



Asymmetric intraguild predation of *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) on a native Coccinellidae guild

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ABSTRACT

Harmonia axyridis is an exotic species that can cause serious impacts on native biodiversity in areas where it is introduced to act as a biological control agent. This predator is often involved with intraguild predation, in most cases with aphids predators. Here, we report the results of experiments designed to understand the outcome of the invasion of *H. axyridis* on native coccinellid aphidophagous guilds using intraguild predation as a theoretical framework. Tests were conducted to determine the interactions between 1st and 4th instars larvae and *H. axyridis* adults, with *Cycloneda sanguinea*, *Cycloneda pulchella*, *Eriopis connexa* and *Hippodamia convergens* larvae, as well as eggs predation rate. Two treatments were used: absence and presence of food, *Cinara atlantica*. The results showed that 1st and 4th instars larvae and *H. axyridis* adults are able to prey on larvae and eggs of four Coccinellidae species analyzed, and also their own eggs, showing that this exotic species is a dominant predator in this guild of aphidophagous coccinellids. Adding to these results, studies are recommended, especially the monitoring of these species in the field in the long run, reporting their abundance and diversity as well as their behavior and impact within aphidophagous guilds, especially in Coccinellidae species.

Introduction

Invasive species enter a novel environment and have to established and spread while interacting with native species in the communities (Simberloff, 2015; DiTomaso et al., 2017). This new scenario implies new trophic interactions and a rearrangement of community structure, that may be facilitated by the life history strategy of the invader (Raak-van den Berg et al., 2017). However, these interactions can be negative, for example, the introduction of non-native species, aiming biological control is considered a major threat to native biodiversity (Van Lenteren et al., 2003; Brown et al., 2015) because destabilizes native prey and predator populations dynamics, especially in agronomic systems (Van Lenteren et al., 2003; Crowder and Snyder, 2010; Brown et al., 2015; Björklund et al., 2016).

A common hypothesis that explains the successful establishment of invasive predators (naïve prey hypothesis, Cox and Lima, 2006) suggests

that after the introduction, the effect of a non-native predator on prey dynamics will be increased because of the lack of effective anti-predator strategies and of a common evolutionary history (Michel et al., 2016). However, a non-native predator would also interact with competitors and predators (top predators). As predator community dynamics are thought to be based on negative interactions, such as intraguild predation (IGP) (the killing and eating of species that use similar, often limiting, resources and are thus potential competitors – Polis et al. (1989)), non-native predators growth rate would be constrained by such interactions promoting coexistence (Polis et al., 1989; Hin et al., 2011; Fonseca et al., 2017). But, when the non-native predator did not suffer a negative effect from a native predator (enemy release hypothesis), either by absence or ineffectiveness of a top predator, or by a competitor it would not be controlled and the structure of this system could be destabilized (Crowder and Snyder, 2010; Björklund et al., 2016; Roy et al., 2016).

Harmonia axyridis (Pallas, 1773) (Coleoptera: Coccinellidae) is a predatory invasive species which cause serious impacts on native

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biodiversity in areas where it was introduced as a biological control agent of aphids. In particular, aphidophagous Coccinellidae suffer heavy negative effects of *H. axyridis* through competition for prey and predation (Pell et al., 2008; Brown et al., 2014; Roy and Brown, 2015; Roy et al., 2016; Raak-van den Berg et al., 2017). This species may displace native species in agricultural habitats in North America (Colunga-Garcia and Gage, 1998; Michaud, 2002; Evans et al., 2011) and in various regions of Europe (Brown et al., 2011; Roy et al., 2012; Roy and Brown, 2015). Also, in Brazil, there was a decrease in Coccinellidae diversity associated with an increase of *H. axyridis* abundance, suggesting a negative effect upon native species through competition and/or intraguild predation (Martins et al., 2009).

