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Density-dependent dispersal in biological control agents: a reflexion on the side-effects of mass-rearing conditions

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Abstract High-density rearing conditions for the mass-production of biological control agents are known to affect individual quality and performance. However, complex phenotypic traits like dispersal behaviour and their response to rearing conditions are rarely investigated, although they are likely to affect directly biocontrol efficiency in the field. In this study, we develop an original experimental design to evaluate two complementary components of dispersal behaviour in *Trichogramma*. Then, we investigate how these components respond to variations in rearing density, and their correlation with traits related to parasitoid fitness. We find that under high-density conditions, a large proportion of individuals display reduced mobility and fecundity, indicative of a lower-quality phenotype. These interactive effects between dispersal performance and individual fitness highlight the need to develop integrative experimental designs to easily quantify complex phenotypic traits related to the field performance of biological control agents.

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Introduction

One of the first steps in the development of a biological control programme is often the screening of several populations of candidate agents for traits related to host control efficiency, like foraging behaviour, thermal tolerance or realized fecundity (Hopper et al. 1993; Ruberson et al. 1999; Coupland and Baker 2009). Then, these traits and others related to mass-production (e.g., development on factitious hosts, sex-ratio, pesticide resistance, diapause or resistance to storage procedure) can eventually be further enhanced through artificial selection (Rosenheim and Hoy 1988; Hopper et al. 1993; Heilmann et al. 1994).

In addition to such genetic differences in life-history traits between individuals or populations, lab-rearing conditions are also known to induce non-genetic phenotypic changes that might strongly impact individual performance in the short-term (van Lenteren et al. 2003; Luczynski et al. 2007; Pereira et al.

2007). Phenotypic plasticity refers to the capacity of a given genotype to exhibit a range of phenotypes in response to variation in the environment (Pigliucci et al. 2006). In particular, processes like diapause induction, host recognition or habitat choice, that can all deeply affect the performance of a biological control agent in the field, are known to be sensitive to environmental influences (Rietdorf and Steidle 2002; Davis and Stamps 2004; Bean et al. 2007).

Classically, phenotypic plasticity is expected to occur during development, as this is the time during which most of the individual phenotype is determined, and development is known to be particularly susceptible to deviating perturbations and environmental influences in general (Whitman and Agrawal 2009; Fusco and Minelli 2010). Maternal effects can also be considered as a special case of phenotypic plasticity, where the individual phenotype is affected by the environment experienced by its mother (e.g., through reproductive allocation or more directly through cytoplasmic factors, Mousseau and Fox 1998).

For adaptive plasticity, early determinism provides the double advantage of (i) producing phenotypes that are pre-adapted to their future environment, and (ii) inducing complex, coordinated ontogenic shifts that affect multiple traits (Pigliucci and Preston 2004; Shingleton et al. 2007). For hymenopteran parasitoids used as biological control agents, developmental plasticity is expected to strongly affect performance in the field, as environmental conditions experienced during larval development (i.e., laboratory environment) will be dramatically different from those experienced during adult life, the latter being the determinant life stage for biocontrol efficiency. Indeed, many studies have documented strong downstream effects of early environment (e.g. host species, host condition, temperature) on adult phenotype and fitness in parasitoids (Boivin 2010; Cicero et al. 2011; Mawela et al. 2013).

Among the factors most likely to impact parasitoid's phenotype, density is a crucial one as it is directly related to mass-rearing productivity and thus should be maximized for commercial purposes. High-density conditions are likely to deeply affect individual phenotype, the most frequent effects being a reduction in size, a decreased emergence rate and a male-biased sex ratio (van Lenteren 2003; González et al. 2007; Tormos et al. 2012). Such effects are expected not only in gregarious but also in solitary parasitoids, where female reproductive allocation or

the frequency of superparasitism can depend on local density (Wylie 1965; van Baaren et al. 1995). Adult size is a strong determinant of several performance traits in insects, including female fecundity (Boivin 2010), while emergence rate and sex ratio directly affect parasitism pressure and thus the potential for host control. Therefore, the effects of rearing density on these traits are usually investigated quite early when setting up an industrial production of parasitoids, and rearing density is then chosen to optimize the trade-off between parasitoid quantity and quality (i.e., phenotype, Nunney 2003).

