Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae, Aphidiinae) in Benin: its potential as a biological control agent for integrated aphid management in vegetable systems

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae, Aphidiinae) i Benin: artens egenskaper for biologisk kontroll av bladlus i grønnsaksystemer

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List of papers

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III. Tepa-Yotto GT, Hofsvang T, Sæthre M-G. Effect of mating, energy and host age on the oviposition behavior of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae). *Manuscript submitted to Journal of Insect Behavior.*

IV. Tepa-Yotto GT, Hofsvang T, Sæthre M-G. Intraguild predation of an alien aphid parasitoid *Lysiphlebus testaceipes* by three indigenous predators. *Manuscript submitted to BioControl.*

V. Tepa-Yotto GT, Hofsvang T, Godonou I, Sæthre M-G. Responses of *Lysiphlebus testaceipes* (Cresson) to varying densities of *Aphis gossypii* Glover: laboratory and field investigations including effect of indigenous predators. *Manuscript.*

Summary

In Benin, vegetable producers' reliance on toxic synthetic pesticides threatens the environment and human health. However, the recent discovery of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) in Benin offers new options for integrated pest management (IPM), particularly with a view to biological control of aphid host pest species in vegetable agroecosystems in the country. The current work therefore discusses the potential of the alien parasitoid *L. testaceipes* to control aphids within the framework of integrated pest management.

The results demonstrate that the wasp increases its stabbings in order to increase the chance of oviposition on *Aphis gossypii* Glover and *Aphis craccivora* Koch (Homoptera: Aphididae). *Aphis craccivora* and especially fourth instars exhibited more intensive defense behavior against *L. testaceipes* compared to second instar-nymphs. The data proved that energy (honey as sustenance) had a significant effect on the wasp's oviposition performance, compared to that observed in females supplied with water. A single sting of *L. testaceipes* was sufficient to induce successful parasitism on its hosts, although the rate for achieving this was low. The wasp performed less than four stings on aphids younger than third instar prior to oviposition. These stabbings were increased to around seven stings on older nymphs. This was to counter stronger aphid defense and therefore increase the probability of successful oviposition.

Parasitism negatively affected the survival and fertility of *A. gossypii*. The life-time fecundity of the aphid at third instar decreased dramatically to 4.66 times (only 7.569 ± 2.381 nymphs per female) as a result of parasitism by *L. testaceipes*. A decrease of 7.33 and 2.45 times of the net reproductive rate (2.119 ± 0.272) and the intrinsic rate of increase (0.110 ± 0.018),

respectively, was also observed among parasitized aphids. Furthermore, the wasp displayed a type II functional response on *A. gossypii* in the laboratory at 26 ± 1ºC.

Field trials proved that the wasp is effective in aphid suppression, despite the fact that the attack of the indigenous hyperparasitoid *Syrphophagus africanus* (Gahan) (Hymenoptera: Encyrtidae) was observed. In addition, investigation in the field during both rainy and dry seasons, in 2011 and 2012 respectively, showed that the parasitoid along with three native predatory species that feed on aphids *Cheilomenes propinqua* (Mulsant), *Cheilomenes sulphurea* (Olivier) (Coleoptera: Coccinellidae) and *Ischiodon aegyptius* (Wiedemann) (Diptera: Syrphidae) responded positively in numerical terms to changing densities of *A. gossypii*.

Laboratory work carried out at 26 ± 1ºC provided evidence that the three recorded predators are potential competitors to *L. testaceipes.* The three native predatory species had substantial consumption rates of *A. gossypii*, the aphidophagous larvae of *I. aegyptius* being the most voracious compared to their corresponding instars of the ladybirds *C. propinqua* and *C. sulphurea*. Daily consumption of unparasitized aphids by first instars of predators was 9.58 ± 2.89; 12.03 ± 4.36 and 17.40 ± 7.18 for *C. propinqua*, *C. sulphurea* and *I. aegyptius*, respectively. The consumption rate increased 7.99 and 7.38 times for fourth instars *C. propinqua* and *C. sulphurea*, respectively; and 5.00 times for third instar *I. aegyptius*.

The results also indicate that the predators provide an important ecosystem service regarding the natural control of the aphid. However, the data revealed an existence of asymmetrical omnivorous intraguild predation of *A. gossypii* mummies parasitized by *L. testaceipes*, by *C. propinqua* and *C. sulphurea*. Fourth instar of *C. sulphurea* was the most destructive omnivorous intraguild predator. They killed 46.06% of *L. testaceipes* pupae within 24 hours. In contrast, *I. aegyptius* larvae did not attack the aphid mummies parasitized by *L. testaceipes*.

These findings indicate that the intraguild interactions between the alien parasitoid *L. testaceipes* and indigenous aphid predators probably play an important role in determining community structure, and they also act as regulators of both the aphid populations and the alien wasp populations.

In conclusion, the study's results demonstrate that *L. testaceipes* is a promising biocontrol agent for the two major vegetable aphid pests *A. gossypii* and *A. craccivora* and this insight provides important background information for sound decision-making with regard to implementing sustainable measures for aphid management in Benin.

Sammendrag

Grønnsakprodusenter i Benin er avhengig av å bruke syntetiske pesticider, noe som utgjør en risiko for både miljø og helse. Bladlussnyltevepesen *Lysiphlebus testaceipes* (Cresoon) (Hymenoptera: Braconidae, Aphidiinae) ble nylig oppdaget i Benin, og dette kan gi nye muligheter for integrert plantevern (IPM), særlig med tanke på biologisk kontroll av bladlus i ulike grønnsaksystemer. Dette doktorgradsarbeidet diskuterer derfor hvilket potensiale den fremmede arten *L. testaceipes* har for å holde bladlus under kontroll innenfor rammene av en IPM strategi.

Resultatene viser at snyltevepsen intensiverer angrepene (antall stikk) med den hensikt å øke mulighetene for egglegging i *Aphis gossypii* Glover og *Aphis craccivora* Koch (Homoptera: Aphididae). *Aphis craccivora,* og da særlig det fjerde nymfestadium viste en mer intens forsvarsadferd sammenlignet med andre nymfestadium. Forsøkene har vist at energi (honning-løsning) førte til en signifikant økning i egglegging, sammenlignet med hunner som ble foret med bare vann. Ett enkelt stikk med eggleggingsbrodden var nok til å parasittere en vert, men sannsynligheten for vellykket parasittering var liten med bare ett stikk. Snyltevepsen brukte mindre enn fire stikk med brodden på nymfer yngre enn tredje stadium, men måtte øke frekvensen til rundt sju stikk på eldre nymfer. Denne endringen i adferd skyldes at snyltevepsen måtte bekjempe den økende motstanden som eldre nymfer/bladlus gir i forhold til unge nymfer for å lykkes med eggleggingen.

Parasitterte bladlus hadde dårligere overlevelse og nedsatt fertilitet sammenlignet med uparasitterte. Den totale fertiliteten (livsløpsfertiliteten) hos bladlus parasittert av *L. testaceipes* i tredje nymfestadium ble redusert med 4,66 ganger (kun 7,569 ± 2,381 nymfer per hunn). Netto reproduksjon ble redusert med 7,33 ganger (2,119 ± 0,272), mens

populasjonsveksten (intrinsic rate of increase) ble redusert 2,45 ganger (0,110 ± 0,018) hos parasitterte bladlus. I laboratorieforsøk viste snyltevepsen type II funksjonell respons til *A. gossypii* ved 26 ± 1°C.

Feltforsøk viste at snyltevepsen er effektiv som bladlus regulator til tross for at angrep fra den innfødte hyperparasitten *Syrphophagus africanus* (Gahan) (Hymenoptera: Encyrtidae) ble observert under naturlige forhold. I tillegg viste feltforsøk gjennomført både i regntida og i tørketida i henholdsvis 2011 og 2012, at snyltevepsen og de tre innfødte bladlus predatorene *Cheilomenes propinqua* (Mulsant), *Cheilomenes sulphurea* (Olivier) (Coleoptera: Coccinellidae) og *Ishiodion aegyptius* (Wiedemann) (Diptera: Syrphidae) reagerte positivt i antall til ulike tettheter av *A. gossypii*.

Forsøk utført i laboratoriet (26 ± 1°C) viste at disse tre predatorene er potensielle konkurrenter til *L. testaceipes*. Alle de tre innfødte predatorene konsumerte *A. gossypii* i betydelige mengder, der larvene av *I. aegyptius* spiste mest sammenlignet med tilsvarende larvestadier av de to marihønene *C. propinqua* og *C. sulphurea*. Daglig konsum av uparasitterte bladlus for første larvestadium av de tre predatorene var 9,58 ± 2,89; 12,03 ± 4,36 og 17,40 ± 7,18 for henholdsvis *C. propinqua*, *C. sulphurea* og *I. aegyptius.* Konsumraten økte med 7,99 og 7,38 ganger for fjerde larvestadium av henholdsvis *C. propinqua* og *C. sulphurea,* og 5,00 ganger for tredje larvestadium av *I. aegyptius.*

Resultatene viser at predatorene er viktige i økosystemet med hensyn på naturlig regulering av bladluspopulasjoner. Forsøkene avslørte en asymmetrisk omnivor intraguild predasjon på mummier av *A. gossypii* parasittert av *L. testaceipes*. Fjerde larvestadium av *C. sulphurea* var den mest ødeleggende omnivore intraguild predatoren, og drepte 46,6% av puppene av *L. testaceipes* i løpet av 24 timer. Larvene av *I. aegyptius* derimot angrep ikke bladlusmummier parasittert av *L. testaceipes*. Disse resultatene indikerer at intraguilde interaksjoner mellom den fremmede arten *L. testaceipes* og innfødte predatorer sannsynligvis spiller en viktig rolle når det gjelder artsstrukturen i/formingen av økosystemet, og at disse samspillene også fungerer som regulatorer av både bladluspopulasjonen og populasjonen av den fremmede snyltevepsarten.

For å konkludere, resultatene viser at *L. testaceipes* er en lovende organisme for biologisk kontroll av de to viktigste bladlusartene i grønnsaker, *A. gossypii* og *A. craccivora*. Dette er viktig basiskunnskap for å utvikle og implementere bærekraftige kontrollmekanismer for bladlus i Benin.

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1 Introduction

1.1 Background

The damage caused by aphids results in economic losses in a variety of ecosystems. However, a number of predatory and hymenopteran arthropods have the potential to kill aphids in the fields. Aphid predators comprehend various species from the Coccinellidae, Syrphidae, Chrysopidae and Anthocoridae families, and the majority of the species from Aphidiidae and a few in the family formed by the Aphelinidae are known as aphid parasitoids.

There is increasing evidence that a biological control approach is needed to reduce farmers' reliance on toxic chemicals. In effect, chemical pest control includes the use of a range of organophosphate-, carbamate-, pyrethroid- and neonicotinoid-based insecticides, and increasingly of pymetrozine (a pyridine azomethine) to fight aphids. However, growing aphid resistance to toxic synthetic insecticides issues along with environmental and human health risks have resulted in serious concerns regarding the use of such chemicals.

In Benin, vegetable production is economically important and carried out intensively predominantly in urban and peri-urban areas. In the country, vegetable producers grow a diversity of crop species belonging mostly to the plant families Brassicaceae, Cucurbitaceae, Malvaceae and Solanaceae (James et al. 2010). Unfortunately, a number of aphid species, including some Aphidini and Macrosiphini (Fig. 1), pose serious pest infestations problems and are recognized as one of the major constraints to vegetable production (Sæthre et al. 2011). Recent surveys conducted in the vegetable agroecosystems in the country have identified a diversity of aphid natural enemies, which can be classified into two main categories: indigenous predators (Fig. 3) and alien primary parasitoids (Fig. 2) (Sæthre et al. 2011).

There is broad recognition that biological options in an integrated pest management (IPM) approach may provide a solution for sustainable control. Even though the risks related to the environmental effects of biological control have raised some concerns, to date the approach has not, to our knowledge, often resulted in negative impacts in the fields. Biological control is commonly defined as the actions or use of a living beneficial organism (natural enemy) to maintain a pestiferous organism at low density. The approach basically includes four options, namely classical biocontrol, inundation, inoculation and conservation.

The recent discovery of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) provides new alternatives for integrated pest management (IPM), particularly for the biological control of aphid host pest species in vegetable agroecosystems in Benin (Sæthre et al. 2011). Therefore, this work aimed to examine the potential of the alien aphid parasitoid *L. testaceipes* as a possible biocontrol agent against aphids in vegetable agroecosystems in Benin.

Fig. 1 Key pestiferous aphid species (a) *Aphis craccivora*, (b) *Aphis gossypii* and (c) *Lipaphis erysimi* in vegetable agroecosystems in Benin (Photo: Arnstein Staverløkk, Bioforsk). Both *A. craccivora* and *A. gossypii* belong to the tribe Aphidini, while *L. erysimi* is a Macrosiphini.

Fig. 2 Mummies of *Aphis gossypii* (a) parasitized by the alien primary parasitoid (b) and (c) *Lysiphlebus testaceipes* in vegetable agroecosystems in Benin (Photo: Arnstein Staverløkk, Bioforsk).

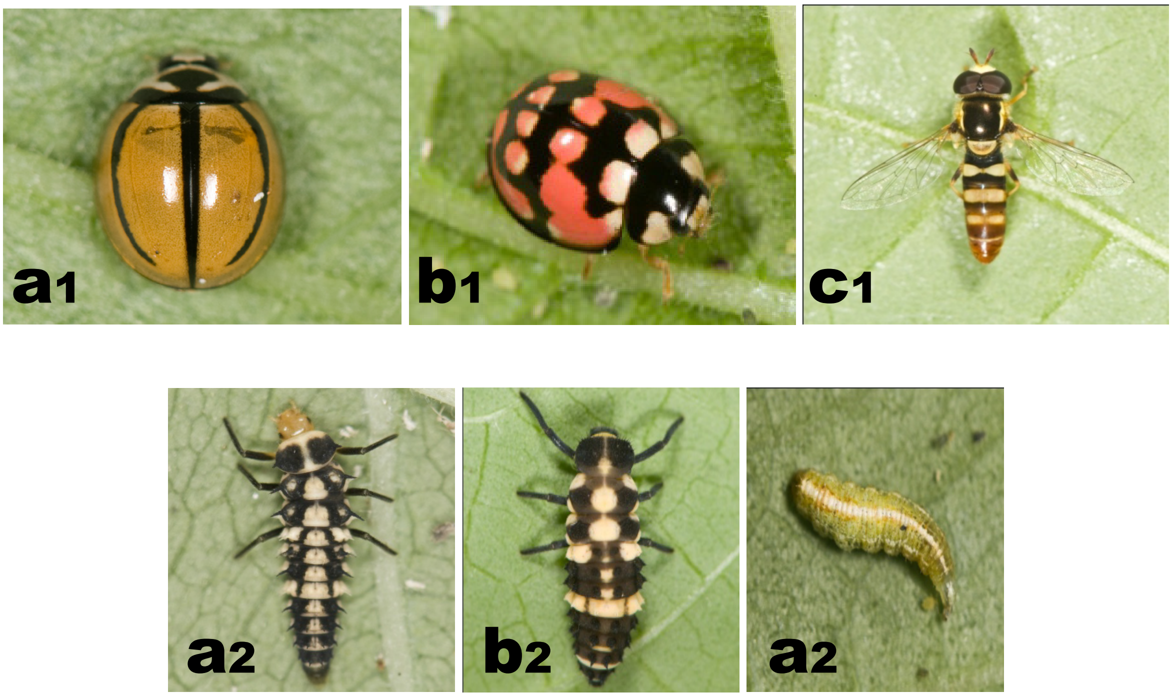

Fig. 3 Adults (1) and larvae (2) of key indigenous aphid predator species (a) *Cheilomenes propinqua*, (b) *Cheilomenes sulphurea* and (c) *Ischiodon aegyptius* in vegetable agroecosystems in Benin (Photo: Arnstein Staverløkk, Bioforsk). *Cheilomenes propinqua* and *C. sulphurea* are coccinellids, whereas *I. aegyptius* is a syrphid. Only the larval stages of *I. aegyptius* are aphidophagous.