Predators of aphids exploit ephemeral but abundant resources. Aphid colonies are considered ephemeral because they hardly last longer than the time it takes the consumers to develop from egg to adult. Many predators feed on these resources when they are abundant and as a consequence there is a high risk of cannibalism and intraguild predation (Hemptinne et al., 2012; Hemptinne and Magro, 2015). The hypothesis that native coccinellids decline in neotropics through competition or predation (Martins et al., 2009) has been used because it has been shown that aphidophagous coccinellids interact through intraguild predation, in most cases with predators of aphids (Pell et al., 2008), but also with parasitoids and entomopathogens (Roy et al., 2008; Kenis et al., 2010; Meisner et al., 2011; Ingels et al., 2015; Castro-Guedes and Almeida, 2016). Laboratory and field studies have suggested that *H. axyridis* may be the top predator inside the guild of aphidophagous coccinellids and, consequently, the driver of the guild diversity reduction (Nault and Kennedy, 2003; Roy et al., 2006; Snyder and Evans, 2006; Pell et al., 2008; Martins et al., 2009; Brown et al., 2011; Rondoni et al., 2012; Brown et al., 2015). However, there is evidence of cannibalism in *H. axyridis* (Yasuda et al., 2001; Rondoni et al., 2012; Santos et al., 2014) and also that aggressiveness and predation rates could be size-dependent toward older, and larger instars (Yasuda et al., 2001). Both characteristics, size structure and cannibalism, are theoretically mechanisms that leads to coexistence in IGP models (Polis et al., 1989; Rudolf, 2007).

There are some native Coccinellidae species that are part of aphidophagous guild with *H. axyridis*, such as *Cycloneda sanguinea* (Linnaeus, 1763), *Cycloneda pulchella* (Klug, 1829), *Eriopsis connexa* (Germar, 1824) and *Hippodamia convergens* Guérin-Méneville, 1842. All of these species were already observed co-occurring with *H. axyridis* in different areas, but specially in pine trees, feeding on *Cinara atlantica* (Wilson, 1919) (Martins et al., 2009). These species are considered potential agents of biological control, mainly of aphids. However, some evidence suggests that declining biodiversity among natural enemies usually weakens biological control, and this can usually be attributed to enemies killing, like intraguild predation and its ecological subset, cannibalism (Snyder, 2019).

Thus, given the importance of IGP regarding biological control success, here, we report the results of experiments designed to understand the outcome of the invasion of *Harmonia axyridis* on native coccinellid aphidophagous guilds using intraguild predation as a theoretical framework. We tested: 1) whether predation was symmetric or asymmetric between guild members; 2) whether predation is age structured; 3) Whether the presence of extra food source (aphids) would change predation rate and cannibalism. From field data sampling (Ware and Majerus, 2008; Ware et al., 2008; Martins et al., 2009; Katsanis et al., 2013; Roy et al., 2016; Sloggett, 2017) we hypothesize that *H. axyridis* will show strong asymmetric predation rates, behaving as a top predator in all instars. Also, we expected that cannibalism would not stabilize predation rates unless when interacting with the presence of an extra food source (Rudolf,

2007). We also predicted that the presence of aphids (extra food source) would reduce intraguild predation rate (Lucas et al., 1998; Yasuda et al., 2004; Rudolf, 2007).

Material and Methods

Our system is composed by an aphid (*Cinara atlantica* (Wilson, 1919)) and five predators, *Harmonia axyridis* (Pallas, 1773), *Cycloneda sanguinea* (Linnaeus, 1763), *Cycloneda pulchella* (Klug, 1829), *Eriopsis connexa* (Germar, 1824) and *Hippodamia convergens* Guérin-Méneville, 1842. All species we tested are multivoltine in Brazil and co-occur temporal and spatially. Therefore, it is likely that the abundance and diversity of this guild of aphidophagous predators are influenced by competition or with *H. axyridis*.

Cinara atlantica (Wilson, 1919)

Aphids were collected in the field, on branches of *Pinus taeda* Linnaeus and *Pinus elliottii* Engelm. infested with *Cinara atlantica*, in Curitiba, PR and taken to the insect rearing laboratory, Zoology Department, Universidade Federal do Paraná. They were kept at $21 \pm 1^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 24-hour photoperiod to be maintain a sufficient amount for the bioassays.