In contrast, many other traits likely to impact efficiency in the field but less easily measured in the laboratory may also be plastically affected by density conditions during development. Among these traits, dispersal is likely to be a key determinant of biological control efficiency in the field (Heimpel and Asplen 2011), in particular for augmentative releases. Indeed, low dispersal rates will result in highly heterogeneous parasitism rates over space (e.g., Greatti and Zandigiacomo 1995; McDougall and Mills 1997; Fournier and Boivin 2000), while excessive dispersal may lead to significant emigration away from the target area (Bellamy et al. 2004; Collier and van Steenwyk 2004; Pineda and Marcos-García 2008). Therefore, the level of host control achieved in the field is likely to be sensitive to individual dispersal rate, and any mechanism that allows the manipulation of the dispersal patterns of natural enemies could be used to increase biological control efficiency in the field depending on the ecological context (e.g., level of landscape fragmentation, Heimpel and Asplen 2011).

Dispersal is known to be a complex trait open to the influence of many external and internal factors (Clobert et al. 2001; Bowler and Benton 2005), and density-dependent dispersal responses have been reported in many taxa, including insects (Harrison 1980; Zera and Denno 1997). Rearing density during development might thus be a convenient trigger to adjust the dispersal rate of parasitoids at will to optimize biocontrol efficiency. However, density-dependent dispersal can be either positive (i.e., higher dispersal rate in more crowded populations) or negative, depending on the selective forces acting on dispersal evolution in a given population or species (Bowler and Benton 2005; Matthysen 2005). In addition, dispersal abilities are usually determined by a large set of correlated traits (e.g., morphological,

physiological and behavioural) so that density-dependent dispersal responses are likely to affect global population performance. Dispersal is often considered a costly behaviour (Ronce 2007), both because of increased mortality risk and high energy costs associated with mobility (e.g., flight-fecundity trade-offs in insects, Zera and Denno 1997; Roff and Fairbairn 2001), so that individuals in higher-dispersing populations might have lower average fecundity or longevity. Alternatively, when dispersal is condition-dependent and requires a minimum level of energetic reserves (Ims and Hjermann 2001; Chaput-Bardy et al. 2010), individuals from higher-dispersing populations should be on average in better physical condition and achieve higher fitness.

In that respect, assessing whether the dispersal of natural enemies might be efficiently manipulated through phenotypic plasticity by tuning the level of rearing density during larval development requires to determine: (i) the nature of the density-dependent dispersal response (i.e., positive or negative), and (ii) its consequences on other performance related-traits. We specifically addressed these points by investigating the impact of rearing density on dispersal rate, female fecundity and longevity for three different species of *Trichogramma* that are commonly used as biological control agents against several Lepidopteran pests. Parasitism rate from *Trichogramma* parasitoids in the field has been found previously to be sensitive to dispersal distance (Fournier and Boivin 2000; Wright et al. 2001; Kölliker-Ott et al. 2004), so that the efficiency of augmentative releases might directly benefit from the control of plastic dispersal responses during the production process. In addition, *Trichogramma* have consistently been used as model species in behavioural ecology (Wajnberg et al. 2000; Boivin et al. 2004; Huigens et al. 2010; Kruidhof et al. 2012) and population dynamics (Vercken et al. 2013, 2015), and as such represent a particularly relevant focus group for the study of ecological and evolutionary processes related to biological control.

Materials and methods

Biological model

We used three different species of *Trichogramma* wasps (Hymenoptera: Trichogrammatidae) as model

species: *T. achaeae*, *T. chilonis* and *T. euproctidis*. *Trichogramma* are minute solitary parasitoids of Lepidopteran eggs widely used as biological control agents against noxious species (Smith 1996). The species *T. achaeae*, *T. chilonis* and *T. euproctidis* are respectively released against the tomato leafminer *Tuta absoluta* (Chailleux et al. 2012), the sugar cane spotted borer *Chilo sacchariphagus* (Tabone et al. 2010), and for the integrated protection of several stored products (Schöller 2010). The location and date of sampling of the different species are given in Table 1. While some strains and species of *Trichogramma* can be thelytokous (Stouthamer 2003), the species used in our study are all arrhenotokous, i.e. fertilized and unfertilized eggs respectively develop into diploid females and haploid males (Quicke 1997).

Rearing conditions

The three species were maintained in the laboratory on the factitious host *Ephestia kuehniella* (flour moth). Host eggs were irradiated previous to parasitoid exposure, thus preventing their development while maintaining their suitability for the *Trichogramma*. Temperature and light conditions were set on a cycle of 16 h daylight (25 °C)/8 h dark (20 °C) with constant 70 % RH. Generation times for each species under these conditions are given in Table 1.

