

Leucopis glyphinivora, a potential aphidophagous biocontrol agent? Predation and comparison with the commercial agent *Aphidoletes aphidimyza*

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Abstract The silver fly *Leucopis glyphinivora* Tanasijtshuk (Diptera: Chamaemyiidae) is an aphidophagous predator during its larval stage. Few studies have examined the predation habits of this species for biological control. Larval voracity of *L. glyphinivora* was measured under laboratory and controlled greenhouse conditions and compared with a commercially available biocontrol agent, *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae). Laboratory tests were conducted in Petri dishes using *Myzus persicae* Sulzer (Hemiptera: Aphididae) on potato leaves. In greenhouse tests, predator voracity was evaluated with various plant-aphid treatments. In the laboratory, silver fly larvae consumed 39% more aphids than *A. aphidimyza* throughout their larval development. In the greenhouse, *L. glyphinivora* consumed more aphids than *A. aphidimyza* regardless of treatment. The highest voracities were obtained on

tomato and bell pepper infested with *M. persicae*. No antagonistic predatory effects were observed when predators were used together. This study provides useful insight on *L. glyphinoivora* as an efficient aphid predator but more research is needed to establish its potential for biological control.

Keywords *Aphidoletes aphidimyza* · Cecidomyiidae · Chamaemyiidae · Furtive predation · Greenhouse · *Leucopis glyphinivora* · Voracity

Introduction

Aphids are major crop pests in many greenhouses (Blackman and Eastop 2007; Sorensen 2009). The use of chemical pesticides has led to pest resistance and environmental problems by having adverse effects on non-target species. Biological pest control is used as an alternative to traditional chemical pesticides to avoid these risks while still maintaining pest populations low (van Lenteren and Woets 1988; Blackman and Eastop 2007; Hoddle and van Driesche 2009; Sorensen 2009). While the market for biological control is growing and many species have been tested, only a fraction of the potential present in nature has been studied for biocontrol evaluations (Sloggett 2005; Begum et al. 2017; van Lenteren et al. 2018). It is therefore likely that many interesting species have been overlooked for biocontrol programs in

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greenhouses. Various criteria are used to determine if a predatory insect has the potential to become an effective biocontrol agent. Specificity to the target pest, ability to suppress pest populations, numerical response, searching ability, reproductive behaviour, generation time and ability to be mass-reared for commercial production are all important factors that need to be well understood before a biocontrol program can be established (Coppel and Mertins 1977; Stiling and Cornelissen 2005).

The silver fly *Leucopis glyphinivora* Tanasijtshuk (Diptera: Chamaemyiidae) is an aphidophagous predator in its larval stage (Rad et al. 2003; Satar et al. 2015; Mehrparvar et al. 2016). Very little information is currently available about the biology and ecology of this species, specifically on its efficiency as a biological control agent. *L. glyphinivora* has a Holarctic distribution, being found throughout much of Europe and North America (Carroll and Hoyt 1984; Brewer and Noma 2010; Rakhshani et al. 2010; Natshuk and Bagachanova 2013; Kahanpää 2014; Satar et al. 2015). Larvae of this silver fly feed on a wide variety of aphid species, many of which are agricultural pests such as the black bean aphid *Aphis fabae* Scopoli (Rad et al. 2003; Mustață et al. 2010), the soybean aphid *Aphis glycines* Matsumura (Kaiser et al. 2007) and the green apple aphid *Aphis pomi* DeGeer (Carroll and Hoyt 1984). Other species in the *Leucopis* Meigen genus have been studied as potential biological control agents in both agriculture and forestry. *L. gaimarii* Tanasijtshuk and *L. ninae* Tanasijtshuk were used against the Russian wheat aphid *Diuraphis noxia* Mordvilko (Hemiptera: Aphididae) (Gaimari and Turner 1996a, 1997; Mohamed et al. 2000; Brewer and Elliott 2004; Noma et al. 2005). In vineyards, *L. simplex* Loew was observed to be an important natural enemy of *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae) (Stevenson 1967). In forestry, *L. hennigrata* McAlpine was used against the balsam woolly adelgid *Adelges picea* Ratzeburg (Hemiptera: Adelgidae) (McAlpine 1978; Humble 1994) while *L. argenticollis* Zetterstedt and *L. piniperda* Malloch were used to control hemlock woolly adelgid *A. tsugae* Annand (Hemiptera: Adelgidae) (Wallace and Hain 2000; Preisser et al. 2014; Kohler et al. 2016).