1.2 Biology of *L. testaceipes*

Lysiphlebus testaceipes is among the most abundant and important aphid parasitoids belonging to the Aphidiidae, a highly specialized family in which all species parasitize only aphids (Hågvar and Hofsvang 1991).

1.2.1 Life cycle and developmental rate

Like all Aphidiinae species, *L. testaceipes* is an exclusive solitary parasitoid. After having investigated the quality of its prey, the endoparasitoid bends its abdomen and inserts its ovipositor in the aphid host for oviposition (Fig. 4). The oviposition act of this wasp is short and often lasts about one second (Marullo 1987; Gross 1993; Völkl and Mackauer 2000). On *Toxoptera citricida* (Kirkaldy), *L. testaceipes* eggs hatch within 54.3 hours (Persad and Hoy 2003a). The length of the time from egg to pupa in *L. testaceipes* varies depending on the aphid host species (Table 1) and is on average five days on *Aphis gossypii* Glover at 26 ± 1ºC (Tepa-Yotto et al., unpublished data). From the brown-colored mummy an adult will emerge through a circular hole cut either between the cornicles or laterally on the aphid abdomen or dorsally near the mesothorax (Hofsvang et al., unpublished data). Non-fertilized eggs produce males (Michaud and Mackauer 1995; Fauvergue et al. 2008).

The immature mortality of the parasitoid was found to be higher at 25ºC compared to 20ºC: 29.6% and 9.5% respectively (van Steenis 1994). The threshold temperatures for development from egg to adult were estimated by Royer et al. (2001) for various colonies of *L. testaceipes* collected in the native distribution area, that is, Nebraska, Oklahoma, and Texas. Threshold temperatures were 5.64; 6.61; and 6.42ºC with corresponding degree-day requirements of 181.2; 169.5; and 188.0 for Nebraska, Oklahoma, and Texas isolates, respectively. These findings were comparable to those determined by Elliott et al. (1999). Hughes et al. (2010)

recently established that *L. testaceipes* stops locomotion at temperatures below -0.1ºC and above 41.4ºC; and suffers from chill coma at -8.0ºC and heat coma at 44.1ºC.

Supercooling points (SCP) for *L. testaceipes* were determined with a view to understanding the overwintering of the parasitoid by Jones et al. (2008), who found that less than six-hour old female adults of the wasp including mummies on average had SCP < -26ºC. This was consistent with the results of Hughes et al. (2011), demonstrating that the SCP of *L. testaceipes* life stages were between -24.6ºC and -17.7ºC for both non-acclimated and acclimated individuals. Hughes et al. (2011) suggested that in most parts of Europe *L. testaceipes* overwinters either as larva in the living aphid hosts or as pupa in mummified aphids.

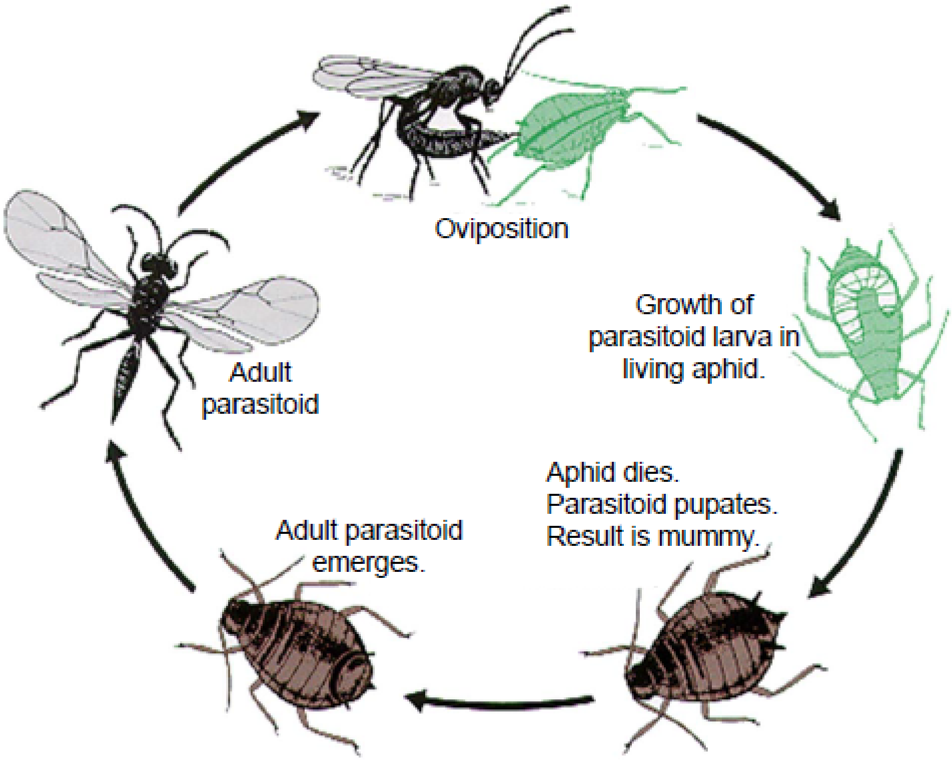

Fig. 4 Schematic life cycle of *Lysiphlebus testaceipes* (Adapted from Knutson et al. 1993).

Table 1 Developmental period in *Lysiphlebus testaceipes*.

Aphid species	Aphid instar	Temp. (ºC)	Egg stage (days)	Oviposition to mummification (days)	Mummification to emergence (days)	Oviposition to emergence (days)	References
Schizaphis graminum	Average 1-7 days	21	-	9.1	4.1	13.2	Hight et al. 1972
S. graminum	Average 1-5 days	27	-	7.4	3.4	10.8	Hight et al. 1972
S. graminum	Average 1-5 days	21-32	-	7.4	3.7	11.1	Hight et al. 1972
Pentalonia nigronervosa	3rd	21	-	-	-	9.4(♂) 9.8(♀)	Völkl et al. 1990
Aphis gossypii	2nd	20	-	-	-	12.9	van Steenis 1994
A. gossypii	2nd	25	-	-	-	9.5	van Steenis 1994

Table 1 (*continued*).

Aphid species	**Aphid instar**	**Temp. (ºC)**	**Egg stage (days)**	**Oviposition to mummification (days)**	**Mummification to emergence (days)**	**Oviposition to emergence (days)**	**References**
Toxoptera citricida	3rd	22	2-3	-	-	-	Persad and Hoy 2003a
A. gossypii	2nd and 3rd	25	-	6.3	-	10.1	Silva et al. 2008
Myzus persicae	2nd and 3rd	25	-	7.0	-	10.2	Silva et al. 2008
Rhopalosiphum maidis	2nd and 3rd	25	-	6.7	-	10.2	Silva et al. 2008
S. graminum	2nd and 3rd	25	-	6.8	-	10.3	Silva et al. 2008

1.2.2 Oviposition period and fecundity

The ovipositional data of *L. testaceipes* is rather scanty (Table 2). However, it is recognized that the aphid host species/instar used to rear a parasitoid along with the physiological status of females as well as biotic (for instance, interspecific competition in case of multiparasitism) and abiotic factors may have a significant effect on parasitoid fecundity. The daily egg-production of *L. testaceipes* declined with increasing wasp age and total fecundity was 128.2 (within seven days) and 180.0 eggs (within five days) per female on *A. gossypii* at 20 and 25ºC respectively (van Steenis 1994). However, Persad and Hoy (2003b) found that the number of progeny produced by 25-hour old *L. testaceipes* (27.4 ± 12.8) was higher than that generated by females that were one hour of age (6.5 ± 3.6) on *T. citricida*. The fecundity of *L. testaceipes* less than 24-hour old was greater on *S. graminum* (257.8) when compared to many other host species (Table 2), making it one of the most suitable aphid host for the parasitoid (Rodrigues et al. 2003).

Table 2 Oviposition period, fecundity, female longevity and parasitism of *Lysiphlebus testaceipes*.

Aphid species	**Temp. (ºC)**	**Oviposition period (days)**	**Mean fecundity**	**Mean longevity (days)**	**Parasitism**	**Method**	**References**
Aphis gossypii	20	7	128.2	2.7	76.7 of 200 aphids	Aphid dissection	van Steenis 1994
A. gossypii	25	5	180	2.6	104.6 of 200 aphids	Aphid dissection	van Steenis 1994
A. gossypii	25	-	-	-	46%	Mummies and emergence	Rodrigues et al. 2001
Schizaphis graminum	25	-	-	-	67%	Mummies and emergence	Rodrigues et al. 2001
S. graminum	25	7	257.8	-	-	Aphid dissection	Rodrigues et al. 2003
A. gossypii	25	-	-	-	132 larvae from 280 aphids	Aphid dissection	Bueno et al. 2003

Table 2 (*continued*).

Aphid species	Temp. (ºC)	Oviposition period (days)	Mean fecundity	Mean longevity (days)	Parasitism	Method	References
Myzus persicae	25	-	-	-	29 larvae from 280 aphids	Aphid dissection	Bueno et al. 2003
A. gossypii	25	-	-	5.4	55.7%	Mummies and emergence	Silva et al. 2008
Rhopalosiphum maidis	25	-	-	3.8	76.7%	Mummies and emergence	Silva et al. 2008
S. graminum	25	-	-	3.7	66.7%	Mummies and emergence	Silva et al. 2008

1.3 Host selection

Aphidiids' host selection involves several behavioral steps with a view to locating and parasitizing hosts. As the parasitoid pursues these steps, the area of search is reduced, and the likelihood of finding a host that can be successfully parasitized increases (Hågvar and Hofsvang 1991). Although these steps may be adjusted or differ slightly across species, the different steps of host selection may be basically divided as follows: host habitat location, host location, host acceptance, host suitability and host regulation.

1.3.1 Host habitat location

Habitat complexity structures parasitoid-aphid-plant association (Brewer et al. 2008; Starý and Havelka 2008) and may determine host-parasitoid interaction (Thies et al. 2005). It has been established that aphid-induced plants selectively attract parasitoids (Hatano et al. 2008; Brewer and Noma 2010). This involves the emission of specific volatiles by the plants that are colonized by aphids, making them attractive to parasitoids. In the field, the mechanism for *L. testaceipes'* detection of host patches is not well established (Tentelier et al. 2006). However, a behavioral wind tunnel experiment surprisingly revealed that experienced *L. testaceipes* females are not more attracted by infested plants (*Cucumis sativa* L./*A. gossypii*) than by uninfested ones, which sets them apart from the behavior of many other parasitoid species (Lo Pinto et al. 2004). It is argued that distance and a number of other factors including plant synomones are important cues. In addition, the effects of mating, oviposition experience and aphid density mediate host habitat location in *L. testaceipes* (Grasswitz and Paine 1992; Pérez et al. 2007; Fauvergue et al. 2008; Hatano et al. 2008). In addition, it is suggested that environmental factors such as temperature, humidity, wind and light intensity generally determine macrohabitat (forests and fields) location by aphidiids (Hågvar and Hofsvang 1991). Moreover, olfactory cues of plant volatiles, hosts or other associated organisms are thought to regulate microhabitat (host plants) location (Hågvar and Hofsvang 1991). Araj et al. (2009) experimentally demonstrated the role of floral nectar of buckwheat on host habitat location and successful location and parasitism of the aphid host *Acyrthosiphon pisum* (Harris) by the parasitoid *Aphidius ervi* (Haliday).

1.3.2 Host location, acceptance and suitability

There is some data to show that aphid cornicle secretion is used by *L. testaceipes* as a kairomonal cue to find its hosts (Grasswitz and Paine 1992). Parasitoids respond to kairomones (volatiles and non-volatile contact kairomones) by changing their searching behavior, thus improving their chances of finding a host (Hågvar and Hofsvang 1991). After randomly searching on a plant leaf, the parasitoid usually detects aphids through antennal contact (Hågvar and Hofsvang 1991). Upon encountering the aphid, the parasitoid may accept or reject the host for oviposition. *Lysiphlebus testaceipes* is a generalist with a large range of aphid hosts, encompassing more than 100 species (Pike et al. 2000). Extensive field surveys suggest the polyphagy of *L. testaceipes*, but very few adequate studies have measured the suitability of the aphids to the wasp. In other words, little information exists on *L. testaceipes'* preference for aphid host species or host instars. However, it is argued that some non-host Macrosiphini such as *Brevicoryne brassicae* (L.) and *Lipaphis erysimi* Kalt. might not be nutritionally suitable to or physiologically compatible with *L. testaceipes* (Silva et al. 2008). Host suitability and the extent to which the wasp regulates host development are poorly documented for this widespread aphidiid. Examination and corporation of these questions under natural conditions is challenging (Dib et al. 2010).

1.4 Use of *L. testaceipes* in biological control

A variety of attributes privilege the use of parasitoids in biological control. Among these attributes are: host-specificity, a single host needed for development, secured control at low densities, easy handling and distribution over large areas. Despite all these characteristics not being completely met in the case of *L. testaceipes*, with regard to host-specificity, this species proved high potential in establishing itself as a biocontrol agent worldwide. In fact, *L. testaceipes* has been introduced for the biological control of aphids in several countries around the world (Fig. 5). To our knowledge, out of 12 countries/islands worldwide where the wasp was introduced in fields, the establishment of the species was successful in seven regions. At least one successful establishment of *L. testaceipes* has been reported from each of the five continents (Fig. 5). Finally, some effective releases of the parasitoid species in greenhouses have been reported (Wei et al. 2005; Dimitrov et al. 2008).

1.5 Distribution and recent spread of *L. testaceipes*

The parasitoid *L. testaceipes* is a native nearctic species and a typical species of the North America prairies faunistic complex of aphidiids (Starý 1970) and is distributed throughout the USA, and found in Mexico and Southern Canada (Krombein 1958, Schlinger and Hall 1960). *Lysiphlebus testaceipes* is very common throughout temperate North America (Mackauer and Starý 1967), and its distribution extends far into Neotropical America (Starý et al. 1993).

In Europe, the species was introduced to France and established itself in 1973-74, spreading rapidly to the coastal mediterranean areas (Fig. 5). In the past decade, the only known instance of *L. testaceipes* spreading in Europe is known from Slovenia (Kos et al. 2010), indicating a northwards spread of the species in Europe (Fig. 5). In addition, Hughes et al. (2011) argued that due to its greater ability to tolerate cold conditions, *L. testaceipes* will be able to establish itself in the cool, temperate climates typical of Northern Europe.

The introduction of the species in fields had been successful in one Asian country, namely India (Sankaran 1974; Agarwala et al. 1981), but not in China (Wei et al. 2005). While the background to the species’ appearence in Iran in 2001 (Rakhshani et al. 2005) is unknown, the spread of *L. testaceipes* to New Zealand in 2007 was suggested by Teulon et al. (2008) to be an instance of ‘self-introduction’ of the species to the country.

