (in km), flight duration (in min), number of breaks (a break being defined as no passage for more than 5 s), average flight velocity (in m s^{-1}; total distance divided by total time) and maximum flight velocity (in m s^{-1}) were recorded. Each individual was attached to a flight mill for a 1 h time period and was used only once.

Statistics

The flight parameters (flight distance, flight duration, number of breaks, average flight velocity and maximum flight velocity) were analyzed using a three-way analysis of variance (ANOVA) with the following factors: species, food source and gender. The means were separated using Tamhane tests because a Levene test indicated heteroscedasticity. When a significant twofold interaction between species and food source was detected, the data were analyzed for the interacting factors separately and means were subsequently separated using Tamhane tests. The flight parameters of the different morphotypes of *H. axyridis* and *A. bipunctata* were compared using Student's heteroscedastic *t* tests as the Levene test indicated unequal variances. *P* values below 0.05 were considered significant. The relationship between body weight and the flight parameters was assessed with a Pearson's correlation test. All data were analysed using SPSS 21.0 (SPSS Inc. 2009).

Results

A three-way ANOVA showed no three-factorial interactions between the factors species, diet and gender for the parameters flight distance ($P = 0.868$), flight duration ($P = 0.867$), number of breaks ($P = 0.809$), average velocity ($P = 0.747$) and maximum velocity ($P = 0.054$; Table 1). Further, no twofold interactions between species and gender, and food and gender were observed. The twofold interactions between species and food source, however, were significant for all flight parameters tested, except for the number of breaks ($P = 0.066$).

Because gender had no influence on the parameters flight distance, flight duration and average velocity (all $P > 0.172$) and because the interaction between species and food source was significant for these parameters (all $P < 0.001$; Table 1), data of males and females were pooled and subsequently analyzed for food and species separately (Fig. 2). ANOVA showed significant differences for flight distance ($F = 155.86$, df = 5, 463, $P < 0.001$), flight duration ($F = 90.79$, df = 5, 433, $P < 0.001$) and average flight speed ($F = 37.75$, df = 5, 432, $P < 0.001$). *Harmonia axyridis* outperformed *A. bipunctata* in total distance flown irrespective of gender and food source (all $P < 0.001$, Tamhane post-hoc tests). *Cryptolaemus montrouzieri* ladybirds showed an intermediate flight distance between *H. axyridis* and *A. bipunctata*. When *C. montrouzieri* was reared on *E. kuehniella* eggs its total distance flown matched that of *H. axyridis* fed on *A. pisum* ($P = 0.916$). On the other hand, when *C. montrouzieri* was offered *P. citri* mealybugs its flight distance was similar to that of *A. bipunctata* fed *E. kuehniella* eggs ($P = 0.212$). The total distance flown of *H. axyridis* and *C. montrouzieri* fed *E. kuehniella* eggs exceeded that of the ladybirds fed their natural prey (both $P < 0.003$).

Harmonia axyridis had a greater flight duration than *C. montrouzieri* and *A. bipunctata* irrespective of food source (all $P < 0.001$). The flight duration of *C. montrouzieri* fed *E. kuehniella* eggs matched that of *A. bipunctata* fed aphids, whereas *C. montrouzieri* reared on its natural prey spent the same time flying as *A. bipunctata* provided with *E. kuehniella* eggs (both $P > 0.955$).

Harmonia axyridis fed *E. kuehniella* eggs outranked all *C. montrouzieri* and *A. bipunctata* populations in terms of flight velocity (all $P < 0.001$). *A. bipunctata* females maintained on *A. pisum* flew 5.5 times slower than *H. axyridis* females given *E. kuehniella* eggs. When the ladybirds were reared on their natural food sources, *C. montrouzieri* had a higher average

Table 1 Three-way ANOVA results indicating the effect of species, food source and gender on the flight parameters of *H. axyridis*, *C. montrouzieri* and *A. bipunctata*