We have selected the predatory gall midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) as a reference species to evaluate the potential of

L. glyphinivora as a biological control agent. The midge is extensively used in greenhouses around the world to control aphid outbreaks (Malais and Ravensberg 2006; Völkl et al. 2007; Alotaibi 2008). This species was chosen for its resemblance with *L. glyphinivora* due to taxonomic proximity (dipteran), size similarity, presence of a predatory larval stage and dietary preference for aphids. *Aphidoletes aphidimyza* larvae use a so-called furtive predation strategy. Such a strategy enables *A. aphidimyza* larvae to both live and feed within the aphid colony without causing significant disturbance (Lucas and Brodeur 2001). This furtive strategy is a rare trait which allows *A. aphidimyza* to not only reduce the risk of food shortage by limiting aphid dispersal (Fréchette et al. 2008), but also to hide from intraguild predators through both a dilution effect generated by aphids (Lucas and Brodeur 2001) and a selfish herd effect by selecting a central position within the colony (Dumont et al. 2015). Commercial use of *A. aphidimyza* for control of crop pests in greenhouses began in the early 1980s and has remained a popular alternative to chemical pesticides (van Lenteren and Woets 1988; Malais and Ravensberg 2006; Powell and Pell 2007). *Aphidoletes aphidimyza* does, however, present certain undesirable characteristics. To avoid drying out, larvae pupate in the soil if RH is low, thus making them vulnerable to ground dwelling predators (van Schelt and Mulder 2000; Yukawa et al. 2008; Le Goff et al. 2016). There is also a high mortality rate amongst *A. aphidimyza* pupae and emerging adults are often unable to reach the soil surface (Yukawa et al. 2008). *Aphidoletes aphidimyza* larvae are also unable to disperse more than 63 mm before dying from starvation (Wilbert 1973) meaning once eggs are laid, biological control happens on a very local scale on the host plant. It is therefore important to study other potential biological control agents that might lack such negative traits while still sharing the same ecological niche.

The aim of this study is to evaluate the potential of *L. glyphinivora* as a new biological control agent by establishing its predation ability against aphid populations. This trait is then compared to that of a similar commercially available biological control agent, *A. aphidimyza*. While voracity is just one aspect of what makes an effective biocontrol agent, this information will provide a first estimation of its overall efficacy against aphid pests in a greenhouse environment. First,

daily and life time voracity of larvae of the two predators were evaluated and compared under laboratory conditions. Second, we observed the in situ impact of larvae against aphids in a greenhouse using different aphid-host plant combinations. Finally, the combined use of the two predators together was evaluated to determine the possibility of synergistic or antagonistic effects. The main hypothesis is that, due to the slower development time of *L. glyphinivora*, this species is more voracious than *A. aphidimyza* (Canale et al. 2002; Rad et al. 2003; Barriault et al. 2018). Moreover, *L. glyphinivora* is known to be a rather polyphagous species inhabiting a wide variety of host plants (Tanasijtshuk 1986; Satar et al. 2015). We can then expect that the specific crop plants or pest aphids will not significantly alter its predation efficacy. This behaviour is also observed throughout the *Leucopis* Meigen genus (Brewer and Noma 2010; Zhou et al. 2014; Colares et al. 2015; Satar et al. 2015; Kohler et al. 2016).

Materials and methods

Laboratory experiment

Insect material

Wild *L. glyphinivora* specimens were collected from greater burdock (*Arctium lappa* L.) infested with the black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae) found on campus grounds at the *Université du Québec à Montréal* (Montréal, Canada) (43°30'34"N; 73°34'08"O) throughout July 2016. Specimens were mostly collected as larvae and pupae. *Aphidoletes aphidimyza* were commercially supplied as pupae from Plant-Products Québec (Laval, Quebec, Canada). Both predators were reared in the same growth chamber under identical conditions, and for multiple generations using the Gaimari and Turner (1996b) method. Mass rearing cages consisted of a cubic polyvinyl chloride (PVC) frame (35 × 35 × 35 cm³) covered with a sheet of white muslin. Potato plants, *Solanum tuberosum* (var. Norland), infested with the green peach aphid *M. persicae*, were introduced into rearing cages and served as the main host plant and prey aphid for both predators. A saturated solution of water and table sugar (sucrose) was supplied as an additional food source for adults. A

dry mixture of table sugar and brewer's yeast (*Saccharomyces cerevisiae*) (1:1 ratio) was also added into the *L. glyphinivora* cages as another food source for adults. Every week, a single non-infested potato plant was introduced into each rearing cage until adult emergence began, after which adults were transferred into new rearing cages. Old cages were kept for seven days before discarding plant material to ensure all adults had emerged. Rearing cages were kept in a growth chamber at 24 °C, RH 75%, and L:D 16:8.