Laboratory populations were grown in glass tubes (diameter 10 mm, length 80 mm). At the beginning of the experiment, each population was initiated with five mated female parasitoids, and hosts were provided following two density treatments: 50 host eggs, i.e. a ratio of 10 eggs per female (high density, representative of density conditions in industrial rearing for mass-production) or 250 eggs (50 eggs per female, low density). Food for female parasitoids was provided ad libitum as drops of honey placed on the tube walls. Host eggs were exposed 48 h to female parasitoids and then put aside until emergence of the next parasitoid generation. Individuals emerging from the next generation were then evaluated for their dispersal abilities and fitness traits.

Measurement of dispersal abilities

Dispersal abilities were evaluated according to either ambulatory dispersal (short distance, within-plant dispersal) or aerial dispersal (long distance, between-

Table 1 Description of *Trichogramma* populations

Species	Sampling location	Sampling date	Generation time[a] (days)
T. acheae (A.02)	Canaries Islands	2010	8
T. chilonis (ChiSB)	Reunion Island	2004	8
T. euproctidis (34-Me-a1-36)	France (Hérault)	2011	9

[a] Under standard laboratory conditions

plant dispersal), both of which were assessed using original laboratory systems. These measures of dispersal are uncorrelated and aerial distance was found to be a significant predictor of parasitism rate in larger-scale environments representative of the level of spatial complexity typically encountered in greenhouse cultures (E. Vercken and E. Tabone, pers. com.).

For ambulatory dispersal, the emergence tube was connected to a series of eight glass cylinders (diameter 10 mm, length 100 mm) fastened together with soft plastic seals. Parasitoids were allowed to walk within the experimental set-up for 30 min (temperature: 25 °C, RH: 75 %), at the end of which the number and sex of individuals inside each cylinder was recorded to establish the distribution of dispersal distances for each experimental population (Fig. 1). Individuals retrieved from the different cylinders were then used to measure their fitness traits. *Trichogramma* tend to climb up and to orientate towards light, so the experimental set-up was positioned vertically with a light source on the top while the emergence tube was darkened with black tape to enhance dispersal behaviour. In addition, to limit back-and-forth movements between the cylinders, each connection between successive cylinders was made through a funnel-shaped piece (1 mm-diameter on the narrowest end). Between three and nine replicates were done for each species and each density level, with an average of 37 individual positions recorded in each replicate population.

For aerial dispersal, as *Trichogramma* are usually considered to have limited capacities for active flight, the scale of the set-up was chosen to measure jumps or short flights in absence of any air flow. In this set-up, the emergence tube was connected to a circular platform (50 mm diameter, 50 mm height), from which individuals could jump into a large arena (600 mm diameter, the platform being located in the

Fig. 1 Experimental design for estimation of ambulatory dispersal of *Trichogramma*. Individuals are allowed to explore the set-up during 30 min, at the end of which their position (*tube number*) is recorded
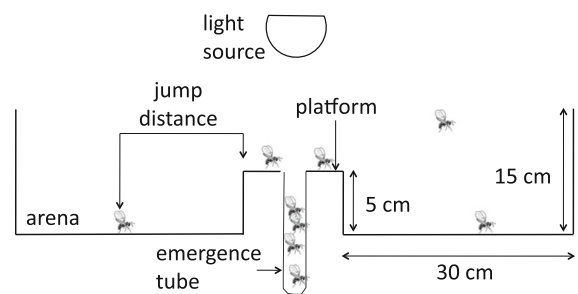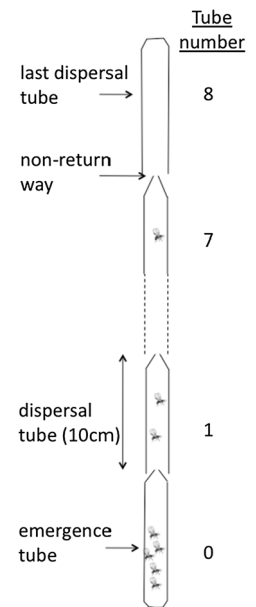

Fig. 2 Experimental design for estimation of aerial dispersal of *Trichogramma*. Each trial lasts 30 min. Every time an individual jumps into in the arena, its landing position is recorded and the individual is removed

center, Fig. 2). The edges of the platform were coated with petroleum jelly in order to prevent individuals from walking down the sides. Parasitoids were allowed to explore the experimental set-up for 30 min (temperature: 25 °C, RH: 75 %). The landing position was recorded for individuals that jumped

from the platform (more than 80 %), after which individuals were put aside for measuring fitness traits later on. Similarly to the set-up for the evaluation of ambulatory dispersal, a light source was positioned 20 cm above the platform and the emergence tube was darkened with black tape to enhance dispersal behaviour. Between four and ten replicates were done for each species and each density level, with an average of 11 individual positions recorded in each replicate population.

