


Factors influencing the dispersal of a native parasitoid, *Phasgonophora sulcata*, attacking the emerald ash borer: implications for biological control

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Abstract High parasitism by a native parasitoid, *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), has been reported on emerald ash borer (hereafter EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in North America. Use of this parasitoid in an augmentative biological control program has been proposed to slow the spread of EAB, yet information is lacking on key aspects of this parasitoid's dispersal. We document the flight capacity and walking activity of *P. sulcata*, its potential fecundity, and describe how age, body size, temperature, and time of day affect these parameters. Wasp flight capacity, measured using flight mills, increased with temperature and decreased with age. Unexpectedly, age and body size did not affect wasp walking activity, and we saw no relationship between walking activity and flight capacity. Older wasps had lower potential fecundity than younger wasps. These results suggest that *P. sulcata* should be released as pupae

near EAB-infested ash trees to improve efficacy and potential biological control success.

Keywords *Agrilus planipennis* · Flight capacity · Flight mills · Walking activity · Wasp age · Fecundity

Introduction

Understanding the dispersal of an insect parasitoid is an important consideration for successful biological control (Hopper and Roush 1993; Heimpel and Asplen 2011; Mills and Heimpel 2018). Knowing the dispersal of a biological control agent can help optimize distances needed between release sites and the appropriate number of agents released in order to avoid negative impacts from Allee effects (Hopper and Roush 1993; Shea and Possingham 2000). Generally, parasitoids with an intermediate dispersal rate are more likely to establish than species with low or high dispersal rates because, for example, those with low dispersal rates may have low success locating hosts while those with high dispersal rates have increased risks of Allee effects (i.e. mate limitation at low population densities) (Heimpel and Asplen 2011). Active dispersal capacities, or a species' propensity and potential ability to disperse (i.e. speed, distance, and activity) without assistance, vary among parasitoid taxa. For example, on a flight mill, *Cotesia*

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glomerata (L.) (Hymenoptera: Braconidae) was observed to have a mean flight distance between 0.05 (\pm 0.01) km and 0.75 (\pm 0.20) km depending on the food source provided prior to flight (Wanner et al. 2006) whereas *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) had a mean flight distance of 5.27 (\pm 0.51) km after 24 h (Yu et al. 2009). The level of parasitism exerted on the target pest population, which ultimately will determine its ability to establish and control that population, is in part driven by its dispersal capacity. Therefore, characterizing the dispersal capacity of a biological control agent is an important consideration when developing any biological control program.

Morphological and life history traits, such as age and body size, as well as abiotic factors, such as temperature, can affect insect dispersal capacity. For example, in *Sirex noctilio* F. (Hymenoptera: Siricidae), body size, temperature, and sex all influence flight speed, distance flown, and frequency of flight (Gaudon et al. 2016). Flight capacity has been reported to decrease with increasing age in many insect orders, including Coleoptera [e.g. *Tribolium castaneum* (Herbst) (Tenebrionidae), Perez-Mendoza et al. (2011)], Diptera [e.g. *Aedes aegypti* L. (Culicidae), Rowley and Graham (1968)], Hemiptera [e.g. female *Oncopeltus fasciatus* (Dallas) (Lygaeidae), Dingle (1965)], and Hymenoptera [e.g. *Tetrastichus planipennis* Yang (Eulophidae), Fahrner et al. (2014)]. In general, larger wasps have a greater flight capacity than smaller ones (e.g. Bruzzone et al. 2009; Fahrner et al. 2014), perhaps related to their greater energy stores and muscle mass. Other studies have shown that older parasitoids, especially those without access to carbohydrates and water, have reduced flight capacity and are less likely to survive than younger ones after being tethered to a flight mill for 24 h (e.g. Fahrner et al. 2014). Thus, body size, access to food, and other traits may all impact parasitoid performance, and having more information as to how they affect an individual species' ability to disperse will help to better select the most appropriate biological control agent.

Similarly, insect fecundity is well documented to be affected by body size, age, and temperature. Within many parasitoid species, fecundity is known to increase with body size (e.g. Rosenheim and Rosen 1991; Waage and Ng 1984), although this is often much more complex because other factors, such as

longevity, can affect it and lead to an unclear relationship between these parameters (Leather 1988). Further, maximum fecundity occurs at an optimal temperature and decreases toward the upper and lower limits around that optimum (Ratte 1985). A parasitoid with high maximum lifetime (i.e. potential) fecundity should be able to parasitize more hosts than a parasitoid with low potential fecundity, and thus could be predicted to have higher realized fecundity under field conditions.