Laboratory tests

Laboratory tests were conducted in small Petri dishes (diameter = 5 cm) with agar gel and a potato leaf lining the bottom of the dish. A single egg (*L. glyphinivora* or *A. aphidimyza*) was collected from a rearing cage and introduced into the Petri dish. A colony of 20 first stage (N₁) *M. persicae* was also introduced into the Petri dish, allowing larvae to feed as soon as they hatched. Once larvae hatched, larval voracity was noted every 24 h until pupation occurred. Observations continued until each larva had pupated. Additional aphids were added daily into the Petri dishes to re-establish the colony at 20 aphids.

A predator free control treatment was conducted under identical conditions as those with a predator. The control treatment was used to consider natural aphid mortality. The following model was used to calculate voracity while taking natural prey mortality into account (Soares et al. 2003):

$$V_0 = (A - a)ra \quad (1)$$

where V_0 is the number of prey eaten by a given predator, A is the number of prey available at the start of the test, a is the number of prey remaining after a certain time frame (24 h) and ra is the ratio of prey alive in the control treatment after the same time frame.

Greenhouse experiment

Insect material

Leucopis glyphinivora specimens came from a pre-existing rearing colony held at the biological control laboratory at the *Université du Québec à Montréal*. Wild specimens were collected on campus grounds (Montréal, Canada) from apple trees (*Malus pumilla* Miller) infested with the green citrus aphid *Aphis*

spiraecola van Der Goot (Hemiptera: Aphididae) throughout July 2009. Wild *L. glyphinivora* were regularly collected and added to the experimental rearing colony. As with the laboratory tests, *A. aphidimyza* specimens came from Plant-Products Québec as pupae. Both predators were reared in cubic PVC cages ($35 \times 35 \times 35 \text{ cm}^3$) covered with a sheet of white muslin. Insects used for the greenhouse tests were reared with the same procedure used for the laboratory test rearing. Rearing cages for both predators were kept in a growth chamber at $24 \text{ }^\circ\text{C}$, RH 75%, and L:D 16:8.

Greenhouse tests

Two types of tests were carried out: single predator and combined predator. All greenhouse tests were held at the Fermes Lufa Inc. © greenhouse in Montréal, Canada. Greenhouse conditions were $23 \text{ }^\circ\text{C}$, RH 73%, and L:D 16:8. Single predator greenhouse tests were conducted from April to August 2013. The crop plants used were eggplant *Solanum melongena* L. (var. Jaylo), tomato *S. lycopersicum* L. (var. Rebelski), bell pepper *Capsicum annuum* L. (var. Red Knight) and cucumber *Cucumis sativus* L. (var. Camaro). All crop plants used were at the fruit bearing stage and had between 15 and 20 leaves. Three pest aphid species were used in these tests as well: the potato aphid *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae), the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) and the peach-potato aphid *M. persicae*. Various crop plant-pest aphid combinations were used: eggplant with *M. euphorbiae*, eggplant with *M. persicae*, tomato with *M. euphorbiae*, tomato with *M. persicae*, bell pepper with *M. persicae* and cucumber with *A. gossypii*.

In the *M. euphorbiae* and *M. persicae* treatments, aphid colonies were established at 20 aphids. For the *A. gossypii* treatment, aphid colonies were set to 30 aphids due to the smaller size of this species. All colonies were made up of N_1 stage aphid nymphs. Aphid colonies were placed on a crop plant leaf and confined to a clip cage (diameter = 5 cm) (MacGillivray and Anderson 1957). A single predator larva having hatched less than 24 h prior was released into each clip cage at the start of tests. A predator free control treatment was also established. Aphid mortality was recorded seven days after predator releases.