Laamari et al. (2012) suggest that the spread of *L. testaceipes* to North Africa resulted from the wasp being air-borne across the Gibraltar strait. In eastern Africa, the first attempt at biological control of an insect pest took place in Kenya in 1911, and was directed against an aphid, *Schizaphis graminum* (Rondani), which had first appeared in 1909-1910, damaging the wheat crop. The government entomologist returned from a visit to the USA with a parasitoid, *L. testaceipes*, and a coccinellid predator, *Hippodamia convergens* (Guérin-Méneville). Both natural enemies were released, but neither is known to have become established (Greathead 2003). To date, *L. testaceipes* has been reported in six African countries (Fig. 5), i.e. Kenya (Starý et al. 1985; Greathead 2003), South Africa (Starý et al. 1985), Burundi (Autrique et al. 1989), Tunisia (Ben Halima-Kamel 2011), Algeria (Laamari et al. 2010) and Benin (Sæthre et al. 2011). We do not know how *L. testaceipes* entered Benin and West Africa, but the species' successful establishment in the region is proven (Sæthre et al. 2011).

Fig. 5 Global distribution map of *Lysiphlebus testaceipes* (Illustration: Erling Fløistad, Bioforsk).

1.6 Hyperparasitism and intraguild interaction

Hyperparasitoids may attack either living parasitized aphids or their mummies (Ganyo et al. 2012). Hyperparasitism on *L. testaceipes* may be of significance (Bernal et al. 1993; Rosenheim 1998; Wright and James 2001; Ganyo et al. 2012) or not (Yokomi and Tang 1996). Despite this, hyperparasitism does not appear to disrupt the potential of braconid parasitoid as biological control of aphids in the fields, although this remains to be proven (Morris et al. 2001).

The effects of intraguild interaction (including coccinellids and other hymenopterans) for a given aphid-food source are among the decisive factors determining the survival and adaptation of aphid-parasitoids (Brodeur and Rosenheim 2000). The possible coexistence of *L. testaceipes* with *Lipolexis scutellaris* (Mackauer) on *T. citricida* (Persad and Hoy 2003a) in citrus crops has been suggested; and that of *L. testaceipes* with *Aphidius colemani* Viereck on both *Pentalonia nigronervosa* (Coq.) and *Aphis fabae* Scop. (Völkl and Stadler 1991); while the intrinsic superiority of *L. testaceipes* on *A. colemani* in *A. gossypii* was reported by Sampaio et al. (2006). A common concern is that invasive parasitoids may be potential competitors with native species. It is argued that invasive alien parasitoids could displace native parasitoids, thus leading to loss of indigenous biodiversity. However, to our knowledge, to date there are very few data reports on the ability of *L. testaceipes* to displace native species in the fields as an invasive alien species (IAS). Inversely, there have been reports on the coincidental and/or asymmetrical omnivorous intraguild predation of *L. testaceipes* by aphid predators. Royer et al. (2008) found that *S. graminum* mummies previously parasitized by *L. testaceipes* are attacked by second and fourth instars of both *Coccinella septempunctata* L. and *H. convergens* (Coleoptera: Coccinellidae).

2 Knowledge gaps

Lysiphlebus testaceipes is an alien aphid parasitoid species to Benin, making it the first recorded instance of the species in West Africa (Sæthre et al. 2011). The origin of *L. testaceipes* in Benin is unknown. To date, for an aphidiid species used so widely in biocontrol, there is little ovipositional and host selection data available on *L. testaceipes* (Table 2). Very few field investigations have attempted to document the foraging behavior and potential of the parasitoid. The continuing spread of *L. testaceipes* is deserving of further study, specifically as regards the interactions of the species with indigenous species. Therefore, new data is needed to increase our understanding and to enable us to predict the full potential of this widely distributed wasp species.

3 Objectives

The overall objective of the present work was to examine the potential of *L. testaceipes* as an alien biological control agent of aphids in vegetable agroecosystems in Benin. More specifically, the aim of the study was to contribute towards filling the gaps regarding the oviposition behavior of *L. testaceipes* and its interactions with other aphidophagous arthropods. Therefore, the specific objectives were to investigate on the:

- ✓ Host suitability of three key pestiferous aphid species as constraints on vegetable production for *L. testaceipes* (Paper I);
- ✓ Effect of host age of *A. gossypii* on the oviposition behavior of *L. testaceipes* and parasitism effect on the aphid population growth (Paper II);
- ✓ Combined effect of mating, energy and host age on the oviposition behavior of *L. testaceipes* on *A. craccivora* (Paper III);

- ✓ Intraguild predation potential of three indigenous aphidophagous predators on *L. testaceipes* (Paper IV);
- ✓ Prey-density effect on *L. testaceipes* including the numerical responses of three aphid predators in the field (Paper V).

4 Materials and methods

Aphid species (*A. gossypii*, *A. craccivora* and *L. erysimi*) and host instar (*A. gossypii* and *A. craccivora*) suitability investigations were conducted in the laboratory at 26 ± 1ºC in both Petri dishes and cages to increase our understanding of host preference by *L. testaceipes* under choice and no-choice conditions (Papers I, II and III). The oviposition behavior of this parasitic hymenopteran of less than 24-hour old females without oviposition experience was also examined under a stereoscopic microscope at 40x magnification (Papers I, II and III). In addition, second and fourth instars *A. craccivora* were compared with regard to aphid defense behavior against the parasitoid (Paper III). The number of parasitoid larvae in the aphid hosts was established by dissection under a stereomicroscope two days after exposure and this was taken as a measurement of the parasitism by *L. testaceipes* (Papers I, II, III and V).

The life table parameters of *A. gossypii* parasitized by *L. testaceipes* were computed to analyze the effect of parasitism on aphid growth (Paper II). The mummification rate of aphids according to the days elapsed after parasitization was also investigated (Paper II). Further measurements of the wasps' responses to host density were carried out in the laboratory at 26 ± 1ºC and in the field during both rainy and dry seasons (Paper V). The numerical responses of three aphid predators in the field, *C. propinqua*, *C. sulphurea* and *I. aegyptius*, to prey density were also analyzed (Paper V). The daily feeding rates of the three predators were measured on unparasitized third instar *A. gossypii*. Besides, the asymmetrical omnivorous intraguild predation of *A. gossypii* mummies, parasitized by *L. testaceipes*, by the two ladybirds *C. propinqua* and *C. sulphurea* and the aphidophagous larvae of the syrphid *I. aegyptius* was examined in the laboratory (Paper IV).

Finally, three statistical software packages, Minitab (2011) (Papers I, II, III and V), R Core Team (2012) (Papers I, III, IV and V) and SAS (2010) (Paper II), were used for data analysis. In addition to descriptive statistics (means, standard deviations and errors, and frequencies) of the variables examined, binary logistic regressions were performed to model the oviposition behavior (Papers I, II and III) and the functional response (Paper V) of *L. testaceipes*. Linear fixed effects (Papers I, II, III and IV) and mixed effects (Paper V) models analysis of variance (ANOVA type II and III sum of squares for fixed effects and mixed effects models, respectively) were also conducted on data to investigate on the potential of *L. testaceipes* as a biological control agent of aphids. The Fisher's exact tests with Bonferroni correction (for binary regressions) and the Tukey's post hoc tests (for ANOVAs) at the 5% level were used to test for significant differences among treatment means, followed by pairwise comparisons.

5 Results and discussion

Parasitoid oviposition and aphid defense behavior

Lysiphlebus testaceipes readily attacked and successfully oviposited in *A. craccivora* and *A. gossypii* (Papers I, II and III). The wasp performed less than four stings on aphids younger than third instar prior to oviposition (Papers II and III). These stabbings were increased to around seven stings on older nymphs to counter more intense aphid defense and therefore increase the probability of successful oviposition (Papers II and III). In effect, shaking, kicking, walking and cornicle secretion were observed to be more frequent as host defense behaviors in fourth instar when compared to second instar *A. craccivora* (Paper III). However, a single sting by *L. testaceipes* could be sufficient for successful parasitism on the host, although the rate was low (Papers I, II and III). The results demonstrate that energy (honey as sustenance) increased wasp performance (Paper III) and thus also strengthen conclusions from previous reports on the handling mechanism of stronger hosts by aphidiids (Hofsvang and Hågvar 1986; Kant et al. 2008; He et al. 2011). However, *A. gossypii* and its third instar proved the most frequently attacked species/instar by *L. testaceipes* (Papers I and II). This echoes the suitability of mid-aged hosts for a number of aphidiids (Weisser 1994; Chau and Mackauer 2000; Colinet et al. 2005; Tahriri et al. 2007). On the other hand, the data confirms that *L. erysimi* is a non-host for *L. testaceipes* (Paper I); the aphid species is thought to be nutritionally unsuitable to and/or physiologically incompatible with the wasp (Silva et al. 2008). In summary, the findings reveal that the oviposition behavior of *L. testaceipes* involves several fairly complex factors such as mating, energy and host age or quality (Papers II and III).

Potential of *L. testaceipes* as a biocontrol agent

Lysiphlebus testaceipes has the ability to oviposit in all developmental stages of *A. gossypii*. The data demonstrated that the net reproductive rate (*Ro*) and the intrinsic rate of increase (r_m) of *A. gossypii* at third instar decreased to 7.33 (2.119 ± 0.272) and 2.45 (0.110 ± 0.018) times respectively as a result of parasitism by *L. testaceipes*. The life-time fecundity of parasitized aphids decreased to 7.57 ± 2.38 nymphs per female compared to unparasitized counterparts (35.29 ± 2.30) (Paper II). Similarly, the negative effect of parasitism by *A. colemani* on *A. gossypii* reproduction has been demonstrated by van Steenis and El-Khawass (1995); and that of *L. testaceipes* on *S. graminum* by Kring and Kring (1988).

Lysiphlebus testaceipes exhibited a type II functional response on third instar *A. gossypii* at 26ºC in the laboratory with an exposure time of 24 hours (Paper V). Earlier findings showed that the wasp species displayed type II and type III functional responses on *A. gossypii* at 20 and 28ºC, respectively, at observation time of 30 minutes (Bazzocchi and Burgio 2001). However, although the type III functional response was also observed in *Aphidius colemani* Viereck on *Myzus persicae* (Sulzer) and in *Aphelinus asychis* Walker (Aphelinidae) on both *M. persicae* and *Macrosiphum euphorbiae* (Thomas) (Byeon et al. 2011), the type II response by parasitoids is common in biological control (Fernández-Arhex and Corley 2003).

The results showed that *L. testaceipes* responded positively to *A. gossypii* density through increased total parasitism in the field in 2011 and 2012 and proved that the wasp is effective at suppressing aphid populations. Generally, during the rainy season (2011) both rainfall and lower temperatures slowed down the population growth of both aphids and natural enemies of the aphids, thus reducing enemies' attraction to the plants. In addition, heavy tropical rain can wash aphids off the host plants, thereby resulting in reduced population growth, compared to the dry season (2012). In the dry season the temperature is generally higher (except in December-January due to the Harmattan season), favoring rapid aphid population growth. However, the significant performance of *L. testaceipes* which has been demonstrated in this investigation indicates a very real possibility that the wasp may be used in an integrated pest management strategy on vegetables (Paper V). Fernandes et al. (1998) established that *L. testaceipes* can maintain *S. graminum* densities below the economic threshold, in the context of augmentative biological control.

Effect of hyperparasitism and intraguild interactions

Field experiments revealed that *L. testaceipes* was attacked by one species of hyperparasitoid *Syrphophagus africanus* (Gahan) (Hymenoptera: Encyrtidae) in both years 2011 and 2012. Our observations confirm those made by Sæthre et al. (2011) and Hofsvang et al. (unpublished data). Both listed a number of native hyperparasitoids (of *L. testaceipes*) including *S. africanus*. In addition, our findings parallel the data of Ganyo et al. (2012) that the indigenous hyperparasitoid *S. africanus* could parasitize both living aphids (*A. craccivora/L. testaceipes*) and aphid mummies (*A. craccivora/L. testaceipes*), preferring newly formed mummies in the laboratory. However, to date the extent to which hyperparasitism disrupts parasitoid-based biological control of aphids in the field is not well determined (Morris et al. 2001; Acheampong et al. 2012).

Evidence was provided in the laboratory that *C. propinqua*, *C. sulphurea* and *I. aegyptius* are potential indigenous competitors to the alien aphid parasitoid *L. testaceipes*. This is because the three indigenous species consumed substantial amounts of *A. gossypii*, the aphidophagous larvae of the syrphid *I. aegyptius* being the most voracious compared to their corresponding instars of the ladybirds *C. propinqua* and *C. sulphurea*. Daily consumption of unparasitized aphids by first instars of predators was 9.58 ± 2.89; 12.03 ± 4.36 and 17.40 ± 7.18 for *C. propinqua*, *C. sulphurea* and *I. aegyptius*, respectively. The consumption rate increased 7.99 and 7.38 times for fourth instars *C. propinqua* and *C. sulphurea*, respectively; and 5.00 times for third instar *I. aegyptius* (Paper IV). *Cheilomenes sulphurea* therefore had the highest total consumption rate. In addition, the data revealed an existence of asymmetrical omnivorous intraguild predation of *A. gossypii* mummies of *L. testaceipes* by *C. propinqua* and *C. sulphurea*. Third and fourth instars *C. propinqua* and second to fourth instars *C. sulphurea* could chew the mummies and damage *L. testaceipes* pupae inside the mummies, excepting the younger predatory instars. Fourth instar of *C. sulphurea* was the most damaging intraguild predator, killing 46.06% of *L. testaceipes* pupae within 24 hours. In contrast, *I. aegyptius* larvae did not attack the aphid mummies. However, the results indicate that the predators provide an important ecosystem service regarding the natural control of *A. gossypii* (Paper IV). Field investigation showed that the three aphid predators responded positively in numerical terms to changing densities of *A. gossypii*. This was also observed for *L.*

testaceipes. The findings indicate that the intraguild interactions between the alien parasitoid *L. testaceipes* and the indigenous aphid predators are likely to play an important role in determining community structure, and intraguild interactions also help regulate both the aphid populations and the parasitoid populations (Papers IV and V). Coccinellid beetle predation may result in considerable mortality among *L. testaceipes* at immature stages, but this does not necessarily result in disruption of biological control (Colfer and Rosenheim 2001; Janssen et al. 2006; Costamagna et al. 2007). This is also consistent with the conclusions of Snyder and Ives (2003) and those of Bilu and Coll (2007); both support the positive combined effect of aphid parasitoids and predators despite the observation of intraguild predation of parasitoids.

6 Concluding remarks

The background of *L. testaceipes* in Benin and West Africa is unknown, but its successful establishment in the region is now proven. The current findings have elucidated the complex mechanism behind its present success in the country. The demonstration that *L. testaceipes* is a promising biocontrol agent of the two major vegetable aphid pests *A. gossypii* and *A. craccivora* provides relevant background information, which can, in turn, create the basis for making sound decisions on the implementation of sustainable measures for aphid management in Benin. However, the successful and sustainable use of *L. testaceipes* in the country depends to a large extent on moderate pesticide use by the farmers. Besides, intraguild interactions added to cropping systems and abiotic factors are also key features that will regulate the action or full potential of this alien aphid parasitoid as a new biocontrol agent in Benin.

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Appendix – Research Papers

Paper I

Tepa-Yotto GT, Hofsvang T, Godonou I, Sæthre M-G 2013. Host preference of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae, Aphidiinae), an alien aphid parasitoid in Benin. International Journal of Tropical Insect Science 33:127-135.

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Host preference of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae, Aphidiinae), an alien aphid parasitoid in Benin

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Abstract. *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) is a newly discovered species alien to Benin (West Africa) and the dominating aphid primary parasitoid in vegetable agroecosystems. A study on the preference of this parasitic hymenopteran for three key aphid pests of vegetables (*Aphis craccivora* Koch, *Aphis gossypii* Glover and *Lipaphis erysimi* (Kaltenbach) (Homoptera: Aphididae)) was carried out under choice and no-choice conditions at 26 ± 1 °C in both Petri dishes and cages. The aphidiine rejected *L. erysimi* and did not oviposit in this aphid species in any of the trials. In all tests, *A. gossypii* proved to be the aphid host preferred most frequently by *L. testaceipes*. This study contributes to the fundamental knowledge on the oviposition behaviour of *L. testaceipes* and provides information for use in the development of sustainable aphid pest management strategies in Benin.