Measurement of fitness traits

Females retrieved from each of the dispersal set-ups were then evaluated for fecundity and survival following dispersal. Each female was isolated in a glass tube with 100 host eggs to parasitize and without any food source. After 24 h, we recorded whether females were still alive or not, and the host eggs were put aside until emergence of the next generation. For ambulatory dispersal, a maximum of ten females were tested for each combination of species, density level and class of distance walked (i.e., cylinder), for a total of 31–95 females tested for each combination of species and density level. For aerial dispersal, between 37 and 80 females were tested for each combination of species and density level, for dispersal distances ranging from 27 to 248 mm.

Statistical analyses

The distribution of ambulatory dispersal distance followed a zero-inflated negative binomial distribution and aerial dispersal distance followed a Gamma distribution. Both variables were analyzed with generalized linear mixed models (log link function in both cases), with density level, sex of the individual and the interaction between sex and density included as fixed effects. Female realized fecundity and survival after 24 h starvation followed a Gaussian and a binomial distribution. These were analyzed with linear mixed models and generalized linear mixed models (logit link function) respectively, with density level, dispersal distance and their interaction included as fixed effects. In all analyses, species and experimental replicate (nested within species) were modelled as random factors. Significance of fixed effects was estimated by likelihood-ratio tests between nested models using Laplace estimations.

Results

Density-dependent dispersal

Ambulatory dispersal was found to depend on sex (males covering longer distances than females, Table 2), and on the interaction between sex and density treatment. Females, but not males showed reduced ambulatory dispersal when experiencing higher larval densities (Table 2; Fig. 3a). Average aerial dispersal distance was different between sexes, with females covering longer distances than males (Table 2). However, both sexes displayed reduced aerial dispersal when experiencing higher larval densities (single effect of density level, no interaction with sex, Table 2; Fig. 3b).

Dispersal performance and female fitness

For both measures of dispersal, neither dispersal distance, density level or their interaction had any effect on survival after 24 h starvation (all p-values > 0.15). In contrast, female fecundity was strongly influenced by dispersal performance. For both measures of dispersal, fecundity was positively correlated with dispersal distance (Table 2; ambulatory dispersal, Fig. 4a; aerial dispersal, Fig. 4b). In the case of ambulatory dispersal, fecundity was also affected marginally by the interaction between density level and dispersal distance (Table 2; the difference between dispersing and non-dispersing females being highest in the high-density treatment, Fig. 4a).

Discussion

Negative effects of rearing density on *Trichogramma* phenotype

For both measures of dispersal, we found that increased density during larval development resulted in lower dispersal tendencies, at least for females. Such negative density-dependent response could in some contexts be advantageous to the parasitoid, in particular when there is spatial heterogeneity in habitat quality (Hassell and May 1988; Hirzel et al. 2007; Rodrigues and Johnstone 2014). Such an advantage should however strongly depend on the density response for other fitness-related traits, as a density-

Table 2 Results from the statistical models

Variable	Selected model	Estimates	SE	χ^2	df	p-value
Ambulatory distance	Intercept	0.0305	0.4173			
	Density[a]	−0.0355	0.1030	1.4	1	0.23
	Sex[b]	−0.4155	0.2177	37.3	1	1.02×10^{-9}
	Density × sex	−0.1438	0.0662	4.7	1	0.03
Aerial distance	Intercept	4.0622	0.0943			
	Density	−0.0481	0.0193	5.74	1	0.02
	Sex	0.2588		19.02	1	1.29×10^{-5}
Survival	Not significant	NA	NA	NA	NA	NA
Fecundity	Intercept	24.1161	2.4032			
	Ambulatory distance	0.1643	0.4472	6.5	1	0.01
	Density	−0.3448	0.3545	0.19	1	0.66
	Distance × density	0.4467	0.2530	3.1	1	0.07
	Intercept	22.7191	2.3646			
	Aerial distance	0.0334	0.0113	8.1	1	4.44×10^{-3}