Combined predator greenhouse tests took place throughout July and August 2013. Cucumber plants infested with *A. gossypii* were used for these tests. As with the single predator tests, an aphid colony was established in a clip cage set on the crop plant. Colonies were comprised of 60 N_1 stage aphid nymphs. Four treatments were set: a single *L. glyphinivora* larva, a single *A. aphidimyza* larva, one *L. glyphinivora* larva with one *A. aphidimyza* larva combined and a predator free control treatment. The combined treatment had a single larva of each predator simultaneously. All predators were introduced in the clip cage less than 24 h after hatching. Clip cages were observed three days after predator introduction to establish aphid mortality. In order to assess the effect of combining both predators, expected combined voracity was calculated using the following model (Soluk 1993):

$$C = N_p(P_1 + P_2 - P_1P_2) \quad (2)$$

where C is the expected combined consumption, N_p is initial prey density and P_1 and P_2 are the proportion of prey consumed by each predator respectively when alone. As with the laboratory tests, control treatments established in the greenhouse tests were used to account for natural aphid mortality using Eq. 1.

Statistical analysis

Mean consumption was compared between predator species for each treatment. A Shapiro–Wilk test was conducted on the data to test for normality before further analysis. Appropriate statistical tests were selected accordingly. With the laboratory test data, mean daily aphid voracity was compared between predators with a Wilcoxon test since data were shown to not follow a normal distribution. Total larval voracity was compared between predators using Welch’s two sample *t* test. A two-way ANOVA was used to compare mean daily predator voracity between species throughout larval development. Mean proportion of aphids consumed in the single predator greenhouse tests were compared between predators and treatments with a two-way ANOVA. A treatment consisted of a specific aphid pest-crop plant assemblage, which means that six treatments were observed in total. The proportion of aphids consumed was used since not all treatments had the same number of aphids at the start of each test. A Tukey HSD post-hoc test

was conducted to identify significant differences between treatments. A one-way ANOVA was used for the combined predator test to compare mean 3-day voracity between *L. glyphinivora* alone, *A. aphidimyza* alone, both predators together and expected voracity of both predators together. The R statistical software version 3.3.1 (R Development Core Team 2016) was used to conduct statistical analyses with each data set.

Results

Laboratory experiments

There was no significant difference in the mean daily voracity between *L. glyphinivora* and *A. aphidimyza* with *M. persicae* on potato leaves (Wilcoxon test: $U = 9101$; $P = 0.567$). Mean daily voracity was 4.26 ± 0.26 (mean \pm SE) aphids per day for *L. glyphinivora* and 4.43 ± 0.39 aphids per day for *A. aphidimyza*. The mean total amount of aphids consumed throughout larval development was significantly different with *L. glyphinivora* consuming significantly more aphids than *A. aphidimyza* (Welch t test: $df = 31.621$; $t = -4.759$; $P < 0.001$). On average, a single *L. glyphinivora* larva killed a total of 38.13 ± 0.60 aphids while a single *A. aphidimyza* larva killed a total of 23.22 ± 1.29 aphids. Total larval consumption was 39% higher for *L. glyphinivora*.

Silver fly reached peak voracity on the 7th day of larval development, with an average of 7.23 aphids consumed (Fig. 1a). On the 3rd day of larval development, *A. aphidimyza* larvae reached a peak consumption of 9.34 aphids (Fig. 1a). The pattern of mean daily larval voracity varied significantly with larval development (Fig. 1a) (two-way ANOVA: $F_{5,262} = 20.345$; $P < 0.001$). Mean aphid consumption was not significantly different between *L. glyphinivora* and *A. aphidimyza* in the first 48 h after hatching (Fig. 1a). Mean daily voracity was significantly higher with *A. aphidimyza* on the 3rd and 4th days of larval development (Fig. 1a). *Leucopis glyphinivora* had a mean daily voracity significantly higher than *A. aphidimyza* on the 5th and 6th days after hatching (Fig. 1a). *Leucopis glyphinivora* larvae began to pupate on the 8th day of development. All larvae had reached pupation by the 12th day. For *A. aphidimyza* pupation happened between the 4th and 6th days of development.

The cumulative aphid consumption for 20 *L. glyphinivora* reached 763 N_1 stage *M. persicae* over a span of ten days (Fig. 1b). A population of 19 *A. aphidimyza* consumed a cumulative total of 469 *M. persicae* over a period of five days (Fig. 1b). Cumulative prey consumption by *L. glyphinivora* surpassed what was observed with *A. aphidimyza* on the 6th day after hatching (Fig. 1b). This coincided with the end of larval development in *A. aphidimyza* and with peak larval consumption in *L. glyphinivora* (Fig. 1a). Both predator species started attacking aphids less than 24 h after hatching.

