IMPACT OF *HARMONIA AXYRIDIS* (COLEOPTERA: COCCINELLIDAE) ON THE SURVIVAL RATES OF OTHER APHIDOPHAGOUS SPECIES IN SEMI-FIELD CONDITIONS

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SUMMARY

The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), previously introduced as a biological control agent against aphids, is now frequently considered as an intraguild predator, consuming other aphid natural enemies. Interactions between this exotic ladybird and other aphidophagous species present in Belgian agro-ecosystems such as *Coccinella septempunctata*, *Episyrphus balteatus* and *Chrysoperla carnea* are asymmetric to the benefit of *H. axyridis*.

This study focuses on the survival rate of three aphidophagous species *Harmonia axyridis (Ha), Coccinella septempunctata (C7)* and *Episyrphus balteatus (Eb)* placed in cages in biological fields of potato and sugar beet. During June and July, hermetic cages were disposed on the crops with 10 larvae (second stage) of each aphidophagous. Four types of cages were observed (1) Ha+Eb+C7+ aphids, (2) Ha+Eb+C7, (3) Eb+C7+ aphids and (4) Eb+C7. Each combination was repeated three times.

In sugar beet fields, *E. balteatus* was the species with the highest mortality rate. The two coccinellid species had a better survival rate than *E. balteatus*. All aphidophagous species had higher mortality rates in absence of aphids. In potato, when aphids were present in excess, the survival rates of all aphidophagous insects were lower than in sugar beet probably because *A. fabae* is less adapted to the potato morphology. Nevertheless, we have observed in potato no mortality of *C. 7-punctata* during all its development cycle. This suggests that (1) *C. 7-punctata* may use aphids as food sources more efficiently than *H. axyridis* and (2) that interactions between aphidophagous could be less frequent (difficulty of mobility). In potato and sugar beet, *H. axyridis* had the longest development cycle, being still at the larval stage when *C. 7-punctata* and *E. balteatus* reached the pupae stage. We can suppose that pupae of *E. balteatus* and *C. 7-punctata* were an alternative food (intraguild prey) to *H. axyridis* when aphids were lacking. Pupae are immobile and therefore more sensitive to the attack of predators.

INTRODUCTION

Aphidophagous communities present in Belgian agro-ecosystems are dominated by natives species such as *Coccinella septempunctata* (Linné) (Coleoptera: Coccinellidae), *Propylea quatuordecimpunctata* (Linné) (Coleoptera: Coccinellidae), *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and by exotic and invasive species such as *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). In the late nineties, the multicolored Asian ladybird *H. axyridis* was introduced from South-Eastern Asia to Western Europe to control aphid populations (Adriaens *et al*. 2008; Brown *et al*. 2008). *H. axyridis* was preferred to the two-spotted ladybird *A. bipunctata*, commonly used in biological control, for its higher efficiency in aphid predation (Ferran and Dixon 1993). *H.axyridis* was effectively more efficient in searching aphid populations (With *et al*. 2002) and predation (Lanzoni *et al*. 2004; Labrie *et al*. 2006; Soares *et al*. 2008). They are also more voracious and are easier to rear (Specty *et al*. 2003).

H. axyridis impact on the ecosystems does not seem to be always positive because many studies have reported its aggressive and predatory behaviour towards other aphidophagous species such as *C. septempunctata* (Yasuda *et al*. 2001), *A. bipunctata* (Majerus *et al*. 2006; Hautier *et al*. 2008; Pell *et al*. 2008), *Propylea quatuordecimpunctata* (Linné) (Coleoptera: Coccinellidae) (Lynch *et al*. 2001), *E. balteatus* and *C. carnea* (Phoofolo and Obrycki 1998). Intraguild predation (IGP) is defined as the death and consumption of species that use the same resources (Polis *et al*. 1989). *H. axyridis* predation is facilitated by its aggressive behaviour as well as its high rate of successful escape during interactions (Yasuda *et al*. 2001) and by the presence of dorsal cuticular spines on larvae, protecting them from attack (Hautier *et al*. 2010). IGP is influenced by many factors such as environment, predator and prey characteristics and presence or absence of extraguild preys (aphids) (Burgio *et al*. 2002; Lucas 2005). One of the consequences of *H. axyridis* predatory behaviour could be the decline of native aphidophagous species such as *A. 2-punctata*, *C. 7-punctata* and *P. 14-punctata* (Brown *et al*. 2011).