Key words: *Lysiphlebus testaceipes*, alien species, oviposition behaviour, aphid preference

Introduction

Within the family Aphididae, aphids are known as economically important insect pests of global importance. Some aphids cause severe direct damage to crops and may also transmit plant viruses. Both problems result in significant yield losses (Kieckhefer and Kantack, 1988; Hughes and Maywald, 1990; Fabre *et al.*, 2003). Sæthre *et al.* (2011) recently conducted extensive diagnostic surveys in Benin and identified aphids as one of the main biotic constraints to urban and peri-urban (UPU) vegetable production. The major aphid pest species listed by Sæthre *et al.* (2011) were *Aphis craccivora* Koch, *Aphis gossypii* Glover and *Lipaphis erysimi* (Kaltenbach).

In contrast to earlier investigations that did not reveal any hymenopterous parasitoids of *A. craccivora* in southern and central Benin (Tamò *et al.*, 2003), Sæthre *et al.* (2011) showed that aphid parasitoids are common in vegetable agroecosystems across the country and may play an important role in controlling aphids. The polyphagous solitary alien species *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) was identified as a countrywide aphid endoparasitoid on vegetables in Benin (Sæthre *et al.*, 2011).

The origin of *L. testaceipes* is North America (Smith, 1944); however, to date, no information exists on how and when *L. testaceipes* spread or was

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introduced to Benin (Sæthre *et al.*, 2011). In addition, few studies exist on the oviposition behaviour of this polyphagous, much-used species in biocontrol (Tepa-Yotto *et al.*, unpublished data). The present work was therefore carried out not only to increase the knowledge on the oviposition behaviour of *L. testaceipes* but also to examine the host preference and adaptation of this potential biological control agent in its new environment (West Africa). In addition, the aim of this study was to investigate whether there is any shift in the host preference of this aphidiine species new to West Africa compared with the native range. This study is the first step towards including *L. testaceipes* in an integrated pest management (IPM) strategy against aphids in vegetable systems in the country.

Materials and methods

Collection and rearing of aphids

The three aphid species (*A. craccivora*, *A. gossypii* and *L. erysimi*) were sampled from vegetable production sites in Southern Benin on common beans (*Phaseolus vulgaris* L.), sweet pepper (*Capsicum annuum* L.) and cabbage (*Brassica oleracea* L.), respectively. Separate cultures of the collected aphid species were established in insect-rearing cages in a screenhouse. The rearing procedure of the aphids was as follows. Adults of the collected aphid species were placed individually on fresh leaves for 24 h in Petri dishes at 26 ± 1 °C in the laboratory to generate new nymphs. Newly produced nymphs were fed for 2 days on fresh leaves in Petri dishes. Using a tiny brush, these nymphs were then transferred to healthy potted plants grown to the eight true-leaf stage. The different aphid species were maintained on their respective original host plant species, i.e. sweet pepper (Hybrid Yolo-wonder; Technisem, Savigny Sur Orge Cedex, France), common beans (Haricot Phenomene; Technisem) and cabbage (F_1 K-K Cross; Takii & Co., Ltd, Kyoto, Japan). The same varieties of plants were used throughout the experiments.

Collection and rearing of parasitoids

Aphis gossypii mummies were collected from sweet pepper plants in the field in Southern Benin and carried to the laboratory. Samples of emerging adults of *L. testaceipes* were identified and a culture of the aphid endoparasitoid was then established using *A. gossypii* on sweet pepper. Four potted aphid-infested plants were arranged per insect-rearing cage. Mated females were selected with an aspirator and released into cages for 6 h. Ten females were released per cage. Parasitized aphids were mummified within 5 days and the mummies were carefully isolated in plastic boxes (diameter 9.5 cm; height 4.5 cm). The majority of males emerged on the 8th day, followed by the females one day later. Serial cohorts of *L. testaceipes* were produced in a screenhouse at 9-day intervals.

Fertilized female parasitoids for the experiments

Leaves with the attached aphid mummies were cautiously sampled from the rearing cages. These leaves were cleaned of living aphids and kept in plastic boxes (diameter 9.5 cm; height 4.5 cm). The mummies were then observed until *L. testaceipes* female adults emerged. A drop of 2 ml nutritive solution of honey (70%) was applied to the net on the top of the box to feed parasitoids. Mated females were picked out for the experiments after they had been observed copulating. The wasps used in all the experiments were reared on *A. gossypii* and had no oviposition experience.

Parasitoid oviposition behaviour

A no-choice oviposition behaviour study of *L. testaceipes* on *A. craccivora*, *A. gossypii* and *L. erysimi* was performed in a Petri dish (diameter 8.5 cm). The aphids used were collected from laboratory cultures. All instar nymphs and newly moulted apterous adults of each aphid species were randomly selected for the experiments. Ten aphids were exposed to a parasitoid female less than 24 h old, previously mated and without oviposition experience. The number of aphids was minimized to 10 in each trial to avoid the wasp having several encounters with the same aphid(s). Each wasp was tested three times for each aphid species at 26 ± 1 °C. By the time the experiment was concluded, 15 wasps (replicates) had been used per aphid species, making a total of 45 parasitoids and 1350 aphids. Direct observations were carried out in a Petri dish under a stereomicroscope at 40 × magnification. During the oviposition act, the number of stings given to a single aphid by the parasitoid was recorded. Any ovipositor bending that did not touch the prey was excluded. After the 10 nymphs/adults had been probed and/or stung by the female wasp, they were one by one removed with a tiny brush, and 10 new nymphs/adults were added in the Petri dish according to Sampaio *et al.* (2006). It was concluded that *L. testaceipes* definitely rejected the host-patch when the wasp had left or was unresponsive for more than 300 s. The aphids that were probed and/or stung were transferred one by one and kept individually on a leaf of plant placed on a moistened paper tissue in Petri dishes. Some of the aphids were still alive after 2 days. These aphids were dissected under a stereomicroscope to check for the first-instar larvae of *L. testaceipes* according to Persad and Hoy (2003).

Study of parasitoid preference for the three aphid species in Petri dishes

The preference of *L. testaceipes* for *A. craccivora*, *A. gossypii* and *L. erysimi* was studied under choice and no-choice conditions at 26 ± 1 °C in the laboratory. In the choice experiments, four different combinations of the three aphid species were offered to the wasps. Fifty aphids per species, comprising all instar nymphs and newly emerged apterous adults with different morphs, were placed on separate same-sized plant leaves in a Petri dish (diameter 8.5 cm). The total number of aphids offered to each wasp varied with the number of aphid species (either three or two species) included in the different choice experiments. In these trials, a total of 150 aphids were exposed to each wasp when the three aphid species were present and a total of 100 aphids with two aphid species. Each aphid species comprising 50 individuals was also tested in the no-choice experiments. In all cases, one mated female parasitoid less than 24 h old (without oviposition experience) was used. The aphids were exposed to the parasitoid for 8 h of contact under daylight conditions. Each experiment was replicated with 15 females of *L. testaceipes*. The number of larvae of the parasitoid in the hosts was determined by dissecting living aphids 2 days after the wasp was removed from the Petri dish. Dissecting was time-consuming and some of the aphids were kept frozen at −16 ± 0.5 °C for subsequent dissection.

Study of parasitoid preference for the three aphid species in cages

The choice and no-choice experiments were also performed in insect-rearing cages (46 × 45 × 43 cm) to study the preference of *L. testaceipes* on *A. craccivora*, *A. gossypii* and *L. erysimi* in the laboratory. Six-week-old potted plants of sweet pepper, common beans and cabbage were used in these experiments. *Lipaphis erysimi* was kept on cabbage, *A. gossypii* on sweet pepper and *A. craccivora* on common beans. In all experiments, a total of 50 aphids including all developmental stages were placed on the fourth leaf of the different host plants. In the choice experiments, four combinations of the three complexes (host plant species with respective aphid species) were set up. The three complexes were *A. craccivora*/common beans, *A. gossypii*/sweet pepper and *L. erysimi*/cabbage, as mentioned earlier. The first combination consisted of *A. craccivora*/common beans, *A. gossypii*/sweet pepper and *L. erysimi*/cabbage, the other three combinations comprising two different aphid/plant complexes each. In the no-choice tests, one host plant with its respective aphid species was arranged per cage. One female parasitoid (reared on *A. gossypii*, mated and without oviposition experience) less than 24 h old was released per cage for 8 h of exposure under daylight conditions with an average temperature of 26 ± 1 °C, 65 ± 7% relative humidity and a 12 h photoperiod. Afterwards, aphids found on the plants were transferred to Petri dishes for 2 days before being dissected. On average, 70% of aphids were recovered alive in the experiments. The number of parasitoid larvae in the respective hosts was established by dissection. Each experiment was replicated using 15 females of *L. testaceipes*. The three aphid species differed in both size and colour and were easily distinguishable in the choice tests. Cornicles and cauda were also characteristic for each species and aided quick identification/separation (Blackman and Eastop, 2000).

Data analysis

In the oviposition experiments, the response (y-values) was coded into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$), where y is the number of *L. testaceipes* larvae found in the stung aphid hosts after dissection. Superparasitism (when $2 \leq y \leq 4$) was rare. Binary logistic regression with a logit link function was used to predict the effect of the aphid host species as a factor and the number of stings given by the wasp to its host as a continuous explanatory variable on parasitism by *L. testaceipes* (MINITAB Release 16, 2011; Minitab, State College, Pennsylvania, USA).

Table 1. *Lysiphlebus testaceipes* attacks on *Aphis craccivora*, *Aphis gossypii* and *Lipaphis erysimi*, and oviposition, parasitism and superparasitism recorded out of 450 aphids per aphid species

Numbers	*A. craccivora*	*A. gossypii*	*L. erysimi*
Aphids attacked and dissected	412	417	12
Unsuccessful attacks (no larvae observed)	375	327	12
Oviposition (larvae observed)	37	90	0
Superparasitism (more than one larva observed)	5	9	0

Table 2. Binary logistic regression of two aphid host species (*Aphis craccivora* and *Aphis gossypii*) and stabbing stings of *Lysiphlebus testaceipes* on parasitized aphids[1] ($n = 900$)

Predictor	Coefficient	SE coefficient	Z	P	Odds ratio[2]	95% Confidence interval	
						Lower	Upper
Parasitized aphids							
Constant	− 3.83141	0.263873	− 14.52	0.000			
Host species (slope)	1.06080	0.232506	4.56	0.000	2.89	1.83	4.56
Wasp stings (slope)	0.472550	0.0519008	9.10	0.000	1.60	1.45	1.78

[1] Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

[2] Odds ratio means the estimated probability for parasitism to be successful/unsuccessful for *A. gossypii* compared with *A. craccivora*, and for each increase of stings given by the wasp prior to oviposition.

In the parasitoid preference experiments, we transformed the y-values into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$), where y is the number of *L. testaceipes* larvae found in the aphid hosts after dissection. Superparasitism (when $2 \leq y \leq 4$) was infrequent. Binary logistic regression with a logit link function was also used to predict the effect of the aphid host species, the experimental condition (choice and no-choice, involving the aphid host species) and the experimental arena (Petri dish and cage) as factors on parasitism by *L. testaceipes* (MINITAB).

In the parasitoid preference experiments, the number of aphid hosts parasitized and superparasitized by *L. testaceipes* was counted. These data were log-transformed before analysis to meet the assumptions of normality and equal variance. Transformed data were then analysed using a linear model analysis of variance (ANOVA) type II sum of squares with aphid host species, experimental condition (choice and no-choice, involving the aphid host species), experimental arena (Petri dish and cage) and the level of parasitism (parasitism and superparasitism) as factors. Tukey's *post hoc* tests at the 5% level were used to test for significant differences among the groups, followed by pairwise comparisons (R statistical software; R Core Team, 2012).

Results

Parasitoid oviposition behaviour

Lysiphlebus testaceipes never oviposited in *L. erysimi*, but readily attacked and parasitized *A. gossypii* and *A. craccivora* (Table 1). The binary logistic regression lines provided a good description of the effect of the aphid host species and the number of stings given by the wasp *L. testaceipes* to its hosts on oviposition (Tables 2 and 4; Fig. 1). The proportion of aphids with parasitoid larvae increased steadily with the wasp's attacks, the probability for parasitism to be successful with increasing stings being higher in *A. gossypii*. We predicted that the increase of stabbings to 15 stings more probably on stronger (older/larger) aphids had proportions of 0.99 and 0.90 of parasitized aphids for *A. gossypii* and *A. craccivora*, respectively (Fig. 1). There was evidence that both the aphid host species as a factor and the stings of the wasp as a continuous explanatory variable were significantly different from zero for the response investigated (Tables 2 and 4), showing that the two variables affected the oviposition of *L. testaceipes*. However, there was no interaction between these two effects.

Parasitoid preference

The binary logistic regression also provided a good description of the effect of the aphid host species,

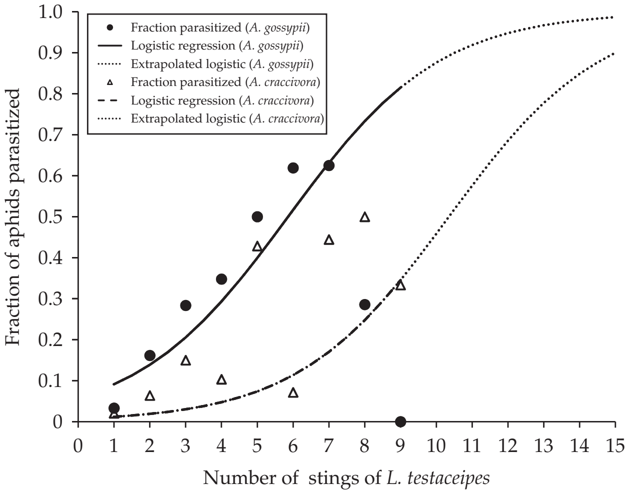

Fig. 1. Parasitism rate of *Lysiphlebus testaceipes* on *Aphis gossypii* and *Aphis craccivora* (in the Petri dish) as a function of stabbing stings of the wasp prior to oviposition. Analysis by binary logistic regression, using the number of stings given by the parasitoid to its hosts: 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 stings (Table 2).

Table 3. Binary logistic regression of two aphid host species (*Aphis craccivora* and *Aphis gossypii*), two experimental conditions (choice and no-choice) and two experimental arenas (Petri dish and cage) on aphids parasitized by *Lysiphlebus testaceipes*[1] ($n = 4313$)

Predictor	Coefficient	SE coefficient	*Z*	*P*	Odds ratio[2]	95% Confidence interval Lower	 Upper
Parasitized aphids							
Constant	−1.57108	0.0841818	−18.66	0.000			
Host species (slope)	0.613212	0.0686769	8.93	0.000	1.85	1.61	2.11
Condition (slope)	0.142086	0.102579	1.39	0.166	1.15	0.94	1.41
Arena (slope)	0.432001	0.0993020	4.35	0.000	1.54	1.27	1.87
Condition × arena (slope)	0.284028	0.137282	2.07	0.039	1.33	1.02	1.74

[1] Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

[2] Odds ratio means the estimated probability for parasitism to be successful/unsuccessful for *A. gossypii versus A. craccivora*, under the no-choice test *versus* the choice test, in Petri dish *versus* cage as the experimental arena and for the interaction between the experimental condition and the experimental arena.

the experimental condition (choice and no-choice), the experimental arena (Petri dish and cage) and the interaction condition × arena on parasitized aphids in *A. gossypii* and *A. craccivora* (Tables 3 and 4). There was evidence that all the factors were significantly different from zero for the response investigated, except for the effect of the experimental condition (choice and no-choice) (Tables 3 and 4). This shows that there was no difference between the choice and no-choice tests with respect to parasitism potential by *L. testaceipes*. There was, however, an interaction between the experimental condition (choice and no-choice) and the experimental arena (Petri dish and cage), showing that the influence of one of these factors on the fraction of aphids parasitized depends on the level of the other factor (Table 3).