Greenhouse experiments

Leucopis glyphinivora consumed significantly more aphids than *A. aphidimyza*, regardless of treatment (two-way ANOVA: $F_{1,286} = 18.310$; $P < 0.001$). There was a significant difference in predator consumption between the various prey aphid-host plant combination treatments used (two-way ANOVA: $F_{5,286} = 7.413$; $P < 0.001$) with *M. persicae* on tomato and bell pepper having the greatest effect (Fig. 2). A higher proportion of aphids was consumed in the aforementioned treatments by both predators since the interaction between predator species and treatment was not significant (two-way ANOVA: $F_{5,286} = 0.994$; $P = 0.422$), (Fig. 2).

There was no significant difference in the 3-day larval voracity between *L. glyphinivora* and *A. aphidimyza* when they were released individually with a colony of 60 *A. gossypii* (Fig. 3). Voracity was significantly higher when both predators were released together and was not significantly different from the expected value (Fig. 3) (ANOVA: $F_{3,105} = 23.38$; $P < 0.001$).

Discussion

Larval voracity for *L. glyphinivora* was observed in both laboratory and controlled greenhouse conditions. The ability of a predator to control its target prey is an essential quality for a biological control agent and the most basic measure of its efficiency (Hoddle and van Driesche 2009). This chamaemyiid species was compared with a similar commercial biological control agent, *A. aphidimyza*, in order to estimate its efficacy in suppressing pest aphids. The mean daily number of

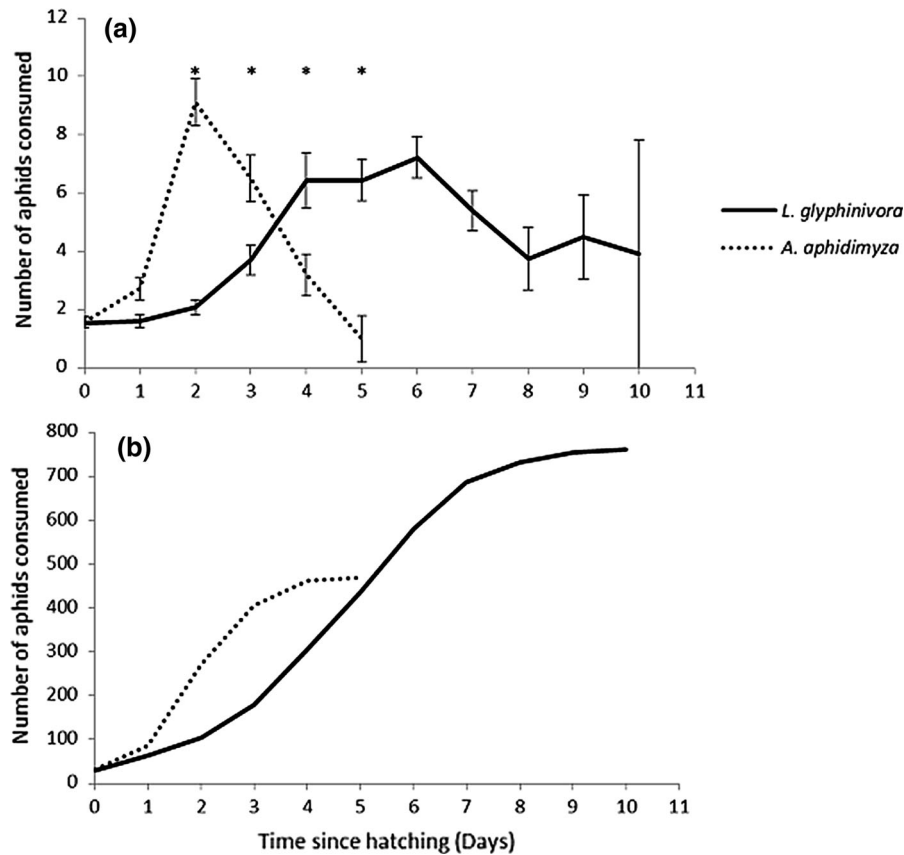


Fig. 1 Predator voracity dynamics for *L. glyphinivora* ($n = 20$) and *A. aphidimyza* ($n = 19$) under laboratory conditions shown as **a** mean daily voracity (\pm SE) and **b** cumulative population consumption throughout complete larval development. Larvae

having reached pupation were progressively removed from the experimental population. Significant differences ($P < 0.05$) between species for each time since hatching are indicated by an asterisk (*). Day 1 = hatch day