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), was accidentally introduced into North America from Asia, and was first discovered in 2002 around the Detroit-Windsor area (Haack et al. 2002). Since then, it has become one of the most damaging and costly insects invading North American forests (Herms and McCullough 2014), with an estimated impact of up to US\$2 billion for the removal, replacement, and treatment of street and backyard ash trees [*Fraxinus* L. (Lamiales: Oleaceae)] in Canadian urban areas throughout the natural distribution of native ash (McKenney et al. 2012). During its larval stage, EAB can kill all five eastern North American ash species it attacks, including *F. pennsylvanica* Marshall (green or red ash), *F. americana* L. (white ash), *F. nigra* Marshall (black ash), *F. quadrangulata* Michx. (blue ash) (Anulewicz et al. 2008), and *F. profunda* (Bush) Bush (pumpkin ash) (Czerwinski et al. 2007). Concerns have also been expressed about the possibility of it attacking non-ash tree species in North America, given that it has been shown to complete development on white fringetree, *Chionanthus virginicus* L. (Lamiales: Oleaceae), a novel host in Ohio, USA (Cipollini 2015), and in the laboratory on cultivated olive, *Olea europaea* L. (Lamiales: Oleaceae) (Cipollini et al. 2017).

Eradication is no longer considered a viable option for managing EAB, so ongoing efforts aim to slow its spread across North America. Biological control is one of the few long-term tools available to incorporate into such a management strategy (Herms and McCullough 2014). *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae), a solitary larval parasitoid native to North America, is known to attack native *Agrilus* spp. (Coleoptera: Buprestidae) (e.g. Barter 1957, 1965; Haack et al. 1981), and has been proposed as a candidate for augmentative biological control (e.g. Roscoe 2014), using it in supplemental releases across the introduced range of EAB. It is thought to

have significant potential because it has been observed parasitizing up to 40.7% of EAB larvae at some sites in southern Ontario, Canada (Lyons 2010).

The biology of many native North American parasitoids is poorly documented, although biotic and abiotic factors clearly affect a parasitoid's ability to disperse in its habitat, and ultimately to locate and parasitize hosts. Thus, it is extremely important to understand the dispersal capacity and fecundity of any parasitoid being assessed for potential use in a biological control program. Critical predictors of a parasitoid's activity, and ultimately successful parasitism in the field, are its natural modes of dispersal (e.g. walking and flying). The basic biology of *P. sulcata* has been recently described by Roscoe (2014), but its dispersal capacity has not been studied nor has its fecundity been verified even though these parameters are important to evaluate its potential as a suitable candidate for augmentative biological control.

Here, we explore the flight capacity and walking activity of *P. sulcata* in relation to biological and environmental conditions in order to predict its potential for dispersal when used augmentatively against EAB. Specifically, we: (1) examine the relationship between parasitoid age and body size, temperature, and time of day on parasitoid flight capacity and walking activity; (2) determine how flight, parasitoid age, and body size affect potential fecundity; and (3) investigate whether there is a trade-off between flight capacity and walking activity in this parasitoid species. We predict that at warm temperatures larger and younger wasps will have a greater dispersal capacity (i.e. fly and walk faster, farther, and more frequently) than smaller and older ones, and that potential fecundity will decrease with increasing wasp age and decreasing body size. Less clear is the relationship between parasitoid flight and walking capacities, which could be either positive, in that wasps walking more also fly more (i.e. active vs. inactive parasitoids), or negative, in that wasps walking more fly less (i.e. a trade-off between the energy expended on flying and walking).

Materials and methods

Wasp collection and rearing

EAB-infested ash trees were identified in three woodlots in southern Ontario, Canada: two from properties managed by the Ausable Bayfield Conservation Authority (43.34573, – 81.5572 and 43.38295, – 81.54295) and another on a private ash plantation near Brooke Line, Alvinston, ON (42.84389, – 81.85438). These trees were felled, cut into ~ 50-cm lengths, brought to the Great Lakes Forestry Centre (Sault Ste. Marie, Ontario, Canada), and put into rearing cabinets at temperatures ranging from 23 to 28 °C depending on cabinet height. Relative humidity (RH) was maintained at 45% and a 16:8 L:D photoperiod. Adult wasps were collected daily from the rearing cages and housed together, separated by sex, in ventilated, clear plastic cups (375 ml) at 24 °C, 60–70% RH, and a 16:8 L:D photoperiod (the latter starting at 7h00 and ending at 23h00) until they were used in the experiments. Wasps were fed using a streak of pure honey on duct tape attached to the inside of each cup. Water was provided in each cup by saturated cotton inside a 12-ml vial.