Aphids were taken from pine branches from the field, with the help of a fine brush and transferred to the new seedlings purchased at a commercial nursery. The seedlings were replaced when required to maintain the stock population, later used to feed the coccinellids, using the same transfer technique. Aphids were separated by size: small (1st and 2nd instar nymphs), medium (3rd and 4th instar nymphs) and adult aphids.

Coccinellidae

The laboratory population was originated from adult individuals of *H. axyridis*, *C. sanguinea*, *C. pulchella*, *E. connexa* and *H. convergens* collected in the field, in pine trees located in Curitiba, PR and taken to the laboratory.

Insects were reared in plastic containers of 500 ml, in brood chambers (BOD), at $25^\circ\text{C} \pm 1^\circ\text{C}$, relative humidity (RH) of $70\% \pm 10\%$ and photophase of 12 hours. The prey, *C. atlantica*, was provided daily *ad libitum* for both larvae and adults. Subsequently, the adults were sexed and, after obtaining the egg masses, they were transferred into Petri dishes of 9 cm diameter. After hatching, the larvae were individualized in Petri dishes lined with filter paper and a moistened cotton swab along with a drop of honey.

After prepupa formation to the emergence of adult, insects were kept under the same conditions, but without food. The exchange and cleaning of the containers were carried every 48 hours and the observations made daily. Third-generation individuals of each species were used for the experiments.

Experimental design

All experiments were performed under the same controlled conditions described above, and with 30 replicates for each combination and for each treatment. The observations were made under stereomicroscope ZEISS Stemi SV6 and microscope, to better distinguish the larvae. In some predation experiments with first instar larvae the survivor could not be identified during the experiments. In these cases, the larvae were raised individually until adult stage to identification.

The outcome of the trials between species was observed after 24 hours of the onset of the experiment. The occurrence of intraguild predation was considered as follows: IGP in favor of *H. axyridis*: *H. axyridis* larvae preyed the larva of another species; IGP in favor of another species: *H. axyridis* larvae was preyed by the larva of another species; IGP absent: both larvae remained alive.

Same instar larvae of different species may be quite different regarding to size and weight. For this reason, in the experiments we tried to combine larvae of the same size to avoid any kind of influence.

In the experiments with an extra food source, the amount of supplied aphids was determined according to the food consumption test reported by Santos et al. (2014), who reported an average of prey consumption of 5.9, 12.7, 22.9, 46.0 and 22.3, respectively, for all four instars and adult.

Experiment 1: Predatory activity of *Harmonia axyridis* 1st instar larvae

The newly hatched 1st instar larvae were fed for a 24 hours period. A 1-day-old *H. axyridis* individual was transferred to a 3-cm diameter Petri dish, and then, was added a 1-day-old individual from other species (one of the four native coccinellids) to the opposite side of the plate.

Two different treatments were used: **Treatment 1**: a first instar larva of *H. axyridis* and a first instar larva of another species, without prey supply; **Treatment 2**: a first instar larva of *H. axyridis* and a first instar larva of another species, with 8 small aphids (1st and 2nd nymphs instars).

Experiment 2: Predatory activity of *Harmonia axyridis* 4th instar larvae

The 4th instar larvae were combined according to the date of the third ecdysis. The larvae were fed for a day and kept without food for twelve hours, until the start of bioassays. A 4th instar larva of *H. axyridis* was transferred to a 9-cm diameter Petri dish and on the opposite side of the plate a 4th instar larvae of another species was added.

Two different treatments were used: **Treatment 1**: a 4th instar larva of *H. axyridis* and a 4th instar larva of another species, without prey supply; **Treatment 2**: a 4th instar larva of *H. axyridis* and a 4th instar larva of another species, with 50 medium aphids (3rd and 4th nymphs instars).

Experiment 3: Predatory activity of *Harmonia axyridis* adult

A seven-days-old adult of *H. axyridis* was randomly chosen from stock population and transferred to a 9 cm Petri dish and combined with 4th instar larvae of another species. Two different treatments were used: **Treatment 1**: an adult of *H. axyridis* and a 4th instar larva of another species, without prey supply; **Treatment 2**: an adult of *H. axyridis* and a 4th instar larva of another species, with 40 adult aphids.