In both choice and no-choice experiments, *L. erysimi* never hosted *L. testaceipes* (Fig. 2A and B). No parasitoid larvae were found after the dissection of *L. erysimi* from all trials in Petri dishes or in cages. *Lysiphlebus testaceipes*, on the other hand, oviposited in both *A. craccivora* and *A. gossypii* in the experiments involving both Petri dishes and cages. In the no-choice experiments in Petri dishes, the mean number of *L. testaceipes* larvae was higher in *A. gossypii* than in any other aphid species with 0.59 ± 0.03 and 0.38 ± 0.03 for *A. gossypii* and *A. craccivora*, respectively (Fig. 2A). The same trends were also observed in the case of the choice experiments with a lower number of parasitoid larvae, relatively speaking, when compared with the no-choice test in Petri dishes (Fig. 2A) or in cages (Fig. 2B). In the cage experiments, the number of *L. testaceipes* larvae was significantly low compared with the experiments in Petri dishes (Tables 3 and 4; Fig. 2A and B). However, superparasitism was observed in both aphid host species in both Petri dishes and cages and was significantly lower than parasitism in all cases (Table 5; Fig. 3A and B). In general, superparasitism was usually significantly frequent in *A. gossypii* compared with *A. craccivora*, except in the case of the no-choice condition in the Petri dish where the two aphid host species were equally superparasitized (Fig. 3A and B). The interaction between the aphid host species and the level of parasitism (parasitism and superparasitism) was significant, showing that the number of aphids parasitized to a level of parasitism depends on the species of host attacked by the wasp (Table 5).

Discussion

Lysiphlebus testaceipes has a large host range (Pike *et al.*, 2000; Starý *et al.*, 2004). According to Mackauer *et al.* (1996), host choice is based on three steps: host recognition (change in the female's behaviour), host evaluation (by antennation and ovipositor probing) and host acceptance (oviposition). During probing with the ovipositor, aphidiine females may evaluate chemical cues in the aphid's haemocoel

Table 4. Results of goodness-of-fit tests related to the results of binary logistic regressions in Table 2 (parasitoid oviposition behaviour) and Table 3 (parasitoid preference)

Experiment	Goodness-of-fit test	χ^2	df	*P*
Oviposition behaviour				
	Pearson	46.7507	17	0.000
	Deviance	45.7438	17	0.000
Parasitoid preference				
	Pearson	4.56448	3	0.207
	Deviance	4.55774	3	0.207

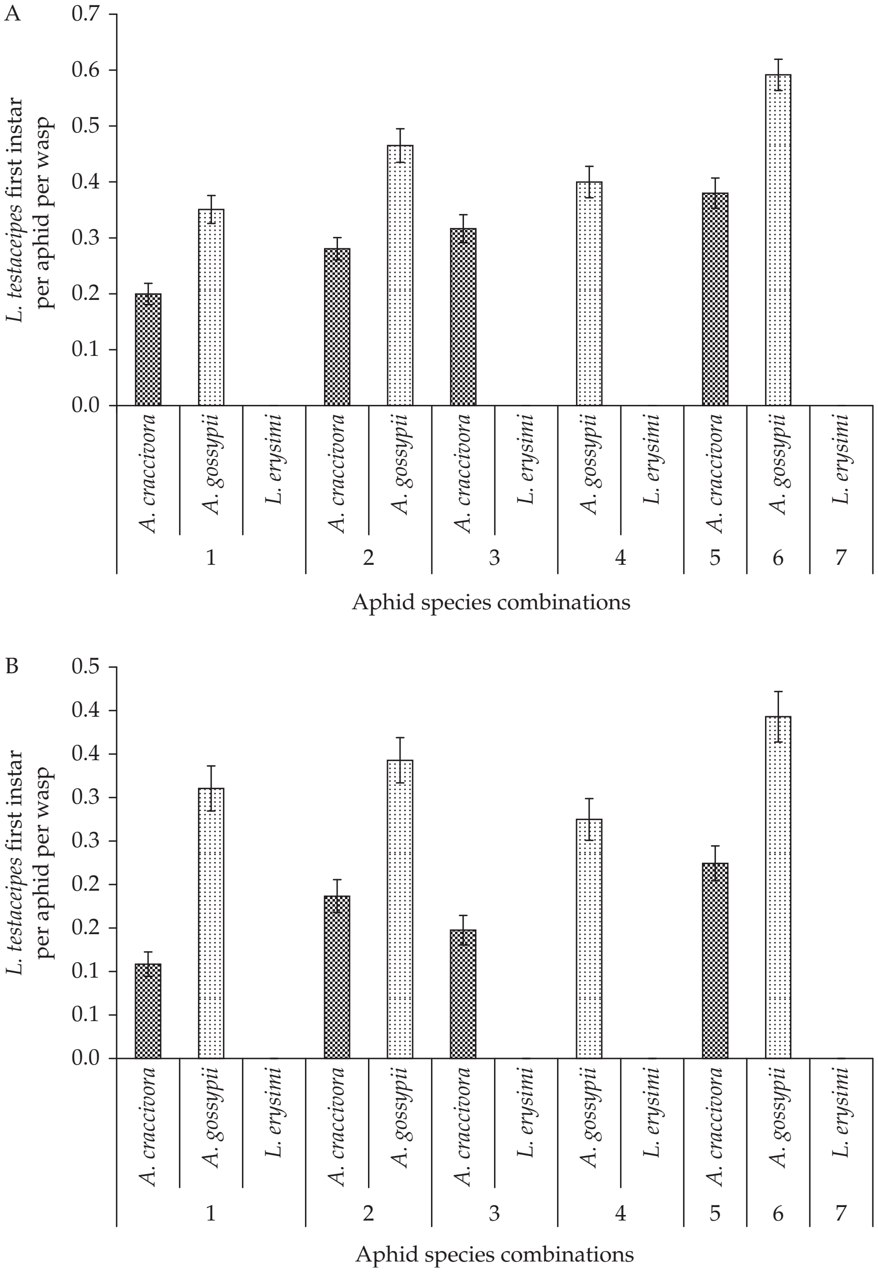

Fig. 2. Mean number (± 1 SE) of parasitoid first instar in the three aphid species under (1, 2, 3 and 4) the choice and (5, 6 and 7) no-choice conditions per female *Lysiphlebus testaceipes* in (A) the Petri dish and in (B) the cage as experimental arenas. *A. gossypii*, *Aphis gossypii* and *A. craccivora*, *Aphis craccivora.*

(Mackauer *et al.*, 1996; Rehman and Powell, 2010). *Lysiphlebus testaceipes* has an average oviposition time of 1.4 s (Marullo, 1987; Gross, 1993; Völkl and Mackauer, 2000), which makes it difficult to observe whether a female is probing or ovipositing. It was observed in this study that *L. erysimi* was quickly recognized and rejected by naive *L. testaceipes*. Similarly, parasitism by *Binodoxys communis* Gahan (Hymenoptera: Braconidae) was not detected in *L. erysimi* by Desneux *et al.* (2009). This partly suggests that some physical characteristics might lead to the recognition of the non-aphid host *L. erysimi* by the two foraging parasitoids. The indifference of *L. testaceipes* to *L. erysimi* may also presume the absence of an attractant in this aphid species. Grasswitz and Paine (1992) showed how kairomonal activity may make *L. testaceipes* attractive to one of its non-aphid hosts (*Acythosiphum pisum* (Harris)). This demonstrated the role of aphid secretion in the olfactory recognition of a host by a parasitoid. An aphid may be unsuitable because the species is physiologically incompatible (Carver and Sullivan, 1988), for instance due to the deficiency/richness of some necessary/harmful

Table 5. ANOVA results related to the number of aphid hosts parasitized and superparasitized by *Lysiphlebus testaceipes* (Fig. 3)

Source	df	SS	MS	*F*	*P*
Host[1]	1	2.15	2.15	42.06	5.2×10^{-10}
Condition[2]	1	0.26	0.26	5.06	0.0253
Arena[3]	1	1.50	1.50	29.28	1.5×10^{-7}
Parasitism[4]	1	19.22	19.22	375.77	2.2×10^{-16}
Host × parasitism	1	0.32	0.32	6.22	0.0133
Residuals	234	11.97	0.05		

[1] Two aphid host species: *Aphis craccivora* and *Aphis gossypii*.
[2] Two experimental conditions: choice and no-choice.
[3] Two experimental arenas: Petri dish and cage.
[4] Two parasitism levels: parasitism (aphids with one parasitoid larva) and superparasitism (more than one larva).
SS, sum of squares; MS, mean squares.

substance or hormonal resource. The present results indicate that *L. erysimi* is probably nutritionally unsuitable to or physiologically incompatible with *L. testaceipes*. Likewise, Silva *et al.* (2008) observed that the wasp did not parasitize the two Macrosiphini species *Brevicoryne brassicae* (L.) and *L. erysimi* in Brazil. Documentation on the rejection or unsuitability of *L. erysimi* to *L. testaceipes* is rather scanty.

This study shows that *L. testaceipes* readily attacked and successfully oviposited in *A. craccivora* and *A. gossypii*. The results reveal that *L. testaceipes* females may attack the same aphid host with up to nine stings before leaving. Furthermore, the increasing number of stabbing stings seems to have a positive effect on oviposition (Fig. 1). However, He *et al.* (2011) suggested that increasing attack attempts and/or ovipositor probings do not result proportionally in the oviposition of the aphidiine *Aphidius ervi* Haliday on its aphid host *Acyrthosiphon pisum* (Harris).

Aphis craccivora and *A. gossypii* were identified as the aphid hosts of *L. testaceipes* in Guam by Miller *et al.* (2002). Nevertheless, little is known about the preference of *L. testaceipes* for these aphid species in the native distribution range of the wasp where the common hosts comprise some Aphidini including *Schizaphis graminum* (Rondani) and *Rhopalosiphum* spp. The present results show that *L. testaceipes* exhibited higher parasitism in *A. gossypii* compared with *A. craccivora* under both choice and no-choice conditions. In all cases, *A. gossypii* was the most preferred host by *L. testaceipes*. Aphid parasitoid preferences for various host species have been investigated previously (Tripathi and Singh, 1995; Chau and Mackauer, 2001; Bueno *et al.*, 2003). There are some data to show that under no-choice conditions, *L. testaceipes* preferred *S. graminum* to *A. gossypii* with a parasitism rate of 76 and 56%, respectively (Rodrigues and Bueno, 2001). In the present experiments, *L. testaceipes* was reared on *A. gossypii*. This could have resulted in a higher susceptibility of *A. gossypii* to the wasp compared with *A. craccivora*. However, the preference for *A. gossypii* to *A. craccivora* is highly significant in all experiments, indicating that *A. gossypii* is a preferred host for *L. testaceipes* independent of the rearing conditions. Our results are consistent with those of Desneux *et al.* (2009) who found a significant preference of the braconid *B. communis* to *A. gossypii* compared with *A. craccivora*, and a higher ability of *A. craccivora* to defend itself at a rate of 53.8% compared with *A. gossypii* (14%) against the wasp.

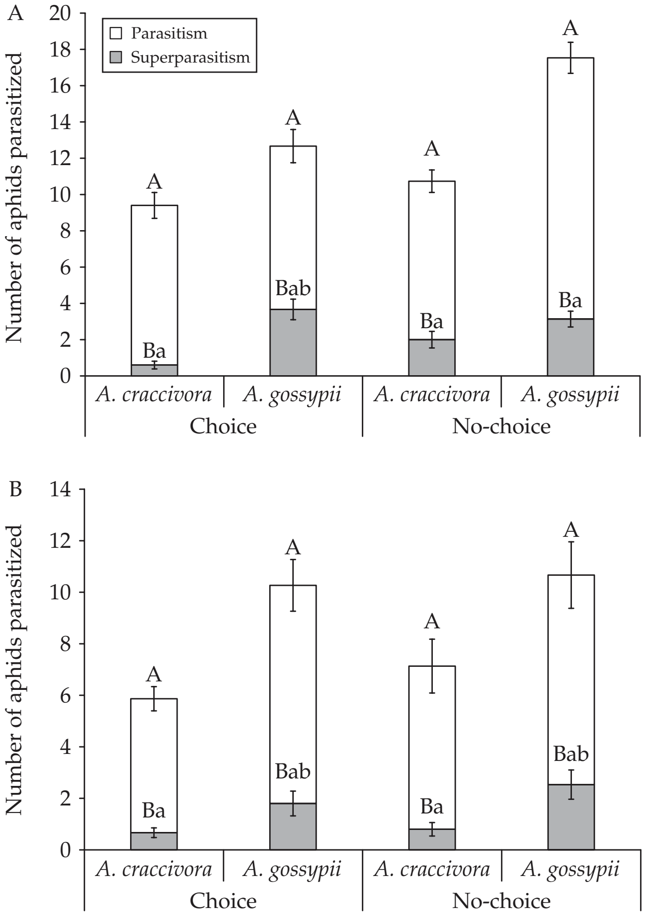

Fig. 3. Mean number (±1 SE) of aphids parasitized and superparasitized by *Lysiphlebus testaceipes* in *Aphis craccivora* and *Aphis gossypii* under the choice and no-choice conditions in (A) the Petri dish and in (B) the cage. Mean values followed by different capital letters are significantly different between the parasitized and superparasitized aphids within each host species, and those followed by the same small letters are not significantly different among the superparasitized aphids of *A. craccivora* and *A. gossypii* under each experimental condition (choice and no-choice), at the 5% level.

It is argued that parasitoids prefer hosts that guarantee optimum conditions for development and growth in immature stages (Godfray, 1994). Since all aphid instars were mixed in these experiments, a closer look on how host age/size might influence the preference of *L. testaceipes* is lacking. This is probably also one of the reasons why parasitism was low in some experiments. The defence mechanism in aphid insects may influence host acceptance by foraging parasitoids across aphid species, although how it does so remains to be established. Moreover, a higher aphid defence is usually presumed to minimize superparasitism (Rodrigues and Bueno, 2001), as it was observed in *A. craccivora*. However, superparasitism was not consistent enough (Fig. 3A and B) to assume a shortage of host discrimination (Jones *et al.*, 2003) by *L. testaceipes* in any of the two aphid host species. Although encapsulation of aphidiine eggs has been reported to be rare (Hågvar and Hofsvang, 1991), this phenomenon may explain host defence against parasitoid offspring in aphids. Furthermore, Oliver *et al.* (2010) clarified that another potential source of resistance to parasitoid is aphid infection by facultative symbionts. This is in accordance with Desneux *et al.* (2009) who identified the bacterial endosymbiont *Hamiltonella defensa* in *A. craccivora*. In the present study, the aphid hosts were dissected either alive or frozen 2 days after parasitization to check for *L. testaceipes* first-instar larvae. A control study for the emergence of parasitoid adults was lacking. In addition, we cannot totally exclude that some eggs of *L. testaceipes* had not hatched before dissection. However, the low number of *L. testaceipes* first instars observed in the cage experiments compared with the Petri dish experiments is likely to be related to the size of the arena as the number of aphids offered was the same in cages as in Petri dishes, where the wasps were confined to a small and narrow environment. Finally, it was observed that in some cages, *L. testaceipes* did not successfully locate the aphid hosts.