Experimental procedures

Wasps were walked and flown 1–26 days following adult emergence. The body mass of each wasp was recorded as a proxy for body size taking pre- and post-walking and pre- and post-flight measurements to the nearest 0.1 mg using a digital analytical balance (Mettler Toledo AG285). After weighing, wasps were gently moved into KIMAX (USA) test tubes (150 mm in length, 18-mm opening diameter) with a drop of a honey-water solution (i.e. 50% honey, 50% water), and the tubes laid out horizontally in the laboratory at ~ 24.5 °C. Walking activity was observed for 70 wasps, with an observation event occurring every 5 min over 2.5 h, from 9h00 to 11h30, recording whether the wasp was 'walking' or 'resting'. After the walking period, wasps were chilled to slow their movement and the head of an insect pin (# 1) was glued (Quick Grip Permanent Adhesive, Beacon Adhesives, Mt. Veron, NY, USA) to the prothorax of each to allow it to be tethered to a flight mill. The flight mills were similar to those used by Jones et al. (2010) and Wiman et al. (2014) [see Haavik et al. (2016) for

more detail]. All wasps were then flown for 24 h at 21.0, 24.0, 24.5, or 25.0 °C under controlled conditions (i.e. 16:8 L:D photoperiod and 50–70% RH). Female wasps were dissected after each flight period to count their total number of eggs.

Data processing and analyses

LabVIEW Full Development System software was used to record in-flight data and Scout 1.6.0.0 (Signal.X Technologies LLC, Commerce Township, MI, USA) to export these data to Microsoft Excel. The R software package, ‘flightmillR’ (developed by CJK MacQuarrie), was used to calculate summary statistics, including mean flight bout speed, total distance flown, and number of flight bouts taken. A successful flight bout was defined as ≥ 30 s of continuous flight. Bouts of flight < 30 s were excluded from the analysis. Time of day was specified as either ‘light’ if wasps were flying during the photoperiod or ‘dark’ if wasps were flying during the scotoperiod. The proportion of time spent walking (i.e. the number of walking events over the total count of walking and resting events) was a binomial outcome (i.e. walking or resting), so we specified the number of ‘walking’ and ‘resting’ counts observed in a two-vector response variable. In all cases, female and male wasps were analyzed separately. All possible interactions were considered. In cases where interaction terms were not significant, they were removed to use the simplest model that, at minimum, tested main effects. All data were analyzed using the R statistical environment (R Development Core Team 2018).

Flight capacity

The effects of wasp age, wasp body size, and temperature on mean flight bout speed, total distance flown, and the number of flight bouts taken were fit to linear models for multi-factor analysis. Each model was assessed using graphical methods for homogeneity of variance and normality of the residuals. Models for total distance flown and number of flight bouts taken violated our assumptions, so both dependent variables were log-transformed to improve the model fit.

The effects of time of day and temperature on distance flown were also tested using a linear mixed-effects model (LMM), with a random effect on each

wasp accounting for repeated measures on individuals in the photoperiod and scotoperiod. Because there was an unequal L:D ratio across the 24-h flight period, we analyzed the mean distance flown per hour in the photoperiod and scotoperiod instead of total distance flown. This LMM violated our assumptions as before, so mean distance flown per hour was log-transformed, which improved the model fit. Pearson’s χ^2 test with Yates’ continuity correction was used to determine whether an abrupt change from photoperiod to scotoperiod or scotoperiod to photoperiod stimulated flight in the wasps.

A paired *t* test examined whether there was a difference in wasp body mass before and after the 24-h flight period. The effects of mean flight bout speed, total distance flown, and the number of flight bouts taken on body mass lost were tested using multiple regression. We observed that residuals had a non-normal distribution and heterogeneity in the variance of mass lost among individual wasps, so body mass lost was log-transformed to improve the model fit. The same effects on post-flight survival were tested using logistic regression with a logit link function, which we assessed for overdispersion by examining the residual deviance, which was considered similar to the residual degrees of freedom.