Factors	Flight distance	Flight duration	Number of breaks	Average velocity	Maximum velocity
Species					
F	172.778	109.265	17.544	42.499	77.026
df	2	2	2	2	2
P	<0.001	<0.001	<0.001	<0.001	<0.001
Food					
F	93.423	5.247	0.484	74.201	34.607
df	1	1	1	1	1
P	<0.001	0.022	0.487	<0.001	<0.001
Gender					
F	1.034	1.872	0.033	1.288	6.554
df	1	1	1	1	1
P	0.310	0.172	0.857	0.275	<0.001
Species × food					
F	32.032	9.725	2.738	17.877	23.490
df	2	2	2	2	2
P	<0.001	<0.001	0.066	<0.001	<0.001
Species × gender					
F	0.024	0.173	0.065	1.177	0.118
df	2	2	2	2	2
P	0.976	0.841	0.937	0.309	0.889
Food × gender					
F	0.003	0.086	0.246	0.029	0.000
df	1	1	1	1	1
P	0.954	0.769	0.620	0.866	0.984
Species × food × gender					
F	0.141	0.143	0.213	0.292	2.947
df	2	2	2	2	2
P	0.868	0.867	0.809	0.747	0.054
Error					
df	452	452	452	452	452

flight speed than *H. axyridis* and *A. bipunctata* ($P < 0.002$).

The twofold interaction between species and food source was also significant for the parameter maximum flight speed ($P < 0.001$). In contrast to former flight parameters, gender affected maximum velocity ($P < 0.001$), with female ladybirds reaching a higher maximum flight speed than males (Table 1). Data were analyzed for the factors species, food and gender separately (Fig. 3). ANOVA indicated significant differences ($F = 37.11$, $\mathrm{df} = 11, 456$, $P < 0.001$). *Harmonia axyridis* males and females fed *E. kuehniella* eggs flew significantly faster than their counterparts reared on aphids and than *C. montrouzieri* or *A. bipunctata* fed natural or artificial food (all $P < 0.003$; Tamhane post-hoc tests).

For the parameter number of breaks, no twofold interactions were detected (all $P > 0.066$). In contrast to gender and food source, species was found to have an impact on the number of breaks ($P < 0.001$; Table 1). ANOVA showed significant differences ($F = 42.08$, $\mathrm{df} = 2, 432$, $P < 0.001$). *H. axyridis* (5.8 ± 0.5 breaks, mean ± SE) flew more frequently than *C. montrouzieri* (17.8 ± 1.6 breaks) and *A.*

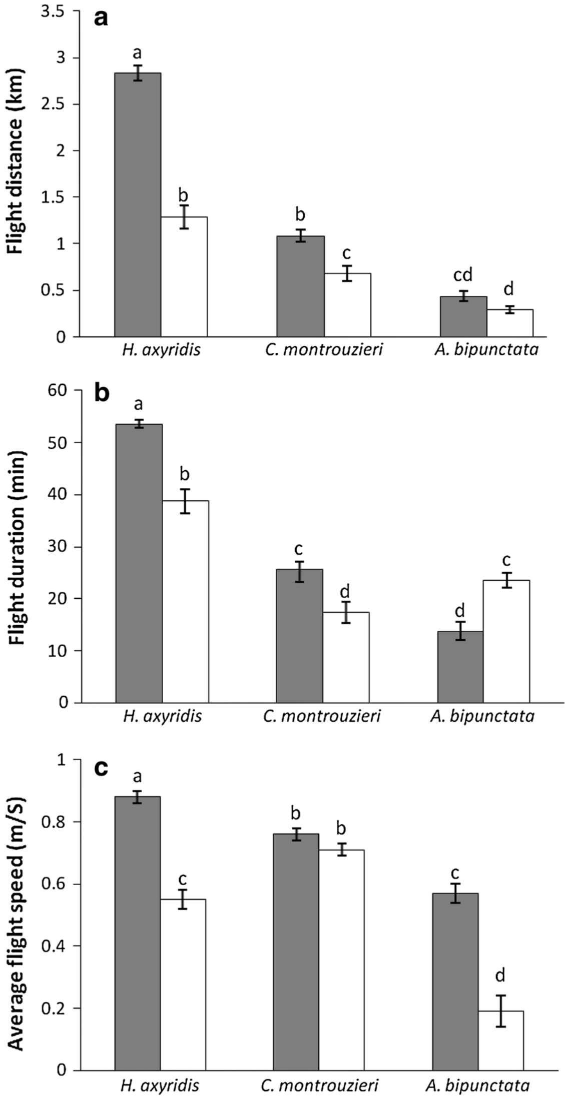

Fig. 2 Flight distance (**a**), flight duration (**b**) and average flight speed (**c**) of *H. axyridis*, *C. montrouzieri* and *A. bipunctata* fed on factitious (grey bar) or natural prey (white bar). Data of males and females were pooled as factor analysis showed no influence of gender. Graph bars (mean ± SE) with the same letter are not significantly different ($P > 0.05$; Tamhane test)