aphids killed does not differ among predator species. *Leucopis glyphinivora* does, however, consume a larger amount of aphids throughout its entire larval development, thus supporting our first hypothesis. This might be caused by its longer larval development (Canale et al. 2002; Rad et al. 2003; Barriault et al. 2018). Although this was not the case in our study, Latham and Mills (2010) observed an unidentified species of *Leucopis* spp. that had an increased daily consumption rate and spent more time feeding compared to *A. aphidimyza* when preying on *Hyalopterus pruni* Geoffroy (Hemiptera: Aphididae) in California plum orchards. Wild populations of *L. americana* in Florida, USA were reported to have a consumption rate of 17 *A. spiraecola* per day (Miller 1928), almost four times as high as what we observed, although aphid size and environmental conditions were not specified in this study. This still suggests that there is

much variation in the predatory habits of different *Leucopis* spp. species.

Observation of a predator's voracity dynamics (mean daily aphid voracity for the entire larval stage) reveals the predation pattern for both predators. Aphid consumption with *L. glyphinivora* increases steadily in the early stages of larval development to reach a sort of plateau, followed by a steady decline of predation before pupation occurs. With *A. aphidimyza*, consumption increases rapidly, peaking two days after hatching, and sharply declines afterwards. Differences in the rates of development for these two predators should lead to a different biological control application. The slow nature of *L. glyphinivora* is best suited for more mid-term biological control while the immediate effect of *A. aphidimyza* seems more optimized for very short term use. Biological control programs using both predators can therefore be

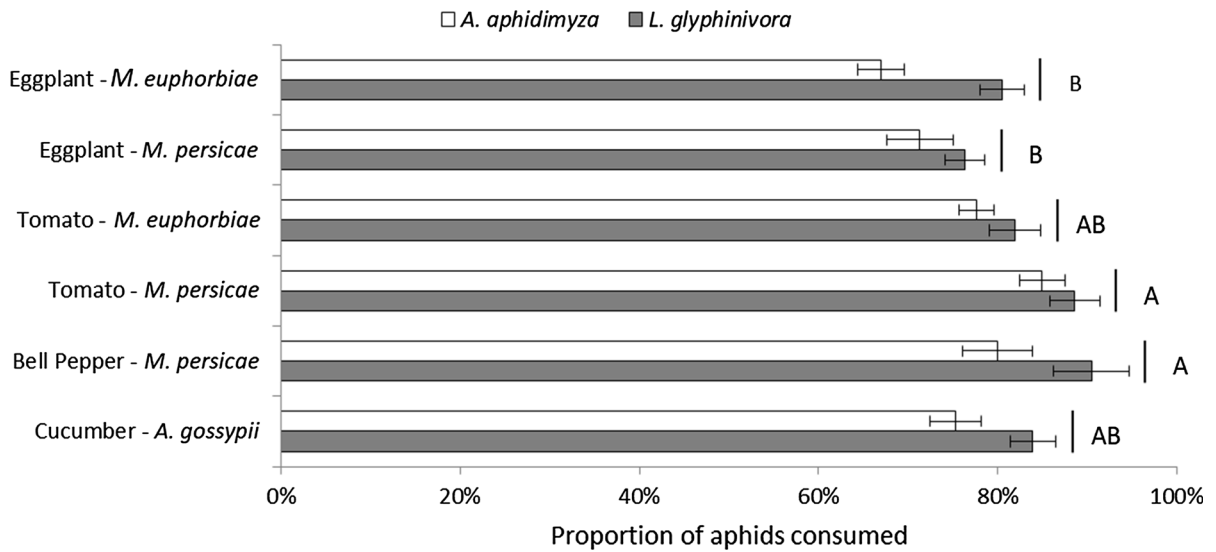


Fig. 2 Proportion of total aphids consumed (\pm SE) by *L. glyphinivora* and *A. aphidimyza* larvae with various host plant and aphid combinations in controlled greenhouse conditions. Different letters indicate a significant difference ($P < 0.05$) between treatments