Walking activity

A generalized linear model (GLM) with binomial errors and logit link function was fit to test the effects of wasp age and body size on the proportion of time spent walking. We diagnosed overdispersion using the same method as above and re-fit the model taking into account such overdispersion. This did not improve the model, so a generalized linear mixed model (GLMM) with binomial errors and logit link function and observation-level random effect was fit to account for the overdispersion by modelling the excess variance. Because this is a relatively novel approach (see Harrison 2015), we also tested the same by adjusting the covariance matrix and fit statistics using the R package ‘dispmod’ and compared the two models using a likelihood ratio test.

Relationship between flight capacity and walking activity

Simple linear models were used to test the effect of walking activity on both total distance flown and the number of flight bouts taken. Both models violated our assumptions, so total distance flown and the number of flight bouts taken were log-transformed, which improved the models.

Fecundity

A linear model was used to test the effects of wasp age, body size, and whether a wasp flew on egg counts. This model met our assumptions of constancy of variance and normally-distributed residuals.

Results

Body size

Female *P. sulcata* body mass ranged from 6.2 to 22.2 mg, with a mean \pm SE of 11.3 ± 0.3 mg ($n = 83$), whereas male *P. sulcata* body mass ranged from 2.1 to 10.2 mg, with a mean of 6.8 ± 0.4 mg ($n = 23$). Female wasps were significantly larger than males in terms of body mass (Welch *t* test: $t = 10.13$, $df = 48.49$, $P < 0.001$), with the largest female *P. sulcata* being 2.18 times heavier than the largest male.

Flight capacity

For female *P. sulcata*, successful flights were recorded for 30 of 83 individuals, with the total distance flown by females averaging 0.32 ± 0.15 km. The farthest flight by a female wasp was 4.05 km, with each taking on average 6 ± 1 bouts of flight. The maximum number of flight bouts taken by a female wasp was 24. Female wasps had a maximum flight speed of 1.18 ± 0.08 km h⁻¹.

Mean flight bout speed was not affected by the age of female wasps ($F = 0.06$; $df = 1, 26$; $P = 0.798$) or temperature ($F = 1.30$; $df = 1, 26$; $P = 0.265$), however body size had a significant effect on mean speed for female wasps, with larger females observed flying significantly faster than smaller ones ($F = 5.71$; $df = 1, 26$; $P = 0.024$) (Fig. 1). Total distance flown was not affected by the age of female *P. sulcata* ($F = 0.85$;

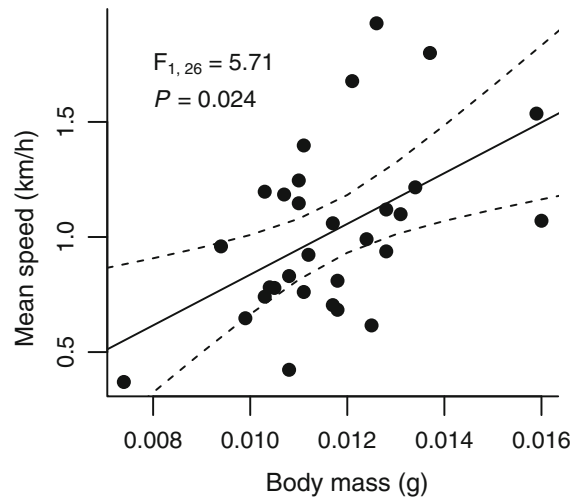


Fig. 1 Relationship between flight speed and female *Phasgonophora sulcata* body size tested on a flight mill for 24 h at 21.0, 24.0, 24.5, or 25.0 °C under controlled conditions (i.e. 16:8 L:D photoperiod and 50–70% RH) ($n = 30$). The solid line shows the fit and the dotted lines show the 95% confidence intervals

$df = 1, 26$; $P = 0.365$), or body size ($F = 1.17$; $df = 1, 26$; $P = 0.290$). However increasing temperature did significantly increase total distance flown ($F = 4.59$; $df = 1, 26$; $P = 0.042$). As age of female *P. sulcata* increased, their number of bouts of flight taken significantly decreased ($F = 5.75$; $df = 1, 26$; $P = 0.024$) (Fig. 2), but the number of bouts of flight was not affected by female body size and temperature ($F = 0.26$; $df = 1, 26$; $P = 0.613$ and $F = 3.04$; $df = 1, 26$; $P = 0.093$, respectively).