[a] Density was calculated as the ratio of parasitoid females on the number of host patches (i.e., one for low density and five for high density)

[b] These estimates are from treatment contrasts. This means that the intercept gives the value for a reference level for factors (in our case, females), and the estimate for "Sex" is the deviation from this reference for the level "males"

Fig. 3 Estimation of dispersal distance (**a** ambulatory dispersal; **b** aerial dispersal) for three species of *Trichogramma* in function of sex and density level during development. Average ambulatory dispersal distances are driven towards low values because of a zero-inflated distribution. *Error bars* represent 95 % confidence intervals
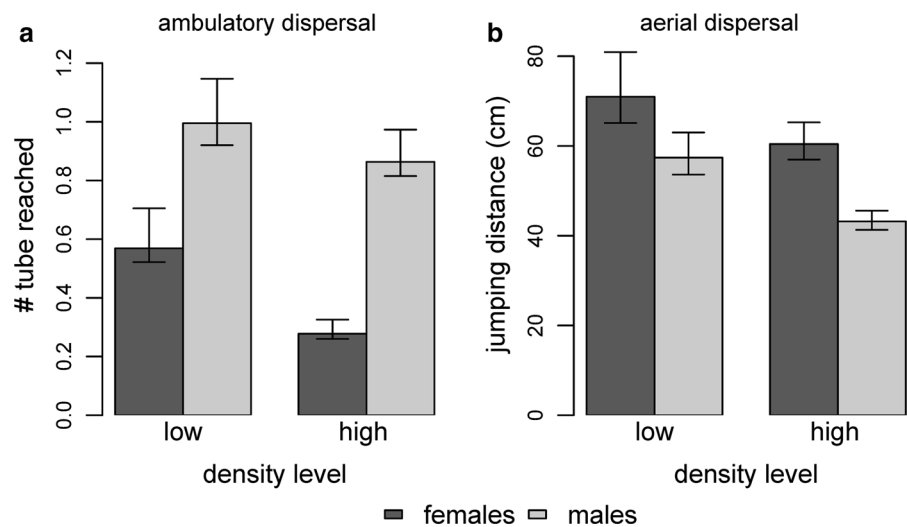
dependent phenotype can arise from two distinct mechanisms: (i) a global energetic or physiological constraint acting on individual development under high density conditions, in which case the whole phenotype of the individual should be deteriorated (Chaput-Bardy et al. 2010; Russell et al. 2011); or (ii) a coordinated ontogenetic switch allowing to produce alternative phenotypes with different life-history trade-offs (Gross 1996).

In our experiment, dispersing females are always more fertile than non-dispersing ones, irrespective of density conditions. This implies that the decreased dispersal rate observed at higher densities is unlikely to be part of a coordinated alternative strategy, but

Fig. 4 Model fit of female fecundity over 24 h after dispersal experiment for three species of *Trichogramma* in function of dispersal distance (**a** ambulatory dispersal; **b** aerial dispersal). *Dots* represent experimental data averaged by tubes for ambulatory dispersal or over 20-mm distance intervals for aerial dispersal (*open circles* low density; *full circles* high density)
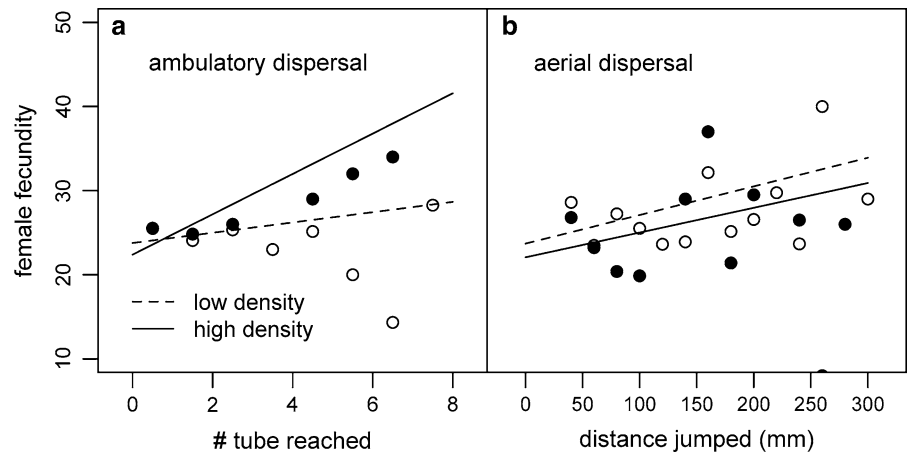
rather reflects the physiological constraints arising from developing in a more competitive environment (e.g., limited access to resources, delayed larval development, Harvey 2005; Chaput-Bardy et al. 2010). Indeed, other experiments conducted in similar dispersal set-ups revealed that low-dispersing individuals have significantly shorter tibias than high-dispersing ones (M. Vilarelle, pers. com.), which supports the hypothesis that mobility is limited by developmental constraints. However, as individual fitness was measured only in laboratory, non-spatial conditions, and only on a subset of fitness-related traits, more integrative measures of individual performance under different levels of spatial heterogeneity would be needed to assess more definitely how density conditions experienced during early development shape individual phenotype and fitness.