In this article we compare the resistance and the survival rates of two native aphidophagous (*C. septempunctata*, *E. balteatus*) and one exotic aphidophagous (*H. axyridis*) in semi-natural conditions (fields of potato and sugar beet in Belgium). Analyze of the survival rates of native aphidophagous was made with or without the predatory Asian ladybeetle and with available food or not.

MATERIALS AND METHODS

Aphidophagous

Three aphidophagous species were used in this experiment: *Harmonia axyridis, Coccinella septempunctata* and *Episyrphus balteatus*. *C. septempunctata* and *E. balteatus* were bought (Katz Biotech AG) at egg stage and reared to second larval stage (L2) under laboratory conditions (19 ± 1 °C, 16-8 L-D photoperiod and 55 ± 5 % RH). *H. axyridis* were reared in 36 × 15 × 8 cm aerated plastic boxes. Each box was provided with sugar, water-impregnated sponge and multiflower pollen. Boxes were placed in a controlled environment presenting 16-hr-light photoperiod; 24 ± 1°C; 60 ± 5% RH. Eggs of the three aphidophagous species were disposed on broad bean (*Vicia faba* L.) previously infested with black bean aphids (*Aphis fabae* Scop.) until reaching L2 stage. 10 larvae (L2) of each species were isolated into a glass Petri dish with *A. fabae* and these boxes were brought to the field.

Field experiment

This experiment was conducted in one potato field and one sugar beet field located near Gembloux (Belgium) during June and July 2010. These fields were not chemically treated with pesticides during the test to avoid mortality on aphidophagous. Four hermetic plastic cages (60 x 60 x 60 cm) covered with net were disposed on the crops over two plants at three leafs stage. The net is buried into the soil to avoid the entry of unwanted insects and to avoid the escape of aphidophagous larvae. 100 aphids were disposed on the two plants in each cage to ensure added aphidophagous to survive. 10 larvae (L2) of two aphidophagous species *C. 7-punctata* (C7) and *E. balteatus* (Eb) were disposed in each cage. Two cages contained also *H. axrydis* (Ha). Half of the four cages contained aphids (*A. fabae)* in excess, throughout the experiment. Four combinations of aphidophagous-aphids were placed inside the cages: (1) Eb/C7, (2) Eb/C7+Ha, (3) Eb/C7+aphids and (4) Eb/C7+Ha+aphids (**Figure 1**).

Each combination was repeated three times. On the field, cages were separated by three meters and the three repetitions were distant of 50 meters. Observation were realised every three days until the adult stage and were focused on the number of larval/pupae/adult individuals alive and dead. The dead bodies of aphidophagous species were removed from the cages.

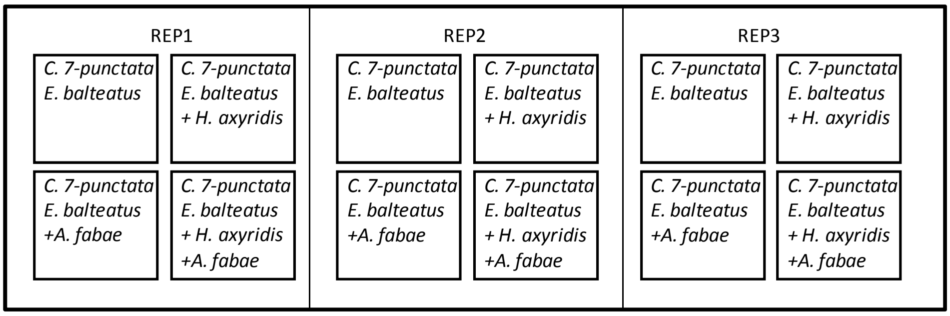

Figure 1. diagram of the experimental setup in field used to study the survival rate of *H. axyridis*

Analyse

The number of alive individuals of the three aphidophagous species observed over time is converted in survival rate [1].

$$\text{survival rate} = \frac{\text{Number of individual alive}}{10} \qquad [1]$$