The present study shows that both *A. gossypii* and *A. craccivora* are readily attacked by *L. testaceipes* and that the species is a good candidate for biological control on vegetables in Benin. However, successful and sustainable use of *L. testaceipes* in Benin depends to a large extent on moderate pesticide use by the farmers. Further studies on intraguild predations and species interactions with other aphidophagous organisms present in the agroecosystem are also needed.

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Paper II

Host instar suitability of *Aphis gossypii* (Homoptera: Aphididae) for *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) and parasitism effect on aphid life table

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Abstract

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae, Aphidiinae) has constituted a well-studied parasitoid insect model but very little is known on the host-instar suitability of aphid for the wasp so far. One of the hosts of *L. testaceipes* is *Aphis gossypii* Glover (Homoptera: Aphididae). The latter is a serious aphid pest to vegetable production in Benin. Therefore, the objectives of our study were to: (i) examine the oviposition behavior of *L. testaceipes* on *A. gossypii*; (ii) investigate the host-instar suitability of *A. gossypii* for *L. testaceipes*; and (iii) compare the life table parameters of *A. gossypii* with aphids parasitized by *L. testaceipes* and unparasitized aphids (control). The study was conducted in a laboratory at 26 ± 1ºC in Petri dishes and revealed that the parasitoid utilized up to seven stabbing stings to handle and oviposit, particularly in older *A. gossypii*. We demonstrated that the net reproductive rate (*Ro*) and the intrinsic rate of increase (r_m) of aphids at third instar decreased to 7.33 and 2.45 times respectively as a result of the wasp's parasitism. The results indicate that the dominant alien wasp *L. testaceipes* has the potential to control the polyphagous aphid *A. gossypii* in vegetable agroecosystems in Benin.

Keywords: *Lysiphlebus testaceipes*; *Aphis gossypii*; instar suitability; parasitism effect

Introduction

The aphidiine *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) is an alien species to West Africa; however, it is the dominating aphid primary parasitoid in vegetable agroecosystems across Benin (Sæthre et al. 2011). The key aphid pest species in these systems are *Aphis gossypii* Glover, *Aphis craccivora* Koch and *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae). Recent studies in Benin have shown that *L. testaceipes* has a higher preference for *A. gossypii* compared to the other two species (Tepa-Yotto et al. 2013). In addition, *A. gossypii* is a serious constraint on vegetable production in this region. The presence of *L. testaceipes* therefore provides new possibilities for biological control of aphids in the region.

The study of host-instar suitability for an aphid parasitoid contributes not only insights into wasp preference with associated fitness costs and benefits, but also provides knowledge for use in biological control (Walker and Hoy 2003; Perdikis et al. 2004). *Lysiphlebus testaceipes* has constituted a well-studied parasitoid insect model but very little is known on the host-instar suitability of aphid for the wasp so far. Furthermore, very few studies have measured the effect of aphidiine parasitism on the aphid host life table.

Therefore, the objectives of our study were to: (i) examine the oviposition behavior of *L. testaceipes* on *A. gossypii*; (ii) investigate the host-instar suitability of *A. gossypii* for *L. testaceipes*; and (iii) compare the life table parameters of *A. gossypii* with aphids parasitized by *L. testaceipes* and unparasitized aphids (control).

Materials and Methods

Parasitoids

Aphid mummies were collected during January-April 2010 in vegetable producers' fields in Southern Benin. The koinobiont aphidiine *L. testaceipes* was then identified and reared in a screenhouse at the International Institute of Tropical Agriculture (IITA-Benin) for use in experiments. The wasp was reared using *A. gossypii* on potted plants of sweet pepper *Capsicum annuum* L. in insect rearing cages (46 x 45 x 43 cm).

Aphid instars

Aphids were collected from sweet pepper grown in the same vegetable producers' fields as for the aphid parasitoid. The species *A. gossypii* was identified and reared on sweet pepper in a screenhouse. The offspring used in the experiments was obtained at one day-intervals from several 24-hour old apterous viviparous female adult aphids in Petri dishes kept at 26 ± 1ºC in the laboratory. Aphid instars were determined on the basis of molts. The different cohorts of the aphid instars were collected at intervals of one day. On average, 24 hours were required for the previous aphid stage to molt to the subsequent stage.

Wasp oviposition behavior

The study of *L. testaceipes* oviposition was performed on all developmental stages of *A. gossypii*. Ten apteriform aphids of each instars-series and newly emerged aphid adults were exposed to a 24-hour old previously mated and inexperienced *L. testaceipes* female in a Petri dish (5.5 cm in diameter) on a section of sweet pepper leaf in the laboratory. Each wasp was tested three times for each aphid stage at 26 ± 1ºC. By the time the experiment was concluded, eight wasps (replicates) had been used per aphid stage, giving a total of 40 parasitoids and 1200 aphids. The oviposition behavior of *L. testaceipes* was observed under a stereomicroscope at x40 magnification during half an hour. During the oviposition act, the number of stings given to a single aphid by the parasitoid was recorded. Any ovipositor bending that did not touch the prey was excluded. After the 10 nymphs/adults had been probed and/or stung by the female wasp, they were removed one by one with a tiny brush, and 10 new nymphs/adults were added to the Petri dish. Aphids were then kept individually on a leaf section of sweet pepper placed on moistened paper tissue in Petri dishes for two days before dissection. The resulting number of *L. testaceipes'* larvae found in the aphid hosts was taken as a measurement of the parasitism of *L. testaceipes*.

Host-instar suitability

The host stage suitability of *A. gossypii* for *L. testaceipes* was studied during an oviposition period of eight hours in Petri dishes in a no-choice test in the laboratory. Each of the four instars and newly molted apterous adults of aphids, i.e. 50 individuals per stage, were kept on a leaf of sweet pepper. One parasitoid female (< 24-hour old), previously mated and without oviposition experience was confined to each Petri dish at 26 ± 1ºC. Parasitoid larvae were counted by dissecting all aphids after two days. Seventeen parasitoid females were used as replicates of each experiment, giving a total of 85 parasitoids and 4250 aphids.

Parasitism effect on aphid host

Ten aphids of each stage were separately exposed to a single 24-hour old *L. testaceipes* female, mated and inexperienced, in a Petri dish (5.5 cm diameter) in the laboratory at 26 ± 1ºC. After a contact period of four hours, the aphids were removed and placed individually in new Petri dishes on a sweet pepper leaf. The aphids were observed every 24 hours until death or mummification occurred. The offspring produced by each aphid was counted and transferred to a new Petri dish during daily inspections. Aphids exposed to the wasp were dissected after they died to check for parasitoid larvae. Only aphids with parasitoid larvae and those which mummified were included in the final data set. The mummification rate was estimated at each developmental stage and was defined as the proportion of mummified aphids relative to the total number of parasitized aphids. A control study was performed parallel to the experiment on unparasitized aphids for a period of 28 days. At two day-intervals, all aphids were transferred to a new leaf using a tiny brush. All the trials were replicated 13 times, giving a total of 65 parasitoids and 650 aphids used.

Statistical analysis

In the oviposition experiments, the response (y-values) was coded into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$); where y was the number of *L. testaceipes* larvae found in stung aphid hosts after dissection. Superparasitism (when $2 \leq y \leq 4$) was rare. Binary logistic regression with a logit link function was used to predict the effect of aphid host stage as a categorical variable and the number of stings given by the wasp to its host as a continuous explanatory variable on the parasitism by *L. testaceipes* (MINITAB 2011).

In the host-instar suitability experiments, we transformed the y-values into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$); where y was the number of *L. testaceipes* larvae found in aphid hosts after dissection. Superparasitism (when $2 \leq y \leq 4$) was infrequent. Binary logistic regression with a logit link function was also used to test for the effect of aphid host stage on the parasitism by *L. testaceipes* (MINITAB 2011). The Fisher's exact tests with Bonferroni correction at the 5% level were conducted to test for significant differences among developmental stages, followed by pairwise comparisons.

In the parasitism effect study, the life table parameters of aphids were computed using the SAS program developed by Maia et al. (2000). Differences in the intrinsic rate of increase values were tested for significance by estimating variances through the jackknife method (Meyer et al. 1986). The life table parameters were compared between parasitized and unparasitized aphids by performing ANOVA using the GLM procedure in SAS followed by paired Student's t-tests (SAS 2010).

In addition, the mummification of aphids was investigated. The response (y-values) was coded into two classes: 0 (when the parasitized aphid did not mummify) and

1 (when the parasitized aphid mummified). Binary logistic regression with a logit link function was used to test for the effect of aphid host stage as a factor and the number of days after parasitization as a covariate on the mummification of aphids parasitized by *L. testaceipes* (MINITAB 2011). The Fisher's exact tests with Bonferroni correction at the 5% level were also performed to test for significant differences among developmental stages, followed by pairwise comparisons.

Results

Wasp oviposition behavior

Lysiphlebus testaceipes used less than four stings to oviposit in younger aphids (first and second instars). The binary logistic regression provided a good description of the effect of aphid host stage and the number of stings given by the wasp *L. testaceipes* to the aphids on oviposition (Tables 1 and 5; Fig. 1). The proportion of aphids with parasitoid larvae increased with the number of the wasps's attacks ($P = 0.001$). Both the aphid host stage as a factor and the number of stings of the wasp as a continuous explanatory variable were significantly different from zero for the response investigated (Tables 1 and 5), showing that the two variables affected the oviposition of *L. testaceipes*. In addition, there was an interaction between these two variables, showing that the increase of the parasitism rate along the number of stings was different among the aphid developmental stages (Table 1; Fig. 1).

Host-instar suitability

In the no-choice host stage suitability study, we found that parasitism increased with aphid age but declined in the fourth and adult stages (Fig. 2). The most frequently accepted stage by *L. testaceipes* was the third instar nymphs of *A. gossypii* (P= 0.0001). In the third instars, the parasitism rate was 0.53 ± 0.02. This was 10.48; 3.27; 1.57; and 2.24 times higher than that observed with the first, second, fourth and adult stages respectively (Fig. 2). The binary logistic regression also provided a good description of the effect of aphid host stage on parasitized aphids (Tables 2 and 5). This factor was significantly different from zero for the response investigated (Tables 2 and 5).

Parasitism effect on aphid host

In aphids parasitized at the third instar, the net reproductive rate R_o as well as the intrinsic rate of natural increase r_m were significantly lower (2.119 ± 0.272 and 0.110 ± 0.018) compared to the control (15.529 ± 1.287 and 0.272 ± 0.008), respectively ($P<$ 0.01). *Aphis gossypii* parasitized by *L. testaceipes* required 6.107 ± 1.111 days to double, which is 2.40 times longer than the doubling time (*Dt*) recorded in unparasitized aphids (Table 3).

Fecundity of *A. gossypii* was severely affected by parasitism (Fig. 3a). Aphids parasitized by *L. testaceipes* produced less than one fourth of the progeny of the control (unparasitized aphids) (Table 3). All the parasitized aphids died or mummified within seven days after having been affected by *L. testaceipes* larvae (Fig. 3b; Fig. 4).

The cumulative rate of mummification was calculated from parasitized aphids. The mummification rate increased with the age of aphids and was relatively higher on older aphids (Fig. 4). There was no difference between the three last developmental stages at the end of observations (Fig. 4), showing that mummification increased only up to third instar. The aphids parasitized at first instar did not mummify. The binary logistic regression lines also provided a good description of the effect of aphid host stage and how many days elapsed from parasitism by *L. testaceipes* to the mummification of aphids (Tables 4 and 5; Fig. 4). Both the aphid host stage as a factor and the number of days after parasitism by *L. testaceipes* as a covariate were significantly different from zero for the response investigated (Tables 4 and 5), showing that the two variables affected the mummification of aphids. However, there was no interaction between these two variables.

Discussion

We found that a single sting of *L. testaceipes* can induce successful parasitism on *A. gossypii*, although the rate is low (< 20%). The results show that an increase of stabbings to seven stings of the wasp can attain 100% parasitism on third instar *A. gossypii* (Fig. 1). Our findings are in line with an earlier report on the handling mechanism of stronger hosts by aphidiine wasps (Kant et al. 2008). We demonstrated that *L. testaceipes* more frequently oviposited in the third instars of *A. gossypii* under no-choice condition (Fig. 2). This suggests that older aphids presumably had stronger defense capabilities, which limited the oviposition of the wasp on these aphids. Mid-aged aphid host instar suitability is common in the subfamily Aphidiinae (Weisser 1994; Chau and Mackauer 2000; Colinet et al. 2005; Tahriri et al. 2007) and usually appears to be the result of a compromise between attacking small and very large hosts. Mummification rate increased with increasing host age (Fig. 4). This means that if the wasp expends time and energy on handling older/larger aphids which usually contain more nutritional resources, this may increase the wasp's fitness gain.

In the present study all aphid stages were attacked (Figs. 1 and 2); and mummified except first instars (Fig. 4). Parasitized first instars died shortly after attack, likely after having suffered from the effect of parasitoid larvae. On the other hand, *A. gossypii* and *Myzus persicae* Sulzer first instar nymphs parasitized by *A. colemani* could still develop and mummify, mainly at fourth instar, with *A. gossypii* instars being the one which developed better to adulthood (Perdikis et al. 2004). This echoes that the effect of parasitoid larvae is likely to vary among host-aphidiid systems. It is also an indication that the food requirements of parasitoid larvae inside their hosts presumably differ from one host-parasitoid system to another.

The net reproductive rate and the intrinsic rate of increase of unparasitized *A. gossypii* at 15-30ºC commonly range between (R_o) 11.5-79.7 aphids per female and (r_m) 0.162-0.527 aphids per female per day respectively (Aldyhim and Khalil 1993; Perng 2002; Razmjou et al. 2006; Satar et al. 2008) on a diversity of host plant species; the highest parameters being at 25ºC from two host plant families Cucurbitaceae and Solanaceae. Our data supports similar conclusions. However, in our experiments parasitism of *L. testaceipes* had a significant effect on the life table parameters of *A. gossypii*. When aphids approach reproductive age, food resources are likely to be partly allocated for reproductive tissue formation or development. The current results indicate that *L. testaceipes* larvae feeding on the aphid host haemolymph and tissue disturbs the reproductive physiology and is also likely to lead to the deterioration of both somatic and gonadal tissue, which plays vital role in aphid host reproduction (e.g. female ovaries).

Similarly, the negative effect of parasitism by *A. colemani* on *A. gossypii* reproduction has been demonstrated by van Steenis and El-Khawass (1995); and that of *L. testaceipes* on *Schizaphis graminum* (Rondani) by Kring and Kring (1988). For future considerations, we expect that parasitism not only creates hormonal disruption but also reduces the sucking and feeding aptitudes of the sick and still living aphid hosts. The plant therefore probably benefits from a reduction in the loss of sap, minerals and vitamins, which may then diminish the viral transmission potential of the vector aphids. The limitation of plant nutrient assimilation may also be another consequence of parasitism, which may in turn negatively affect the growth of aphids. The inclusive knowledge provides an important tool for assessing to which extent the parasitoid species is a good biological control agent.

In conclusion, our data shows that higher number of stabbings of *L. testaceipes* female increased oviposition of the wasp on *A. gossypii*. The parasitoid preferred mid-aged host and third instars under no-choice conditions. The parasitism of *L. testaceipes* had a negative impact on the life table parameters of *A. gossypii*. The results indicate that the dominant alien wasp *L. testaceipes* has the potential to control the polyphagous aphid *A. gossypii* in vegetable agroecosystems in Benin. Nonetheless, further field investigations including environmental and trophic interaction effects will reveal the full potential of the wasp to control the aphid species in the fields.