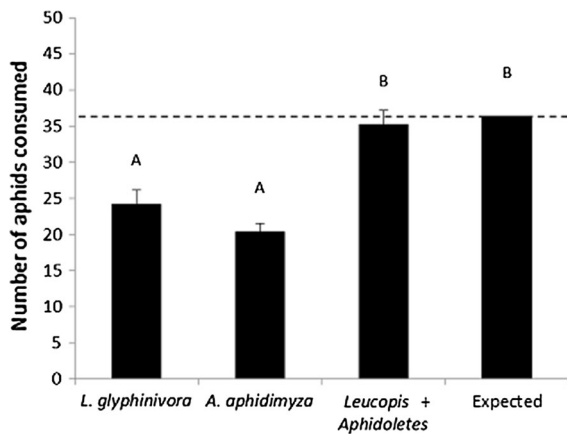


Fig. 3 Mean number of *A. gossypii* consumed (\pm SE) on cucumber by *L. glyphinivora* larvae ($n = 26$), *A. aphidimyza* larvae ($n = 31$) and both predators together ($n = 26$) in controlled greenhouse conditions. The dotted line indicates expected combined consumption. Different letters indicate significant differences between treatments ($P < 0.05$)

tailored to the nature of pest outbreaks. Application of *L. glyphinivora* would be for a moderate and longer sustained pest control due to a longer larval development. *Aphidoletes aphidimyza* would be more suitable for immediate suppression of an intense aphid outbreak. Something else to consider is the timing of peak aphid consumption in both predators and how this would work with aphid population growth dynamics. More research is required in order to

understand how these findings can be implemented into a biological control program.

Greenhouse experiments comparing aphid voracity between *L. glyphinivora* and *A. aphidimyza* demonstrated that *L. glyphinivora* always consumes a higher proportion of aphids, regardless of prey aphid species or host plant used. This means that *L. glyphinivora* is a rather polyphagous predator with the potential for being used on a wide variety of crops. Indeed, *L. glyphinivora* has been reported to attack nearly 80 different species of aphids and is found on over 70 different host plants (Tanasijtshuk 1986; Raspi and Ebejer 2008; Bokina 2009; Brewer and Noma 2010; Mustață et al. 2010; Rakhshani et al. 2010; Satar et al. 2015; Mehrparvar et al. 2016). Interestingly, the best results were observed with *M. persicae* on different crops. These were done on tomato and bell pepper, two structurally different plants. Tomato leaves are rather pubescent, presenting a high density of trichomes. These structures provide an important microhabitat component for a furtive predator such as *A. aphidimyza* by reducing the potential for intraguild predation by some active searching predators such as coccinelids (Lucas and Brodeur 1999; Griffin and Yeorgan 2002). A slow moving vermiform larva like *L. glyphinivora* may also benefit from such protection. Unlike with tomato, bell pepper leaves are glabrous. One would not expect such a crop to be an optimal host plant choice. Surprisingly, the least effective treatments

used eggplant as a target crop, one treatment involving *M. persicae*. Similarly to tomatoes, eggplants have many trichomes and would therefore be expected to make a suitable habitat for *L. glyphinivora* and *A. aphidimyza*. Also, *M. persicae* provided both the best and least effective results. More information concerning preferential host plants and prey aphids is necessary to make an effective biological control program involving *L. glyphinivora*. In our experiment, *L. glyphinivora* was confined to a controlled aphid colony within a clip cage. If *L. glyphinivora* were to be released in a greenhouse for a biological control program, larvae would be free to move around the host plant and even onto other nearby plants. Aphid colony density and age structure varies between colonies on nearby host plants. Rad et al. (2003) observed that *L. glyphinivora* larvae tend to leave small colonies composed of large aphids in search of a new colony, preferring a higher density of small aphids. Further research is needed to define the exact predatory habits of free roaming *L. glyphinivora*, as one would see in an actual biological control setting.

In the combined predator greenhouse test, the observed predatory effect of *L. glyphinivora* and *A. aphidimyza* together was not significantly different from the expected effect. This means there is no antagonistic effect, such as interference, and a biological control program involving the combined use of both predators should generate additive effects on the focal prey. Both *L. glyphinivora* and *A. aphidimyza* larvae were found alive at the end of the test period in 25 of the 26 replicates when used together. Only one replicate contained a single *L. glyphinivora* and no *A. aphidimyza* at the end of the 3-day trials indicating a low potential for intraguild predation. Since *A. aphidimyza* uses a furtive predation strategy and both predators are rather passive slow moving vermiform larvae, this may help them avoid intraguild predation with each other (Lucas et al. 1998; Fréchette et al. 2008). *Aphidoletes aphidimyza* is a known furtive predator (Lucas et al. 1998; Lucas and Brodeur 2001) and this strategy may also occur in *L. glyphinivora*. In fact, *L. glyphinivora* larvae are slow moving and do not seem to actively forage for aphids as one would typically see in an active searching predator. Aphids also did not seem to modify their behaviour when coming into contact with *L. glyphinivora*. Absence of significant defensive behaviour by prey when in the presence of a predator is a key characteristic of furtive