RESULTS AND DISCUSSION

Survival rates in potato

The evolution of aphidophagous survival rates varied according to the species and to the availability of food. *E. balteatus* survival rates decreased rapidly (four days after the beginning of the experiment) and the pupae stage was not reach without additional aphids (**Figure 2**a-c). This species was the most sensitive and showed the highest mortality rates. This may be explained by the presence of trichomes, that were previously shown to interfere negatively with the displacement of syrphid larvae (Verheggen *et al*. 2009). Decreased mobility of hoverfly larvae could decrease escape rates during predation with *H.axyridis* and decrease its aphids consumption. These two factors could lead to high mortality of *E. balteatus* larvae. Another reason explaining the high mortality rates of *E. balteatus* could be the high asymmetric interaction with *C. septempunctata* (Hindayana *et al*. 2001) and *H. axyridis* (Ingels and De Clercq 2009) as IG predators and *E. balteatus* as IG prey. Food quantity and quality are predominant survival factors at immature stage. All larvae of *C. 7-punctata* also died when food was lacking (**Figure 2**c) although 96% survive when food was present (**Figure 2**a). The survival rate of coccinellid immature stage may for example vary from 85.9 % with *A. gossypii* (Kim and Choi 1985) to 88.3% with *Ephestia kuehniella eggs (Abdel-Salam et al. 1997).* When *H. axyridis* was present, *C. 7-punctata* survival rates were also influenced by the food availability because when aphids populations were scarce, the survival rates of this coccinellid was not over 20% and when food was available, the survival rates raised up to 100%. When the exotic ladybird was included in the coccinellid communi-community, we have observed an increase of the survival rates of the two native aphidophagous species. The survival rates of *C. 7-punctata* was higher than *H. axyridis,* with aphids (C7: 100%; Ha: 59%) and without aphids (C7: 16%; Ha: 0%). These results could mean that, *C. septempunctata* was more resistant than *H. axyridis* and that *H. axyridis* could not act as an IG predator in potato on *C. septempunctata*. *The low survival rates of H. axyridis in presence of prey can be explained by the prey species. In their study, Soares et al. (2004) showed that H. axyridis has a feeding preference for Myzus persicae and not for A.fabae. However H. axyridis did not show a difference in relative growth rate, reproductive capacity, fecundity and fertility when feeding on A. fabae (Soares et al. 2004).* We have observed that aphidophagous species mortality occurred essentially during the pupal stage. This stage has no defence systems and therefore is a vulnerable stage (Lucas 2005). Mortality of *H. axyridis* pupae cannot be explained by predation acted by *C. septempunctata* or other aphidophagous because *H. axyridis* appears to be strongly chemically protected (Yasuda *et al*. 2001). Climatic conditions (windy and lot of rain: 3.1 mm/h) could be an explanation to this mortality.

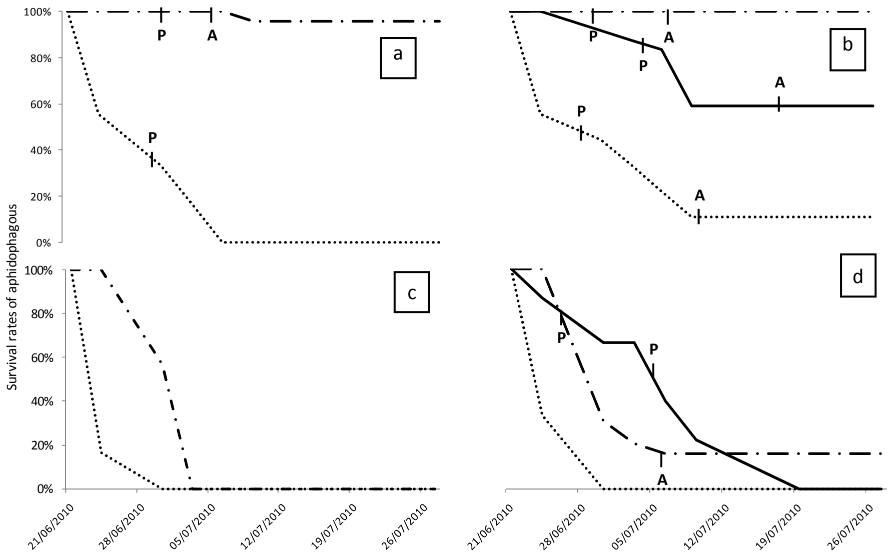

Figure 2. Survival rates of aphidophagous (line: *H. axyridis*, dotted: *E. balteatus*, broken line: *C. 7-punctata*) in potato. (P: beginning of pupal stage, A: beginning of adult stage) (a-b: without aphids, c-d: with aphids, a-c without *H. axyridis*, b-d: with *H. axyridis*)