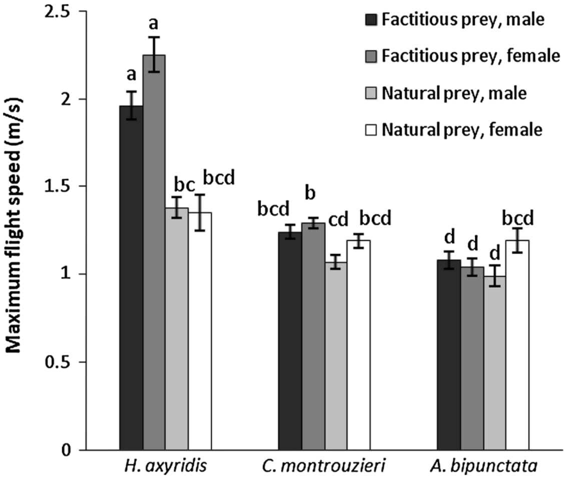

Fig. 3 Maximum flight speed of *H. axyridis*, *C. montrouzieri* and *A. bipunctata* males and females fed on factitious or natural prey. Graph bars (mean ± SE) with the same letter are not significantly different ($P > 0.05$; Tamhane test)

bipunctata (29.2 ± 2.9 breaks; both $P < 0.001$). *C. montrouzieri* was a more frequent flyer than *A. bipunctata* ($P = 0.002$; Tamhane post-hoc test).

The adult body weight of *H. axyridis* and *C. montrouzieri* was positively correlated with flight distance (*H. axyridis*: $n = 156$, $r = 0.278$, $P < 0.001$, *C. montrouzieri*: $n = 188$, $r = 0.210$, $P = 0.004$) and maximum flight speed (*H. axyridis*: $n = 156$, $r = 0.345$, $P < 0.001$, *C. montrouzieri*: $n = 188$, $r = 0.163$, $P = 0.030$) and negatively correlated with flight duration (*H. axyridis*: $n = 156$, $r = -0.224$, $P = 0.005$, *C. montrouzieri*: $n = 188$, $r = -0.151$, $P = 0.045$). A strong correlation was also found between body weight and average flight speed ($n = 156$, $r = 0.287$, $P < 0.001$) in *H. axyridis*. In contrast, no correlations between body weight and the flight parameters were detected for *A. bipunctata* (flight distance: $n = 120$, $r = -0.079$, $P = 0.391$; number of breaks: $n = 120$, $r = 0.032$, $P = 0.727$; flight duration: $n = 120$, $r = 0.021$, $P = 0.834$; average flight velocity: $n = 120$, $r = -0.129$, $P = 0.160$; maximum flight velocity: $n = 120$, $r = -0.113$, $P = 0.219$).

Differences between the flight performances of the morphotypes of *H. axyridis* and *A. bipunctata* were detected (Table 2). The red (or non-melanic) morphs of *H. axyridis* flew longer distances ($t = 3.450$, df $= 154$, $P = 0.001$) with a higher average speed ($t = 3.597$, df $= 154$, $P < 0.001$) and were able to reach a higher maximum flight speed ($t = 7.111$, df $= 153.667$, $P < 0.001$) than the black (or melanic) morphs. Morphotype did not influence the flight distance in *A. bipunctata* but had a role in the activity/rest pattern of the ladybirds. Black individuals of the latter species significantly needed more breaks ($t = -2.094$, df $= 117.972$, $P = 0.038$) while red individuals spent longer time in rest ($t = -2.818$, df $= 119.995$, $P = 0.006$).

Table 2 Flight parameters of (non-)melanic individuals of *H. axyridis* and *A. bipunctata*

Species	Morphotypes	*n*	Flight distance (km)	Flight duration (min)	Number of breaks (#)	Average velocity (m s^{-1})	Maximum velocity (m s^{-1})
H. axyridis	Non-melanic	114	2.6 ± 0.1[a]	50.3 ± 1.2	6.0 ± 0.5	0.83 ± 0.03	2.07 ± 0.07
	Melanic	42	2.0 ± 0.2	47.6 ± 1.98	5.2 ± 0.8	0.68 ± 0.04	1.5 ± 0.04
A. bipunctata	Non-melanic	27	0.2 ± 0.1	22.1 ± 1.57	18.1 ± 4.8	0.61 ± 0.08	1.08 ± 0.07
	Melanic	93	0.4 ± 0.1	11.75 ± 3	33.3 ± 5.4	0.68 ± 0.05	1.08 ± 0.05