There was no effect of time of day on the distance flown for female *P. sulcata* ($F = 3.04$; $df = 1, 29$; $P = 0.092$). We continued to observe a significant effect of temperature on distance flown by wasps, where increasing temperature increased the total distance flown ($F = 5.99$; $df = 1, 28$; $P = 0.021$), although this distance was not affected for females flying immediately after an abrupt shift in light intensity (between photoperiod and scotoperiod and vice versa) ($\chi^2 = 1.70$, $df = 1$, $P = 0.193$).

Significant body mass was lost by female wasps after being tethered to the flight mill for 24 h ($t = 6.42$; $df = 28$; $P < 0.001$). Wasps lost significantly more mass as their mean flight speed increased ($F = 11.34$; $df = 1, 26$; $P = 0.002$) (Fig. 3), but no relationship was observed between mass lost and total distance flown ($F = 0.19$; $df = 1, 26$; $P = 0.665$) or the number

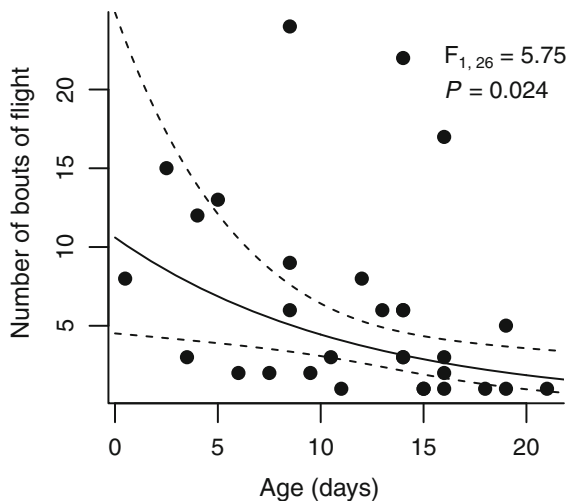


Fig. 2 Relationship between the number of flight bouts taken by *Phasgonophora sulcata* and wasp age tested on a flight mill for 24 h at 21.0, 24.0, 24.5, or 25.0 °C under controlled conditions (i.e. 16:8 L:D photoperiod and 50–70% RH) ($n = 30$). Log-transformed data were back-transformed for presentation. The solid line shows the fit and the dotted lines show the 95% confidence intervals

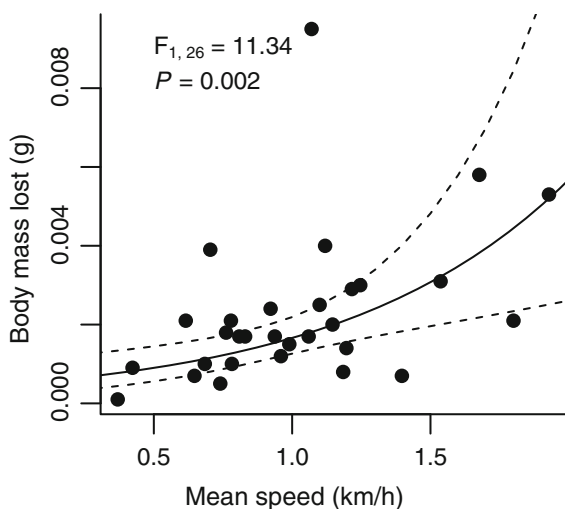


Fig. 3 Relationship between *Phasgonophora sulcata* body mass lost and flight speed tested on a flight mills for 24 h at 21.0, 24.0, 24.5, or 25.0 °C under controlled conditions (i.e. 16:8 L:D photoperiod and 50–70% RH) ($n = 30$). Log-transformed data were back-transformed for presentation. The solid line shows the fit and the dotted lines show the 95% confidence intervals

of flight bouts taken ($F = 0.07$; $df = 1, 26$; $P = 0.796$). Mean flight bout speed, total distance flown, and number of flight bouts taken were not significant predictors of post-flight survival ($\chi^2 = 0.76$, $df = 1$,

$P = 0.38$ and $\chi^2 = 0.09$, $df = 1$, $P = 0.763$ and $\chi^2 = 1.29$, $df = 1$, $P = 0.256$, respectively).

Successful flights were only recorded for five of 23 males, with a total distance flown averaging 0.13 ± 0.07 km. The farthest distance flown by a male wasp was 0.39 km, with an average of 20 ± 11 flight bouts. The maximum number of flight bouts taken by a male was 63. Male wasps had a maximum flight speed of 1.14 ± 0.25 km h⁻¹. No analysis was made of age, body mass, temperature, and time of day on males as successful flights were recorded from too few wasps (five).