Survival rates in sugar beet

In sugar beet, the evolution of survival rates of *E. balteatus* with or without aphids is following the one observed in potato. However, when aphid population was scarce (**Figure 3**a-c), *E. balteatus* were able to reach the adult stage unlike in potato (survival rate without aphids: 5%; survival rate with aphids: 11%). The feeding displacement of the larvae was easier on sugar beet because the leaf cowered and humidity condition highly facilitated displacement. Honěk (1983), showed that syrphid could prefer humid and relatively cool

microclimatic conditions. Another explanation could be that *A. fabae* developed more easily and rapidly in sugar beet and the low aphid quantity disposed on the beginning of the experiment was enough to reach the adult stage. In absence of the exotic ladybird in the community, survival rates of *C. 7-punctata* was higher than those of *E. balteatus* reaching 62% when food was available (**Figure 3**a) and 22% when food was scarce (**Figure 3**c). When *H. axyridis* was present in the coccinellid community, survival rates (with food) of *C. septempunctata* was lower than without *H. axyridis*. Mortality was observed at pupae stage and could be due to IG predation by *H. axyridis*. Many studies focused on IG predation and showed *H. axyridis* as an IG predator on other aphidophagous such as *C. septempunctata* (Yasuda *et al*. 2001), *E. balteatus* and *C. carnea* (Phoofolo and Obrycki 1998).
We observed that when food was scarce, the survival rate of *C. septempunctata* was higher with *H. axyridis* than without this IG predator. We could not explain this behaviour because usually when aphid populations are relatively low, the IGP is high and the survival rates of other aphidophagous is low (Hironori and Katsuhiro 1997).
Finally, observations realised in semi-natural conditions could mean that the availability of food is an important factor which influences the survival rates of aphidophagous species. *H. axyridis* was not the most resistant species and in specific conditions (extra/intraguild prey viability, plant characteristics) could act as an IG-predator.

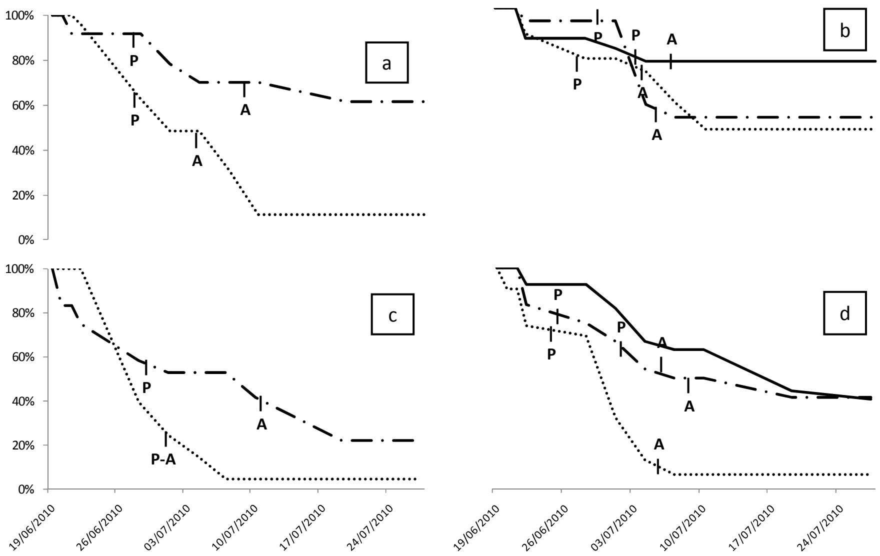

Figure 3. Survival rates of aphidophagous (line: *H. axyridis*, dotted: *E. balteatus*, broken line: *C. 7-punctata*) in sugar beet. (P: beginning of pupal stage, A: beginning of adult stage) (a-b: without aphids, c-d: with aphids, a-c without *H. axyridis*, b-d: with *H. axyridis*)

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