Implications for mass-rearing of biological control agents

We found that density conditions representative of mass-rearing for commercial purposes led to the production of individuals with lower dispersal abilities in *Trichogramma*. These low dispersers were also found to display a reduced fecundity when compared with more mobile individuals. However, we found no difference in mean fecundity when comparing density treatments directly, which means that this effect is mediated through complex interactions with other components of the phenotype, like dispersal abilities. Such interactions at the phenotype level are also likely to involve many other fitness-related traits that were

not investigated in our study, so that rearing density, through its complex effect on phenotype development, might have affected individual quality on many levels.

This might result in higher phenotypic variance in fitness between individuals reared in high-density conditions, even if the population is genetically homogeneous, though not necessarily in lower average values. This could have some significant influence on the performance of the biological control agents in the field. In particular, undetected phenotypic plasticity in performance-related traits could affect the repeatability of host control across different release programs (Lewis et al. 2003). Spatial heterogeneity in performance related to individual variation in dispersal would also strongly affect the reliability and the quality of the commercialised biological control agent (van Lenteren 2003).

However, such indirect effects of rearing density on individual quality and population performance might remain unnoticed at the production stage if complex components of the phenotype, like dispersal, are not investigated. In this context, experimental settings like those used in this study, which allow quantifying complex traits while keeping a small temporal and spatial study scale might prove useful to predict realized performance in the field. While the need for experimental procedures to assess complex components of the phenotype in the context of quality control of commercialised biological control has been advocated repeatedly (Bigler 1994; van Lenteren et al. 2003), these approaches are still under-developed and the relationship between traits measured in the lab and larger-scale performance in the field is rarely

investigated (but see Dutton and Bigler 1995; Silva et al. 2000).

Validation of dispersal protocols

Both dispersal protocols, although involving artificial and simplified environments, seem to provide relevant information regarding the dispersal abilities of *Trichogramma* in the field. For ambulatory dispersal, the distance covered along a vertical axis is likely to be an indicator of the individual tendency to explore its spatial environment at the within-plant scale. Regarding aerial dispersal, *Trichogramma* are not skilled fliers, and most of their between-plant dispersal is assumed to be made through jumps or short flights (Hendricks 1967; Yu et al. 1984; McDougall and Mills 1997). These two measures of dispersal were evaluated as predictors of parasitism efficiency in larger-scale mesocosms including plants and Lepidopteran hosts. While aerial dispersal distance was significantly correlated with host suppression, ambulatory distance was found to have no effect (E. Vercken and E. Tabone, pers. com.). However, this particular trial involved tomato plants, and tomato stems are covered by trichomes that are expected to strongly hamper ambulatory dispersal within the plant. In this particular context, ambulatory dispersal should not have facilitated individual movement.

Therefore, we believe that our measure of ambulatory dispersal is informative, and gives complementary information from aerial dispersal. First, the two measures are uncorrelated, which means that they reflect different qualitative movement patterns. In addition, the results from our experiments were highly consistent across both dispersal measures: in both cases, dispersal was negatively affected by density and female fecundity was positively correlated with dispersal distance, while there was no effect on survival after 24 h-starvation. We are thus confident that the two measures are relevant regarding habitat use at different spatial scales and can be considered as reasonable proxies for dispersal abilities in most agricultural environments. In addition to dispersal abilities, other traits related to spatial performance, like orientation and host detection in a complex spatial environment or habitat use strategies would also bring valuable insight regarding the alteration of parasitism rate when switching from rearing conditions to the field (Lewis et al. 2003; Vet et al. 2003).

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Elodie Vercken studies how landscape characteristics and population-level traits affect the establishment and spread of introduced populations. Her work combines theoretical approaches and experimental work using *Trichogramma* as a model species.