Walking activity

Individual wasps displayed considerable range in walking activity. Female wasps were observed walking between 3 and 100% of the observation events, and none were observed inactive for the entire assay. One 16-day old female weighing 8.0 mg walked 3% of the observation events whereas a 15-day old female weighing 10.0 mg walked 100% of the observation events. On average, female wasps walked $62 \pm 3\%$ of the observation events.

Male wasp walking activity was similarly variable as female wasp walking activity. Male wasps spent between 3 and 80% of the observation events walking, and none were observed inactive for the entire assay. One 16-day old male weighing 8.9 mg walked 3% of the observation events while a 13-day old male weighing 8.0 mg walked 80% of the observation events. On average, male wasps spent $29 \pm 7\%$ of the time they were observed walking.

The time spent walking by female and male wasps was not affected by age ($\chi^2 = 1.73$, $df = 1$, $P = 0.188$ and $\chi^2 = 0.10$, $df = 1$, $P = 0.750$, respectively) or body size ($\chi^2 = 0.27$, $df = 1$, $P = 0.602$ and $\chi^2 = 0.21$, $df = 1$, $P = 0.643$, respectively). Both female and male wasps lost significant body mass after the 2.5-h walking period (paired t-test: $t = 2.64$, $df = 57$, $P = 0.011$ and $t = 5.41$, $df = 11$, $P < 0.001$, respectively). On average, female body mass was reduced from 11.3 ± 0.3 mg to 10.7 ± 0.3 mg and male body mass was reduced from 7.0 ± 0.4 mg to 6.4 ± 0.4 mg.

Results of the model with the adjusted covariance matrix and fit statistics were similar to that of the GLMM (age: $\chi^2 = 1.46$, $df = 1$, $P = 0.226$ and body size: $\chi^2 = 0.24$, $df = 1$, $P = 0.625$). Thus, it was

expected that these two models would be similar ($\chi^2 = 0.00$, $df = 1$, $P = 1.000$).

Relationship between flight capacity and walking activity

There was no evidence of a significant trade-off between the walking activity and total distance flown ($F = 0.41$; $df = 1$, 22 ; $P = 0.527$) or number of flight bouts taken ($F = 0.34$; $df = 1$, 22 ; $P = 0.567$) by female wasps. This was not surprising considering the large variation in wasp walking activity coupled with the low flight capacity of these same wasps. For example, of the wasps that both walked and flew during the experiments, two walking 93% of the observation events also flew 0.02 km over six flight bouts and 0.38 km over three flight bouts, while one wasp walking 3% of the observation events then flew < 0.01 km over one flight bout.

Potential fecundity

The maximum number of eggs observed in a single wasp was 85 eggs at 0.5 days old while the minimum number was 29 eggs at 24 days old. On average, females had 60 ± 3 eggs upon dissection after flying. Whether a wasp flew did not affect its egg count ($F = 2.62$; $df = 1$, 19 ; $P = 0.122$). The relationship between *P. sulcata* body size and egg count was not significant ($F = 2.29$; $df = 1$, 19 ; $P = 0.147$), although egg count decreased significantly with wasp age ($F = 6.22$; $df = 1$, 19 ; $P = 0.022$) (Fig. 4).

Discussion

Dispersal capacity in *P. sulcata*

When developing any biological control program, flight capacity and the factors that influence it are important to understand as these will help identify the most appropriate parasitoid(s) and protocol for their release. Our work is the first to explore the flight capacity of *P. sulcata*, a parasitoid native to North America being considered for augmentative release against introduced EAB. Flight capacity has also been examined for the Asian parasitoid species, *T. planipennisi*, used in classical biological control of EAB. Fahrner et al. (2014) found that female *T. planipennisi*