[a] Mean ± SE; statistical analysis in text

Discussion

The aphidophagous coccinellids *H. axyridis* and *A. bipunctata* disperse locally in response to prey densities and make migratory flights to and from their overwintering sites (Hodek and Honěk 1996; Brown et al. 2008a). Because this feature of long-distance migration is generally less developed in coccidophagous species (Hodek and Honěk 1996; Iperti 1999) and because *C. montrouzieri* is the smallest species tested, we expected *H. axyridis* and *A. bipunctata* to achieve better performances in flight mill experiments than *C. montrouzieri*. Further, *H. axyridis* is known to be a powerful flier (Obata 1986; Hodek et al. 1993; Tourniaire et al. 2000), which is believed to be one of the mechanisms underlying its high degree of invasiveness (Brown et al. 2008a, b, 2011; van Lenteren et al. 2008; Berkvens et al. 2009). For the above reasons, it was expected that *H. axyridis* would outcompete the other tested coccinellid species in the flight mills. This hypothesis was only partially confirmed. Indeed, when the ladybirds were reared on *E. kuehniella* eggs, *H. axyridis* flew further than *C. montrouzieri* and *A. bipunctata*, but when they were fed on natural prey, the total distance flown by *C. montrouzieri* was statistically similar to that of *H. axyridis*. *C. montrouzieri* ladybirds fed *E. kuehniella* eggs also flew further than the larger *A. bipunctata* individuals fed the same factitious prey. Cock (2013) discussed the potential non-target impacts associated with the introduction of *C. montrouzieri* for the biological control of the hibiscus mealybug *Maconellicoccus hirsutus* Green in Grenada and also concluded that the dispersal capacity of *C. montrouzieri* should not be underestimated. Despite the less ephemeral nature of their coccidophagous prey, *C. montrouzieri* ladybirds should be classified as high density predators likely to disperse when prey populations are reduced (Cock 2013; De Clercq et al. 2011).

Our results confirm that body size of a species is not a reliable indicator to compare the dispersal potential of members of the same taxonomic family. The relatively weak performance of *A. bipunctata* compared to *H. axyridis* matches the observation that the former species is characterized by a shorter distance of most dormancy sites from the breeding habitat (Hemptinne 1989; Hodek and Honěk 1996). Prior studies revealed a link between dispersal capacity measured in a flight mill system and capacity for long distance migration to overwintering sites observed in the field. Rankin and Rankin (1980) and Nedvěd et al. (2001) studied the migration behaviour of the convergent ladybird *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and the seven-spotted ladybird *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), a species known for its long distance migrations and a species with a rather short migratory flight, respectively. While Rankin and Rankin (1980) reported that 60 % the individuals of the long distance migrant *H. convergens* flew longer than 30 min, the maximum flight duration observed for the short distance migrant *C. septempunctata* was only 20 min.

For both *H. axyridis* and *C. montrouzieri* a positive correlation between adult body weight, flight distance and velocity, and a negative correlation between body weight and flight duration was discovered. Within each species heavier ladybirds compensated their shorter flight duration by a higher flight speed and flew longer distances than lighter ladybirds. In contrast, no significant correlations between body weight and the flight parameters were detected for *A. bipunctata*. Likewise, positive correlations between adult weight and flight performance have been reported in a number of insect species (Shirai 1995;

Fischbein et al. 2011; Bruzzone et al. 2009; Kaufmann et al. 2013), whereas these were not found in others (Gu and Barker 1995).

Besides information on the total distance flown, flight mill experiments also reveal information on the rest/activity pattern of insects. In the present study, we found that *C. montrouzieri* and *A. bipunctata* needed more breaks than *H. axyridis*, indicating that the former species may disperse more gradually than *H. axyridis*. *C. montrouzieri* and *A. bipunctata* will forage for prey and oviposition sites within a restricted area. If suitable prey is present in sufficient quantities, the coccinellids will only gradually disperse further (Hodek and Honěk 1996). The capacity to fly great distances (measured here as total flight distance) combined with the ability to fly these distances with a minimum of resting pauses (measured here as the number of breaks) could help explain the rapid spread of *H. axyridis* over the European and North-American continent (Koch et al. 2006; Brown et al. 2008a).