Experiment 4: Egg predation by *Harmonia axyridis*

In this bioassay, the egg masses of all four species mentioned have been combined with *H. axyridis*, including its own. Masses containing twenty 1-day-old eggs of prey species were placed in 9 cm diameter Petri dishes, along with a 4th instar larva of *H. axyridis* fed for one day and kept without food for twelve hours, randomly chosen from stock population.

After 24 hours, the number of eggs remaining on the plate was counted, and it was considered preyed only those were completely consumed.

The same experiment was performed with seven-days-old adult *H. axyridis*, fed for one day and kept without food for twelve hours, randomly chosen from the stock population.

In this experiment, four different treatments were used:

Treatment 1: a 4th instar larva of *H. axyridis* and 20 eggs of only one Coccinellidae species, without prey supply; **Treatment 2**: a 4th instar larva of *H. axyridis* and 20 eggs of only one Coccinellidae species, with 50 aphids; **Treatment 3**: an adult of *H. axyridis* and 20 eggs of only one Coccinellidae species, without prey supply; **Treatment 4**: an adult of *H. axyridis* and 20 eggs of only one Coccinellidae species, with 40 aphids.

Statistical analysis

To test what is the direction of intraguild predation we use to each pair of species a chi-square test. To test the effect of an extra food source on IGP we adjusted, to each species pair tested, a generalized linear model (GLM) with a *Quasibinomial* distribution to model the overdispersion. To test the occurrence of eggs predation by *H. axyridis* and the effect of an extra food source we adjusted a generalized linear model (GLM) with a *QuasiPoisson* distribution, to account for overdispersion. All statistical analysis were performed with R 2.1.2 environment (R Development Core Team, 2011) with libraries MASS (Venables and Ripley, 2002), effects (Fox, 2003) and vegan (Oksanen et al., 2013).

Results

We detected in all experiments an asymmetric intraguild predation in favor of *H. axyridis* that was independent of the age, except for adults (Table 1). The absence and presence of another food source played a role only when *H. axyridis* were tested together with *C. sanguinea* (4th instar larvae experiments), in which the predation in the absence of food was 17% significantly higher (Table 1). In all other trials, predation by *H. axyridis* was not reduced (Table 1). In all experiments, two hours

Table 1

Values of the intraguild predation analysis between *Harmonia axyridis* (Pallas) and *Cycloneda sanguinea* (L.), *Cycloneda pulchella* (Klug), *Eriopis connexa* (Germar) and *Hippodamia convergens* Guérin-Meneville, in absence and presence of the food *Cinara atlantica* (Wilson), where df=degrees of freedom, L1=1st instar; L4=4th instar.

Development period	Specie	Extra food source			Predation		
		df	Deviance	P	χ^2	df	P-value
L1	<i>C. pulchella</i>	1.58	0.48	0.48	26.66	1	<0.001
	<i>C. sanguinea</i>	1.58	0.08	0.76	15	1	<0.001
	<i>E. connexa</i>	1.58	0.22	0.64	41.66	1	<0.0001
	<i>H. convergens</i>	1.58	0.16	0.68	35.26	1	<0.0001
L4	<i>C. pulchella</i>	1.58	0.11	0.73	24.06	1	<0.0001
	<i>C. sanguinea</i>	1.58	7.38	0.01	41.66	1	<0.0001
	<i>E. connexa</i>	1.58	1.77	0.18	3.26	1	0.07
	<i>H. convergens</i>	1.58	0.14	0.99	13.07	1	<0.0001
Adult	<i>C. pulchella</i>	1.58	0.27	0.60	2.40	1	0.12
	<i>C. sanguinea</i>	1.58	0	1	0.26	1	0.60
	<i>E. connexa</i>	1.58	1.07	0.30	0.26	1	0.60
	<i>H. convergens</i>	1.58	0.06	0.79	0.60	1	0.43

Table 2
Values of the egg predation of analysis *Cycloneda sanguinea* (L.), *Cycloneda pulchella* (Klug), *Eriopis connexa* (Germar) and *Hippodamia convergens* Guérin-Meneville, by *Harmonia axyridis* (Pallas), in the absence and presence of *Cinara atlantica* (Wilson), where df=degrees of freedom and L4=4th instar.