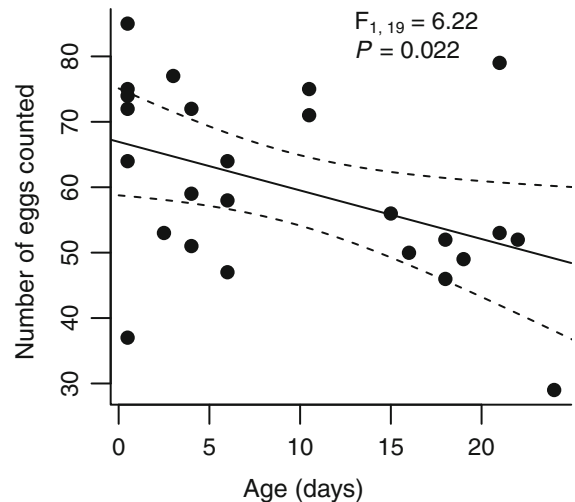


Fig. 4 Relationship between potential fecundity in *Phasgonophora sulcata* after each flight period and wasp age ($n = 25$) tested on flight mills in the lab. The solid line shows the fit and the dotted lines show the 95% confidence intervals

on flight mills in the lab flew 1.26 ± 0.17 km on average, ~ 3.9 times farther than we observed here for native female *P. sulcata*. This suggests that *P. sulcata* has limited dispersal capacity relative to other EAB biological control agents and will spread less rapidly than *T. planipennisi* when released against EAB. It also implies that *P. sulcata* will have a relatively localized impact on EAB since it cannot spread as far as other EAB parasitoids, such as *T. planipennisi*.

Parasitoid age and body size appear to have a variable impact on parasitoid activity, and this will also have implications for the release of *P. sulcata*. We found that younger female *P. sulcata* were more active in terms of flight than older females and that larger female wasps had a greater flight capacity compared to smaller ones as measured by flight bout speed. Fahrner et al. (2014) examined the effect of wasp age and body size on flight capacity in *T. planipennisi*, observing no effect of age. However they did not measure the number of flight bouts taken. Further, we found no effect of body size on the distance flown or the number of flight bouts taken by female wasps, which suggests that body size alone would not reduce the impact of *P. sulcata* in a biological control program against EAB as these parameters likely influence host location more than flight speed.

It is well established that ambient temperature affects the flight behaviour of many poikilothermic organisms, including insects (Taylor 1963), and this effect, along with parasitoid age and body size, should be considered when releasing *P. sulcata* in the field to select the optimal location for dispersal and parasitism of EAB. For example, for *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), maximum flight propensity occurred at 25 and 30 °C, whereas wasps experienced a reduced flight capacity at lower temperatures (Forsse et al. 1992). Similarly, we found that *P. sulcata* wasps flew significantly farther as the ambient temperature increased suggesting that releases will support parasitoid movement and be more effective if made in the summer, especially during periods of warm temperature. In contrast, time of day (as measured by ‘light’ or ‘dark’ period) did not influence wasp flight in our system, and this would mean that *P. sulcata* populations could be augmented and equally effective when released at any point during daylight hours. The implications of parasitoid flight activity as measured here is difficult to interpret in all cases since tethered wasps have no landing cues on a flight mill, and thus, our results may vary somewhat from realized parasitoid activity in the field.

The fact that wasps lost significant body mass after being tethered to flight mills for 24 h and also when their mean flight bout speed increased implies that access to carbohydrates and water is important for parasitoid maintenance, especially for wasps with increased flight capacity. This is true for *T. planipennis* (Fahrner et al. 2014), *C. glomerata* (Wanner et al. 2006), and many other insects in the order Hymenoptera (Beenackers et al. 1984). Carbohydrate sources, such as floral resources, can provide important nourishment for parasitoid maintenance and dispersal in the field (Wäckers 2005), and may be necessary for *P. sulcata* to achieve its maximum flight capacity.

After a parasitoid has located the habitat of its host, walking becomes the next component of its searching behaviour. We observed high variation in walking activity among *P. sulcata* as did Suverkropp et al. (2001) for the egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) at low temperature. The impact of this variation on the ability of parasitoids to establish and control an invasive insect pest population, such as EAB, is not clear. If the time spent walking is positively correlated with the ability to locate hosts, such variation in dispersal could

result in low rates of establishment and host location. Although we observed no differences in the proportion of time spent walking between wasps of varying age and body size, it is possible that younger and larger wasps can walk farther and/or faster than older and smaller ones. Here, the time spent walking was not affected by female wasp age or body size, suggesting that parasitism rates for younger wasps of all sizes would remain relatively consistent across the reproductive season. We did, however, observe reduced potential fecundity in older wasps, which might result in a lower realized fecundity in the field, especially later in the season.