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Table 1 Binary logistic regression of different developmental stages of the aphid host species *A. gossypii* and stabbing stings of *L. testaceipes* on parasitized aphids ($n = 1200$).

Predictor	Coefficient	SE coefficient	Z	P	Odds ratio^{a}	95% confidence interval	
						Lower	Upper
Parasitized aphids							
Constant	-7.21312	1.85231	-3.89	0.000			
Host stage (slope)							
Second instar	3.72316	1.89460	1.97	0.049	41.39	1.01	1696.80
Third instar	5.05263	1.87419	2.70	0.007	156.43	3.97	6160.89
Fourth instar	4.42530	1.87957	2.35	0.019	83.54	2.10	3324.90
Adult	3.73592	1.89683	1.97	0.049	41.93	1.02	1726.12
Stings (slope)	3.02841	0.906803	3.34	0.001	20.66	3.49	122.21

Table 1 (*Continued*)

						95% confidence interval	
Predictor	Coefficient	SE coefficient	*Z*	*P*	Odds ratio[a]	Lower	Upper
Parasitized aphids							
Host x Stings (slope)							
Second instar	-1.56529	0.943674	-1.66	0.097	0.21	0.03	1.33
Third instar	-2.10459	0.919109	-2.29	0.022	0.12	0.02	0.74
Fourth instar	-2.36986	0.913154	-2.60	0.009	0.09	0.02	0.56
Adult	-2.40808	0.912179	-2.64	0.008	0.09	0.02	0.54

[a] Odds ratio means the estimated probability for parasitism to be successful/unsuccessful for the different developmental stages of *A. gossypii* and for each increase of stings given by the wasp prior to oviposition.

Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

Table 2 Binary logistic regression of different developmental stages of the aphid host species *A. gossypii* on aphids parasitized by *L. testaceipes* ($n = 3030$).

						95% confidence interval	
Predictor	Coefficient	SE coefficient	*Z*	*P*	Odds ratio[a]	Lower	Upper
Parasitized aphids							
Constant	-2.93740	0.187350	-15.68	0.000			
Host stage (slope)							
Second instar	1.28953	0.217701	5.92	0.000	3.63	2.37	5.56
Third instar	3.04683	0.203839	14.95	0.000	21.05	14.12	31.39
Fourth instar	2.24672	0.206144	10.90	0.000	9.46	6.31	14.16
Adult	1.75983	0.210503	8.36	0.000	5.81	3.85	8.78

[a] Odds ratio means the estimated probability for parasitism to be successful/unsuccessful for the different developmental stages of *A. gossypii*.

Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

Table 3 Effect of parasitism by *L. testaceipes* on various life table parameters (mean ± standard deviation) of *A. gossypii* parasitized at its third instar stage (*n* = 221). The unparasitized aphid is the control.

Parameters	*A. gossypii*		P*
	Parasitized	Unparasitized	
Net reproductive rate, Ro (female per female)	2.119 ± 0.272	15.529 ± 1.287	0.0001
Intrinsic rate of increase, r_m (per day)	0.110 ± 0.018	0.272 ± 0.008	0.0001
Doubling time, Dt (days)	6.107 ± 1.111	2.545 ± 0.073	0.0017
Mean generation time, T (days)	6.903 ± 0.095	10.091 ± 0.208	0.0001
Finite rate of increase, λ	1.116 ± 0.020	1.313 ± 0.010	0.0001
Life-time fecundity (nymphs per female)	7.569 ± 2.381	35.292 ± 2.296	0.0001

**P*-values indicating statistical differences between unparasitized aphids and those parasitized by *L. testaceipes*, according to the paired Student's *t*-tests at the 5% level.

Table 4 Binary logistic regression of different developmental stages of the aphid host species *A. gossypii* and days after exposure to *L. testaceipes* on mummified aphids ($n = 520$). Aphids at first instar did not mummify and were omitted from the analysis.

Predictor	Coefficient	SE coefficient	Z	P	Odds ratio[a]	95% confidence interval	
						Lower	Upper
Mummified aphids							
Constant	-6.19735	0.235019	-26.37	0.000			
Host stage (slope)							
Third instar	1.92659	0.178733	10.78	0.000	6.87	4.84	9.75
Fourth instar	2.27794	0.180184	12.64	0.000	9.76	6.85	13.89
Adult	2.28459	0.179133	12.75	0.000	9.82	6.91	13.95
Days (slope)	0.729874	0.0302903	24.10	0.000	2.07	1.96	2.20

[a] Odds ratio means the estimated probability for mummification to be successful/unsuccessful for the different developmental stages of *A. gossypii* and for each rise in the number of days after parasitization.

Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

Table 5 Results of goodness of fit tests related to the results of the binary logistic regressions in Table 1, Table 2 and Table 4.

Parameter	Goodness of fit test	*Chi-square*	df	*P*
Oviposition behavior				
	Pearson	43.293	23	0.006
	Deviance	47.915	23	0.002
Instar suitability				
	Pearson	66.104	64	0.404
	Deviance	73.079	64	0.205
Mummification				
	Pearson	184.496	23	0.000
	Deviance	218.156	23	0.000

Figure captions

Fig. 1 Parasitism rate of *L. testaceipes* on different developmental stages of *A. gossypii* as a function of stabbing stings of the wasp prior to oviposition. Analyzed by the binary logistic regression, using the numbers of stings given by the parasitoid to its hosts: 0; 1; 2; 3; 4; 5; 6; and 7 stings (Table 1). The maximum numbers of parasitoid attacks observed on first and second instar nymphs were three and four stings respectively.

Fig. 2 Parasitism rate of *L. testaceipes* on different developmental stages of *A. gossypii*. Analyzed using binary logistic regression (Table 2). Values with different lowercase letters were significantly different among treatments, according to the Fisher's exact tests with Bonferroni correction at the 5% level. Error bars represent one standard error of the means.

Fig. 3 Fecundity (a) and age-specific survival (b) of third instars of *A. gossypii* parasitized by *L. testaceipes* and unparasitized (control) at 26 ± 1ºC.

Fig. 4 Mummification rate of different developmental stages of *A. gossypii* parasitized by *L. testaceipes* as a function of days after exposure to the wasp at 26 ± 1ºC. Analyzed using binary logistic regression (Table 4). Aphids at the first instar did not mummify and were omitted from the analysis. The logistic regression lines of fourth instar and adult overlap. Means with same small letters were not significantly different among aphid developmental stages on days 3 and 7 after exposure, according to the Fisher’s exact tests with Bonferroni correction at the 5% level.

Fig. 1

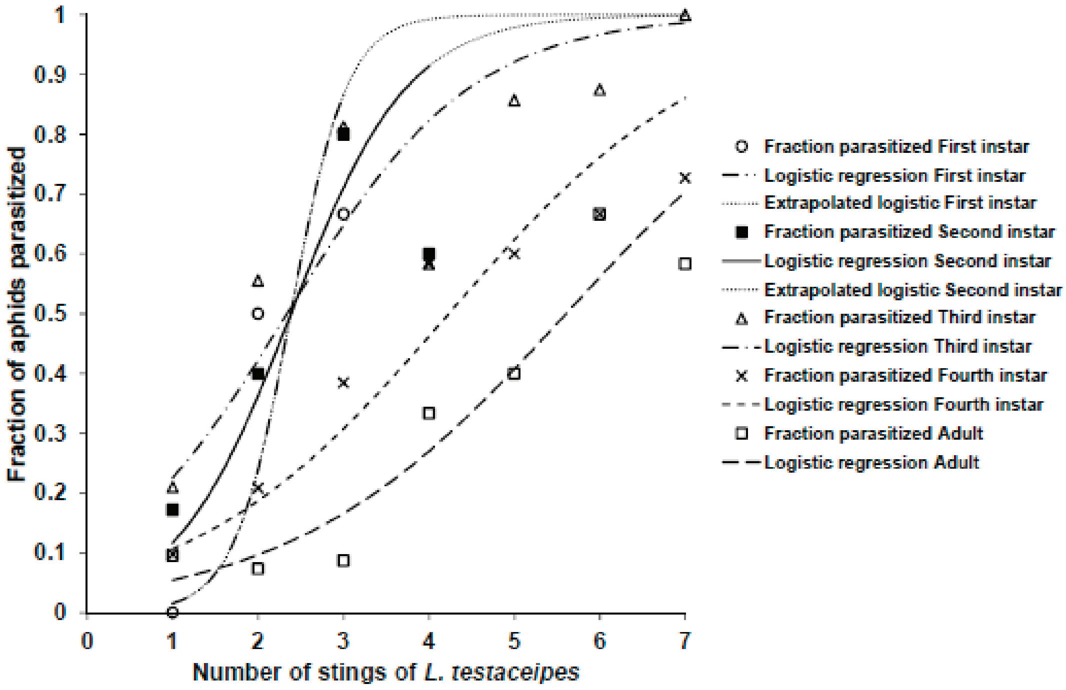

Fraction of aphids parasitized
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5
6
7
Number of stings of L. testaceipes
Fraction parasitized First instar
Logistic regression First instar
Extrapolated logistic First instar
Fraction parasitized Second instar
Logistic regression Second instar
Extrapolated logistic Second instar
Fraction parasitized Third instar
Logistic regression Third instar
Fraction parasitized Fourth instar
Logistic regression Fourth instar
Fraction parasitized Adult
Logistic regression Adult

Fig. 2

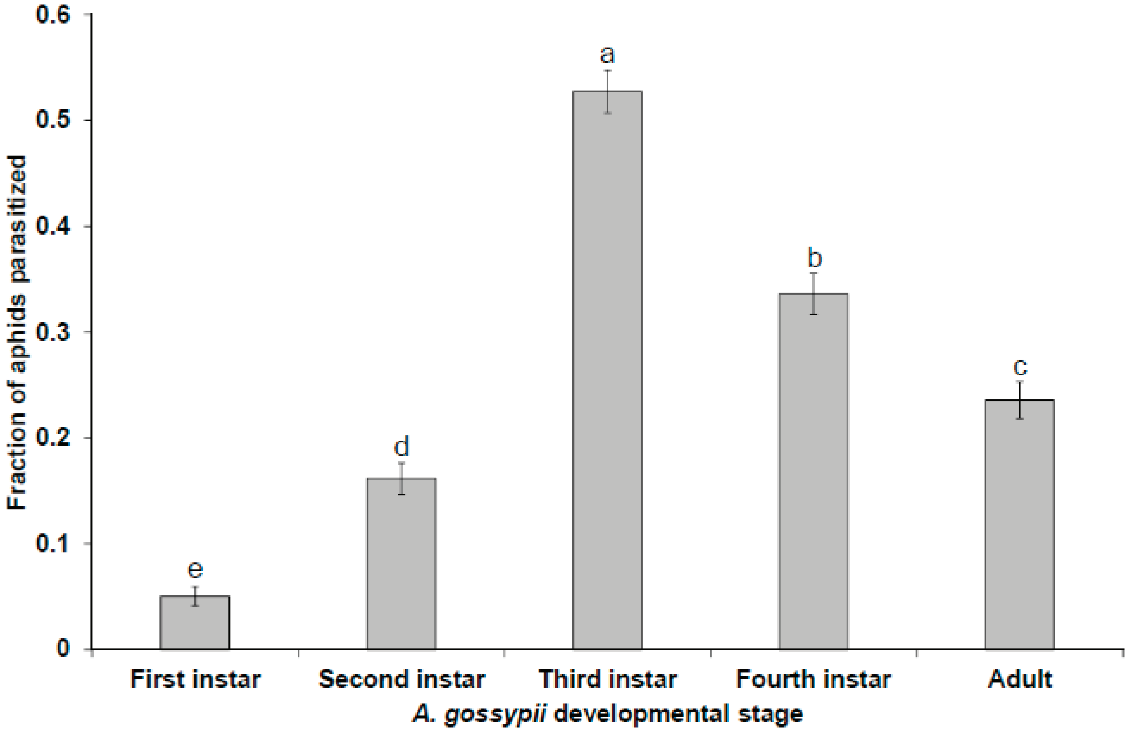

0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of aphids parasitized
a
b
c
d
e
First instar
Second instar
Third instar
Fourth instar
Adult
A. gossypii developmental stage

Fig. 3

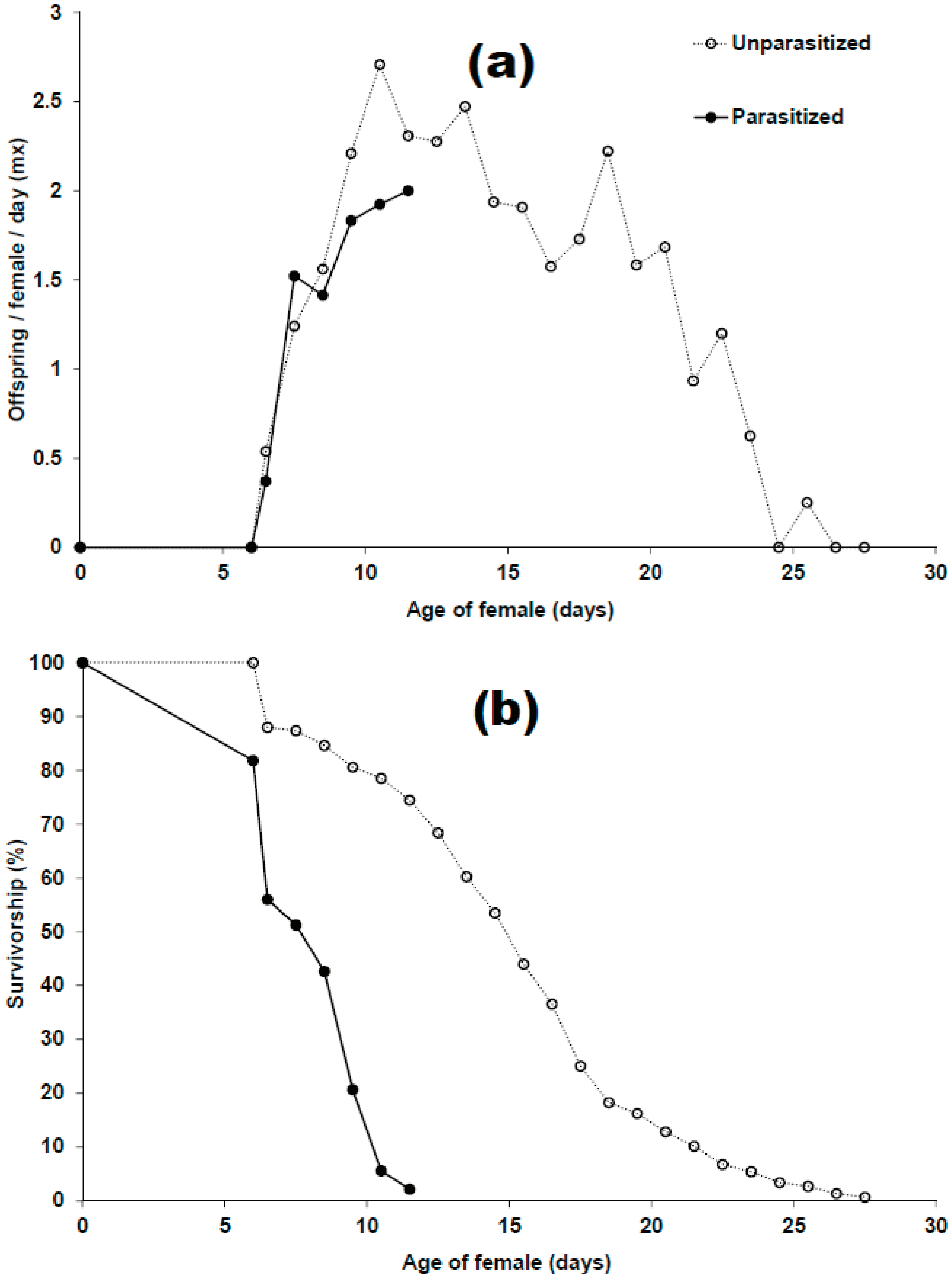
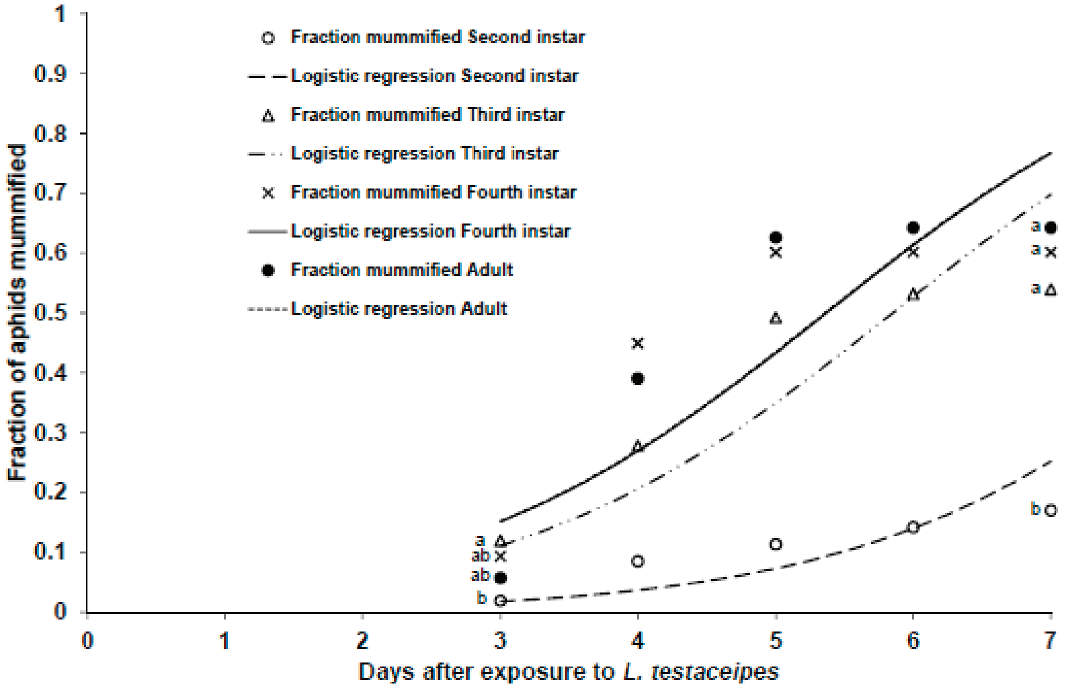