predation (Lucas and Brodeur 2001). Wild *L. glyphinivora* sampled for this study were found within aphid colonies tended by ants, which are known to attack active searching aphid predators (Katayama and Suzuki 2003; Stewart-Jones et al. 2008). While collecting our field samples, ants were never seen attacking *L. glyphinivora* larvae. This is in line with furtive predation behaviour (McLean 1992; Sentis et al. 2012). Furtive predation has also been observed with another close species of silver fly, *Leucopis annulipes* (Fréchette et al. 2008). If intraguild predation between *L. glyphinivora* and *A. aphidimyza* is low, a biological control program using both predators simultaneously could be applied. This could also be extended to other, more active searching aphidophagous predators. Active searching predators, such as ladybirds (Coleoptera: Coccinellidae), can be seen as more efficient predators in that they consume a larger amount of aphids more rapidly (Dixon 1959; Marks 1977; Soares et al. 2001; Cabral et al. 2009). A drawback of these predators is that they are susceptible to engage in intraguild predation (Rosenheim et al. 1993; Hindayana et al. 2001; Völkl et al. 2007) and cannibalism (Osawa 1992; Burgio et al. 2002), which limits their potential at high predator densities. While also susceptible to intraguild predation, furtive predators living within the prey aphid colony benefit from a dilution effect (Lucas and Brodeur 2001) and a selfish herd effect (Dumont et al. 2015) which reduce the negative impact of intraguild predation. At low aphid densities, however, intraguild predation tends to occur more frequently (Polis et al. 1989; Lucas 2005). Predatory mites such as the intraguild predator *Amblyseius swirskii* Athias-Henriot and the hyperpredator *Neoseiulus cucumeris* Oudemans have also been shown to have negative effects on *A. aphidimyza* despite the midge's furtive behaviour (Messelink et al. 2011, 2013). While unable to control large aphid populations, certain zoophytophagous mirids can prevent aphid outbreaks by establishing a population prior to aphid invasion through the use of supplementary food sources (Messelink et al. 2015). Even though these generalist predators engage in intraguild predation, their combined use with *A. aphidimyza* still results in a greater aphid control than *A. aphidimyza* alone (Messelink et al. 2013). Further research on the predatory behaviour of *L. glyphinivora* and on the interactions in a multi-predator environment is still necessary. This may lead to more efficient biological

control programs involving the use of *L. glyphinivora* in conjunction with other beneficial insects against aphids.

Our study shows promising results of *L. glyphinivora* as an effective predator against aphid pests in greenhouses. Further research is, however, necessary before establishing its efficacy as a biological control agent. Searching ability and dispersal capacity are all important traits a good biological control agent would need for use against aphid pests in a greenhouse setting. Fertility, fecundity, oviposition preference and general reproductive behaviour are all key aspects of *L. glyphinivora* biology that remain to be explored and will provide essential information pertaining to mass-rearing conditions and multi-generation population dynamics in this species. All tests in our study were done under very controlled conditions. Actual predator release trials will provide useful information on the behaviour of a free roaming *L. glyphinivora* population in a greenhouse setting. These types of studies, combined with our current study and previous work on the life cycle and survival of *L. glyphinivora* (Barriault et al. 2018) will paint a global picture on how to effectively use *L. glyphinivora* to its full potential.

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- Zhou H, Yu Y, Tan X, Chen A, Feng J (2014) Biological control of insect pests in apple orchards in China. *Biol Control* 68:47–56
- Stephane Barriault** This research is part of the Master’s Project of Stephane Barriault at the Biocontrol lab of the Université du Québec à Montréal (Canada) lead by Eric Lucas. The project aims to study the fundamental ecology of a silverfly species and explore its potential use for aphid biocontrol.
- Marc Fournier** is involved as a senior research assistant in biological control of agricultural pests.
- António O. Soares** is a professor at the University of the Azores (Portugal) and focuses primarily on the ecological bases of biocontrol.
- Eric Lucas** is a professor at the Université du Québec à Montréal and is in charge of the Biocontrol lab since 2002. He studies the applied, theoretical and fundamental aspects of biocontrol.