Relationship between flight capacity and walking activity

The relationship between flight capacity and walking activity in the parasitic Hymenoptera is not well understood, possibly due to their small size and the difficulty in measuring these behaviours, especially under field conditions. Distinct polymorphic differences may explain varying dispersal capacities for some insects as in *Melittobia* spp. (Hymenoptera: Eulophidae), parasitoids attacking solitary bees and wasps. Brachypterous females within this genus develop quickly and remain within their natal patch to mate, lay eggs, and have offspring, while their macropterous counterparts are those that develop slowly, mate, and disperse from the natal patch to look for other hosts (Matthews et al. 2009). Further, host size can also impact the dispersal capacity of parasitoids, where individuals developing in particularly small hosts may emerge as smaller, and even wingless adults than those developing in larger hosts (Salt 1941). We show that the frequency of walking over a 2.5-h walking period did not affect the total distance flown or flight activity (i.e. number of flight bouts taken) during a 24-h flight period. Although the walking assay was limited in time compared to the flight assay, we did observe a reduction in body mass after the 2.5-h walking period, which may indicate that this time interval is sufficient to show depletion of energetic resources in *P. sulcata*. For parasitoids such as *P. sulcata*, where no such morphological differences are apparent, it is less obvious there is a trade-off between flight and walking and more likely that host size and quality impacts their dispersal capacity.

Potential fecundity

Our work shows that *P. sulcata* is pro-ovigenic and emerges with a large complement of eggs as suggested by Roscoe et al. (2016). Similar to other pro-ovigenic parasitoids, eggs are developed from nutritional resources gained during larval feeding, but these resources must be split, in part, between the parasitoid's fitness parameters (i.e. egg development, dispersal capacity, and longevity) (e.g. Innocent et al. 2010; Venkateswaran et al. 2017). Thus, it is expected that pro-ovigenic parasitoids with increased egg loads at emergence will have decreased dispersal capacities and reduced longevity (Jervis et al. 2001). Consequently, pro-ovigenic parasitoids are recommended for environments with high host densities, where they can quickly locate and oviposit on or in hosts following emergence. In contrast to Roscoe (2014), we observed a trade-off between wasp age and potential fecundity in *P. sulcata* during the first 26 days of its life, and a decrease in flight capacity as wasps aged. Thus, we suspect that maximum EAB parasitism by *P. sulcata* would occur soon after adult parasitoid emergence and that wasps consequently should be released before their emergence (i.e. possibly as pupae in EAB hosts) in order to maximize their egg load complement and potential for parasitism.

Several metrics can be used to estimate parasitoid fecundity, with female body size often being a good predictor. However we observed no correlation between potential fecundity and body size in *P. sulcata*. Few studies have found no relationship between female body size and fecundity (e.g. Boggs 1986; Johnson 1990). The fact that we did not see such a relationship may be partly explained by differences in parasitoid age at the time of dissection or possibly by some environmental constraint, such as differences in host quality, where less fecund parasitoids developed from poor-quality hosts and more fecund parasitoids developed from high-quality hosts irrespective of size. As such, it might be important to provision wasps with a food source at release locations in order to increase their potential fecundity and longevity.

Application to biological control

Flight capacity, walking activity, and fecundity are all important determinants of the ability of a parasitoid to

locate and parasitize its host. Our results with *P. sulcata* may be useful to optimize a release protocol against EAB. Poor capacity to fly or walk limits the effectiveness of a parasitoid as a biological control agent. Thus, our findings that flight capacity (i.e. number of flight bouts taken) and fecundity decrease with wasp age suggest that this parasitoid should be released in the field as soon as possible after emergence where mass-rearing in the laboratory is possible or as pupae if it will be released by transporting parasitoid-infested ash material. Further, the weak dispersal capacity of *P. sulcata* observed on flight mills, combined with the reduction in flight capacity and fecundity with increased age, suggests that augmentative releases should occur near EAB-infested ash trees for optimal host location and parasitism. Given personal observations with this system in the laboratory and the fact that wasps walked but not all flew, it appears that *P. sulcata* uses walking and hopping more than flight for dispersal and host finding, and this, combined with the fact that we saw no effect of wasp age or body size on *P. sulcata* walking activity, suggests that the most successful approach for implementation in an augmentative biological control program would be to release it in close proximity to the target host, EAB.

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Author contributions JMG, JDA, and SMS conceived and designed the experiment; JMG conducted the experiment and analyzed the data; and all authors contributed to writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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