Development period	Specie	df	Deviance	P
L4	<i>C. pulchella</i>	1.58	7.08	<0.0001
	<i>C. sanguinea</i>	1.58	13.93	<0.0001
	<i>E. connexa</i>	1.58	5.74	<0.0001
	<i>H. convergens</i>	1.58	35.34	<0.0001
	<i>H. axyridis</i>	1.58	1.75	<0.0001
Adult	<i>C. pulchella</i>	1.58	21.52	<0.0001
	<i>C. sanguinea</i>	1.58	17.40	<0.0001
	<i>E. connexa</i>	1.58	52.60	<0.0001
	<i>H. convergens</i>	1.58	33.83	<0.0001
	<i>H. axyridis</i>	1.58	198.66	<0.0001

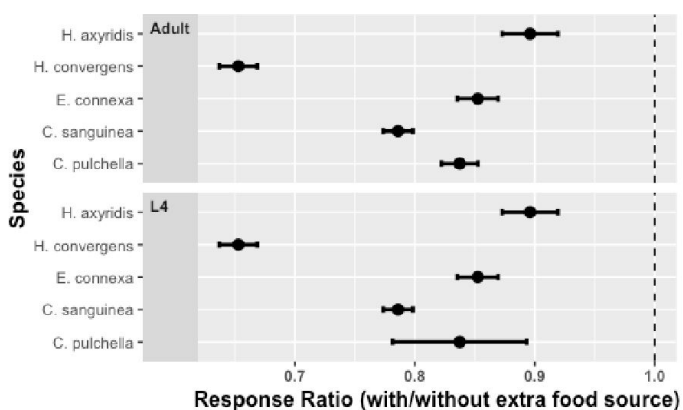


Figure 1 Egg predation response ratio (mean \pm se) estimates of 4th instar larvae (L4) and adults showing the predation rates difference between groups with and without an extra food source. Values below one mean that predation rates are higher when an extra food source is absent.

after starting the experiment, an average of 30% of these larvae had already been preyed.

In egg predation experiments, both 4th instar larvae and adults of *H. axyridis* preyed on the eggs of all species, including their own. Cannibalism of *H. axyridis* was significantly influenced by the presence of an extra food source (aphids), with higher predation rates when aphids were not. It occurred, particularly, when *H. axyridis* adults engaged in cannibalism, preying on their own eggs (Figure 1, Table 2).

Discussion

Our results showed that *Harmonia axyridis* prey upon all native coccinellids we tested and that this pattern holds independently of immature age or the presence of an extra food source. Also, *H. axyridis* preyed upon eggs of all Coccinellidae species, including their own, but it seems to prey more on eggs of other species than on their own eggs, independently of the developmental stage (L4 or adult). Cannibalism is lower when there is an extra food source, particularly for adults of *H. axyridis*. Four instar larvae showed a less pronounced tendency. Overall, our predictions hold, cannibalism was lower with an extra food source and *H. axyridis* preyed more on other species eggs than on its own, suggesting a discriminatory capacity, both as L4 or adults.

Harmonia axyridis behaves as a top predator to the aphidophagous coccinellidae guild we tested because it does not suffer predation in laboratory. This is not occurring only in South America but it is a widespread tendency of *H. axyridis* outcompete and/or prey on other aphidophagous Coccinellidae (Yasuda and Ohnuma, 1999; Yasuda et al.,

2001; Sato et al., 2003; Ware and Majerus, 2008; Pell et al., 2008; Katsanis et al., 2013; Brown et al., 2014; Ingels et al., 2015). So, the key characteristics thought to favor *H. axyridis* in intraguild competition, its larger size, aggressive behavior, extreme polyphagy and the presence of physical and chemical defense strategies, such as the presence of paraescolus throughout its body, benefiting it in confrontation (Félix and Soares, 2004; Pell et al., 2008), are the same that should favor the successful worldwide colonization (Comont et al., 2012). Therefore, the higher predation rate held by *H. axyridis*, independent of stage of development, is related to the greater susceptibility of the other intraguild species, because two hours after starting the experiment, an average of 30% of these larvae had already been preyed. These results showed that native predators did not have negative impacts on *H. axyridis* which will benefit from an enemy free space, becoming a top predator.