The food source offered to the ladybirds affected their total distance flown, duration of flight and flight velocity, but the interactions between food source and species were also found to be significant. Overall, ladybirds fed *E. kuehniella* eggs flew further, at a higher speed and spent more time flying than ladybirds reared on natural prey. However, these differences were only found to be significant for *H. axyridis*. Wanner et al. (2006) showed that nectar with different nutritional values had a different effect on flight activity in the parasitoid *Cotesia glomerata* (L.) (Hymenoptera: Braconidae). In contrast, Fischbein et al. (2011) reported that the flight parameters of another parasitoid, *Ibalia leucospoides* Hochenwarch (Hymenoptera: Ibaliidae), were not affected by prior access to food, but hypothesized that such an effect may manifest itself on subsequent days of flight. The factitious food source used in our experiments, *E. kuehniella* eggs, was considered to be a better food for *H. axyridis* than pea aphids (Specty et al. 2003; Berkvens et al. 2008b). Specty et al. (2003) found that *E. kuehniella* eggs were nutritionally superior to *A. pisum* in terms of amino acid and fatty acid content and composition, which may be essential nutrients to fuel flight in *H. axyridis*.

No significant influence of gender on the flight parameters of the tested ladybirds was detected except that female ladybirds were able to reach a higher maximum flight speed than males. Further, the influence of morph type on the flight capacity of *H. axyridis* and *A. bipunctata* was not straightforward. While non-melanic *H. axyridis* ladybirds outcompeted the melanic individuals in both flight distance and flight speed, no effect of melanism on these parameters was recorded for *A. bipunctata*. However, the rest/activity pattern of latter species was affected: black individuals took more breaks while red individuals spent more time resting. Although the low frequency of melanic morphs in most *A. bipunctata* populations suggests that they are at a considerable selective disadvantage (Majerus and Kearns 1989), our flight mill output indicates that this disadvantage is not due to lower flight performances. Our experiments also showed a greater flight capacity of red *H. axyridis* morphs. Prior studies have indicated that there is variation in the ecological and physiological characteristics among the colour morphs of *H. axyridis*, offering particular morphs a greater fitness than others in specific habitats or at specific times (Osawa and Nishida 1992; Serpa et al. 2003; Wang et al. 2009; Berkvens et al. 2008a). Soares et al. (2001, 2005) and Berkvens et al. (2008b) found that red morphs are nutritionally more adaptive and that their greater nutritional plasticity may offer them a competitive advantage for the exploitation of food sources during establishment. Their greater nutritional plasticity combined with a greater dispersal potential may in part explain the predominance of non-melanic morphs in invaded areas (Koch 2003; Hantson 2004).

Arguably, flight mill experiments like those conducted in this study have their limitations. First, long-term laboratory rearing of natural enemies could induce selective adaptation on their flight propensity (Grenier and De Clercq 2003). Further, data obtained from flight mill experiments allow only for an estimation of flight capacity (Bruzzone et al. 2009). Because insects are forced to fly by lack of tarsal contact, flight mill experiments tend to overestimate their dispersal capacity compared with experiments carried out in flight chambers or MRR experiments conducted at the field scale (Shirai and Kosugi 2000; Yamanaka et al. 2001; Blackmer et al. 2004; Botero-Garces and Isaacs 2004; Edwards 2006). Moreover, the handling of insects when attaching them to the flight mills can reduce or increase their propensity for flight (Cockbain 1961; Kennedy and Booth 1963). Besides, wind-assisted flight is obviously not measured with mills, which could lead to an underestimate of actual dispersal capacities. Nevertheless, recent comparative studies report consistent results between activity patterns measured in a flight mill and flight activity observed in the field (Amat et al. 2012). The use of computer-monitored flight mills has several advantages: flight mills are convenient and relatively inexpensive means to assess a species' flight performance, the analysis of flight mill output data is simple and straightforward and flight mill experiments are less time consuming than traditional MRR experiments which require repeated recaptures and releases over a period of time (Reynolds et al. 1997; Riley et al. 1997; Mills et al. 2006).

Although the calibration of flight mills to obtain absolute estimates of the dispersal capacity of an insect remains an important obstacle, flight mill experiments can yield significant information when used in a comparative approach. The present study indicated the strong flight capacity of the harlequin ladybird, *H. axyridis*, suggesting its role in the rapid invasion of Europe and other parts of the world. In the framework of an environmental risk assessment procedure, candidate biocontrol agents with such pronounced dispersal abilities would immediately be recognised in flight mill studies. Our study indicates that in view of standardizing such a risk assessment procedure, variability related to the mass rearing conditions of the studied biocontrol agent should not be ignored. We demonstrated that predatory ladybirds reared on *E. kuehniella* eggs as factitious food outperformed ladybirds reared on natural prey. Likewise, Maes et al. (2012) showed that the food source offered to the predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) influenced its supercooling capacity and might therefore affect its establishment potential. These findings indicate that factors related to the rearing conditions of biocontrol agents may complicate a risk assessment procedure and thus need to be taken into consideration.

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