Differences in oviposition behavior influences the probability of larvae of two different predators find themselves in the field. Theoretically, females should select the oviposition site in order to raise immatures survival probabilities (Hodek et al., 2012; Hemptinne and Magro, 2015). Aphidophagous Coccinellidae species generally oviposit near aphid colonies, in order to provide to larval stages enough food for their development (Almohamad et al., 2009; Hodek et al., 2012). Besides it, in the presence of a prey a top predator could decrease intraguild predation (Dixon, 2000; Hodek et al., 2012). If this behavior is adaptative it would decrease fitness of intraguild predators, as our experiment have shown even first instar larvae of *H. axyridis* prey upon all other species of aphidophagous coccinellids. Also, predation rates did not change when prey (aphids) were present at experimental arenas (Ware and Majerus, 2008; Pell et al., 2008, but see Santos et al., 2009). So, any aggregative oviposition behavior of the intraguild members would favor *H. axyridis*. It is noteworthy that, in the field, *H. axyridis* and native species are not necessarily in the same instar when they meet. Therefore, there is the possibility that younger instars of *H. axyridis* become prey of more advanced larval stages of other Coccinellidae species (Katsanis et al., 2013). However, Yasuda et al. (2001) observed that even younger *H. axyridis* larvae may prey more advanced larvae instar of other Coccinellidae species.

The egg predation in Coccinellidae is usually associated with immatures stages (Gagnon et al., 2011; Brown et al., 2015; Roy et al., 2016). As it was expected, our experiment showed that *H. axyridis* fourth instar larvae and adults prey heavily on eggs of intraguild coccinellids tested and that the presence of the prey influenced the predation rates: at low aphid densities, larvae will most likely to carry the IGP and cannibalism (Dixon, 2000; Sato et al., 2003; Yasuda et al., 2004; Cottrell, 2007; Hautier et al., 2011; Smith and Gardiner, 2013). Although cannibalism is widespread in *H. axyridis* population (Cottrell, 2004; Ware et al., 2008; Katsanis et al., 2013; Santos et al., 2014; this study) we show that *H. axyridis* prey more on heterospecific eggs than

on conspecific eggs, showing discriminatory behavior. The existence of extrinsic chemicals, present on the surface of the eggs of some species, prevent intraguild predation. This alkaloids concentration can vary within and between species (Hemptinne and Dixon, 2000; Hemptinne et al., 2000; Kajita et al., 2010). In the case of *H. axyridis* eggs, their defensive chemicals have been reported to be essential to explain the resistance of their eggs against intraguild predation by other aphidophagous, which apparently find them unpalatable (Sato and Dixon, 2004; Hemptinne et al., 2012; Martini et al., 2015). More care is required in assessing and describing the likely effects of *H. axyridis*. Our results identified two alternative states predicted by IGP theory (IG top predator and coexistence of IG top predator and IG prey/other predators) in a single system. *Harmonia axyridis* act as a top predator, and although native Coccinellidae populations, which share the same niche as this predator, may be at risk with the increased of distribution area and establishment of *H. axyridis*. Moreover, it is unlikely that these same Coccinellidae species are preying on *H. axyridis*. The stability of aphidophagous Coccinellidae IGP systems is increased in the presence of alternative resources and with increasing habitat complexity. So, it is important to consider the influence of intrinsic and extrinsic factors in the extent of IGP. Nevertheless, with the current environmental scenario, the climate changes and the habitat fragmentation may increase the adverse effects of invasive species by reducing refuges for native species, in which they can avoid competition and direct predation from other invaders.

Thus, it is important monitoring these species in the field in the long run, reporting their abundance and diversity as well as their behavior and impact within aphidophagous guilds, especially in Coccinellidae species, that can be used as biological control agents of aphids. In particular, the comparison between coccinellids groups in invaded and not invaded areas will provide better understanding of the impact of this exotic species.

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Conflicts of interest

The authors declare no conflicts of interest.

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