(a)
Unparasitized
Parasitized
Offspring / female / day (mx)
0
0.5
1
1.5
2
2.5
3
0
5
10
15
20
25
30
Age of female (days)
(b)
Survivorship (%)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
30
Age of female (days)

Fig. 4



Fraction of aphids mummified
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
Days after exposure to *L. testaceipes*
○ Fraction mummified Second instar
— — Logistic regression Second instar
△ Fraction mummified Third instar
— · · Logistic regression Third instar
× Fraction mummified Fourth instar
—— Logistic regression Fourth instar
● Fraction mummified Adult
----- Logistic regression Adult
a
ab
ab
b
a
a
a
b

Paper III

Effect of Mating, Energy and Host Age on the Oviposition Behavior of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae)

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Running head: Oviposition Behavior of *L. testaceipes*

Abstract

A number of studies have been increasingly concerned with host-parasitoid interactions; however, to date no investigation has measured the combined effect of mating, energy and host age on the oviposition behavior of aphidiids. Therefore, the objective of our study is to contribute towards filling this gap using the aphid host *Aphis craccivora* and the parasitoid *Lysiphlebus testaceipes.* The latter is an important biocontrol agent worldwide. The study has proved the higher host defense capabilities of fourth instars compared to those of second instar-nymphs. Kicking, walking away and cornicle secretion were observed as aphid defense behaviors in both instars, while shaking was only performed by fourth instars. We also demonstrated that energy increased the wasp's ability to handle aphids, particularly older/larger/stronger host instar-nymphs. We concluded that the significance of the combined effect of mating, energy and host age is evidence of a rather complex mechanism that mediates the preference behavior of *L. testaceipes*.

Keywords *Lysiphlebus testaceipes*, *Aphis craccivora*, parasitoid oviposition behavior, aphid defense behavior, host preference, intrinsic/extrinsic effects

Introduction

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Braconidae, Aphidiinae) is a koinobiont parasitoid in the group formed by aphidiids. The species was recently found in vegetable systems across Benin, where it was first recorded in West Africa (Sæthre et al. 2011). *Aphis craccivora* Koch (Homoptera: Aphididae) was listed as one of the serious aphid pests hosting *L. testaceipes* in the country. This adds to the global potential of *L. testaceipes* as a biocontrol agent, making it deserving of more attention.

A number of studies have dealt with aphid parasitoid preference. This preference varies from one parasitoid species to another, and also varies with regard to aphid host species/instar. Nevertheless, the mechanisms underlying the preference behavior of aphidiines continue to be poorly understood (He et al. 2011). According to Mackauer et al. (1996) the 'female perceptual assessment of host value' is a function of female state, host quality and host patch. It is well known that host size may be a key factor that regulates the preference of an aphid parasitoid (Kouamé and Mackauer 1991). However, Chau and Mackauer (2001) stated that although nutritionally less suitable, younger pea aphids *Acyrthosiphon pisum* (Harris) were frequently accepted by their wasp *Monoctonus paulensis* (Ashmead). This was inconsistent with the results of Lin and Ives (2003) who demonstrated that *Aphidius colemani* Viereck has a preference for larger *Aphis glycines* Matsamura. On the other hand, a preference for mid-aged *Myzus persicae* (Sulzer) was observed among *Aphidius ervi* Haliday by Colinet et al. (2005); this also echoes the preference of *Aphidius matricariae* Haliday for *Aphis fabae* Scop. (Tahriri et al. 2007); and that of *L. testaceipes* for *Aphis gossypii* Glover (Tepa-Yotto et al. unpublished data). However, apart from aspects of physiological compatibility, such variability among host-parasitoid systems suggests that the ratio of the interspecific 'balance of power' between aphid defense and parasitoid performance may adjust the preference of the wasp for a particular species/instar (Gerling et al. 1990). Therefore, the wasp makes a choice among host instars based on the optimal balance between fitness costs and fitness returns (Chau and Mackauer 2001). Despite this, the interaction between the parasitoid behavioral oviposition and the aphid host defense involves several, more complex factors.

Foraging parasitoids have the ability to adjust their behavior according to the conditions encountered (Rasekh et al. 2010). However, in analyzing the trade-off that the wasp typically faces within an aphid colony, a couple of questions should be addressed. First: is the wasp mostly concerned with the fitness of its future progeny in the larger, high-value aphids and is therefore willing to pay the cost for handling them (Kouamé and Mackauer 1991; He et al. 2011)? Second: does the parasitoid prefer to parasitize the smaller aphids (Chau and Mackaeur 2000; Perdikis et al. 2004) at minimum cost? The two options associated with intrinsic and extrinsic factors are of paramount importance and determine the parasitoid behavior and potential as a biological control agent of aphids.

Wyckhuys et al. (2008) demonstrated that «parasitoids lived significantly longer when fed honey or sucrose than honeydew». On the other hand, Fauvergue et al. (2008) concluded that «virgin female parasitoids stayed motionless more often and for longer periods than mated females and they consequently attacked aphids at a lower rate». Although these facts are well-known, no adequate study has simultaneously measured the combined effect of the various above-mentioned factors on the oviposition behavior of aphidiids.

Aphid parasitoid behaviors mediated by physical/chemical cues are not new (Mackauer et al. 1996; Hatano et al. 2008) as host-parasitoid interactions (Sasaki and Godfray 1999). However, the mechanism by which the wasp handles aphid species/instars with stronger defense skills has not been adequately studied in aphidiines to date. Nonetheless, the counter-defense response in *Ephedrus cerasicola* Starý was demonstrated in the work of Hofsvang and Hågvar (1986), who found that *Myzus persicae* (Sulzer) had greater capability for defensive behavior when older, which was combated by the wasp with higher stabbing attacks. A recent study conducted with the host-parasitoid *A. gossypii*-*L. testaceipes* supports similar conclusions (Tepa-Yotto et al. unpublished data). In the past decade, Völkl and Mackauer (2000) have reviewed the fact that the development of adaptations helps parasitoids counter aphid defense behavior. In addition, the study recently done by He et al. (2011) clearly indicates evidence of counter-defense in aphidiids. These authors have found an increasing number of attack attempts by *Aphidius ervi* Haliday in order to handle older pea aphid *Acyrthosiphon pisum* (Harris).

In this study, we specifically hypothesized that mating in *L. testaceipes* leads to an adjustment of the oviposition behavior of females. Besides, we hypothesized that carbohydrates as resource energy serve to enhance the counter-defense of *L. testaceipes* for successful oviposition. We studied the combined effect of these factors along with the effect of host age on the oviposition behavior of *L. testaceipes*. In this paper, the word 'counter-defense' is defined as the cost or ability of the wasp to overcome aphid defense and successfully perform oviposition.

Materials and Methods

Collection and rearing of insects

Aphis craccivora and mummies of *L. testaceipes* were collected in January-February 2012 from cowpea field at the International Institute of Tropical Agriculture (IITA-Benin) located in the southern coastal area of Benin. Clean cultures of *A. craccivora* and of *L. testaceipes* reared on *A. craccivora* were established in an insect rearing facility using cowpea as host plant.

Production of insects for experiments

Second instars of *A. craccivora* were used to produce parasitoid cohorts for trials and were the basis for standardizing the fitness of experimental wasps. The aphids were placed and fed for two hours on a set of ten fresh cowpea beans on a layer of paper tissue in a plastic box (15 x 12 x 10 cm). Six *A. craccivora*-reared mated females of *L. testaceipes* were then released in the box for two hours. The mummies developed at 26 ± 1ºC and 12 hours photophase and they were split into two main batches. Each batch was assigned for use in experiments with either mated females or virgins. We then carefully cut the plant organs with attached mummies. Next, these were isolated in glass vials (5 x 1cm). In order to obtain fertilized eggs, one female was exposed to a male in a glass vial. After copulation was observed and completed, the monandrous mated female was selected. Virgin females were selected immediately after emergence. The two types of wasps (mated and virgin) were kept separately in glass vials and supplied with either carbohydrates with four drops of 50% honey, or with water. The sustenance was provided through the mesh at the top of the vials for a period of 6 ± 2 hours prior to the experiment.

Experimental procedure

Ten apterous aphids that had previously been given 5 min to settle on a section of cowpea leaf in a Petri dish with a diameter of 5 cm at 26 ± 1ºC were exposed to a single wasp for 30 min. The half-hour observation duration included the time the wasp required to clean its mouthparts and antennae with forelegs and its ovipositor, often with its hind legs or all the cited parts simultaneously, using only its forelegs after frontward bending of abdomen. During pre-trials we observed obstinate stings of wasp on aphid molt exuviae. In this study, the experimental arena was therefore free of aphid exuviae thus avoiding any loss of energy by the wasp. We tested aphids of two different development stages, namely second and fourth instars under no-choice conditions. Thus, a total of eight treatments were set up: 2 aphid ages (second/fourth instars) x 2 wasp states (mated/virgin) x 2 nutritional states (honey/water). In this study, each of the eight treatments was replicated using 30 *L. testaceipes* females without oviposition experience and of 12 ± 4 hours of age.

Observations

The aphid defense behaviors in both second and fourth instars were recorded under a stereomicroscope at 40x magnification with respect to the rate of a) shaking; b) kicking; c) walking away; and d) cornicle secretions. In addition, we documented the behavioral events displayed by the wasp from her encounter with the host until her departure. Here, we divide the wasp's attempts at attack into two main categories, i.e. ovipositor probing that did not touch the hosts, and successful stings that touched aphids. By excluding the probing that did not touch the host, the following direct observations were then carefully recorded: i) total successful wasp stings; and ii) ovipositor insertions for oviposition. Experimental aphids were kept separately in Petri dishes at 26 ± 1ºC and 12 hours photoperiod, supplied with fresh cowpea leaf at intervals of one day and dissected 48 hours after wasp oviposition in order to check for parasitoid larvae under a stereoscopic microscope.

Statistical analysis

The aphid defense behavior data with respect to the number of aphids displaying the different behavioral defense events (shaking, kicking, walking away, and cornicle secretion) was log-transformed before analysis to meet the assumptions of normality and equal variance. Transformed data was then analyzed using a linear analysis of variance model (ANOVA type II sum of squares) with aphid host age as a categorical variable. The number of stings given to aphids by the wasp prior to oviposition was also log-transformed and analyzed in the same way with mating, energy and host age as factors. The Tukey's post hoc tests at the 5% level were used to test for significant differences among groups, followed by pairwise comparisons (R statistical software; R Core Team 2012).

For the parasitoid oviposition behavior observations, the response (y-values) was coded into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$); where y was the number of *L. testaceipes* larvae found in stung aphid hosts after dissection. Superparasitism (when $2 \leq y \leq 4$) was very rare. Binary logistic regression with a logit link function was used to test for the effect of mating, energy and host age as factors on *L. testaceipes* parasitism (MINITAB 2011). The Fisher's exact tests with Bonferroni correction at the 5% level were performed to test for significant differences among groups, followed by pairwise comparisons.

Results

Aphid defense behavior

To escape from an attack of a wasp, the target aphid used its defense strategies. Kicking, walking away and cornicle secretion were observed as aphid defense behaviors by both instars, while shaking was only performed by fourth instars (Fig. 1). In the current investigation, shaking and kicking were often performed by the aphid to prevent the attack of the wasp whereas walking away and cornicle secretion were the most decisive and presumably more costly behaviors to halt the attack. In this study, *A. craccivora* emitted cornicle secretion in order to produce a highly repellent environment to *L. testaceipes* at close proximity of the aphid emitter. Then the wasp invariably runs away from the aphid, which releases cornicle secretion.

The results showed that aphid defense was, in general, more frequent in fourth instars compared to second instars ($F_{1,52}$= 13.75; *P*= 0.000509); ($F_{1,52}$= 11.28; *P*= 0.00148); ($F_{1,52}$= 29.53; *P*= $1.49*10^{-6}$) for shaking, kicking and walking respectively; except the release of cornicle secretion, which did not differ between the two host ages in terms of number of aphids producing the secretion ($F_{1,52}$= 1.216; *P*= 0.275). Walking and cornicle secretion release were the most common defense behaviors followed by kicking and shaking, in that order, for both host ages, ($F_{3,92}$= 27.83; *P*= $6.75*10^{-13}$), ($F_{3,116}$= 35.29; *P*= $2.79*10^{-16}$) for fourth and second instars respectively.

Parasitoid oviposition behavior

Lysiphlebus testaceipes was sometimes deterred by the fourth instar *A. craccivora* with its big size and well developed legs. As a result, she ran away upon the encounter. In other cases, the wasp exhibited more strong behavior by giving several stings to the fourth-instar aphid (Fig. 2), gyrating around the host to find the ideal place for oviposition. The wasp targeted the cauda region, thus risking exposure to kicking from the hind legs. She alternatively targeted either the abdomen laterally or the part lengthening from the mesothorax to the prothorax. In all cases, the wasp needed to succeed in ovipositing before the aphid performed a costly defense such as releasing its cornicle secretion. This form of defense was always powerful enough to keep the wasp away. On the other hand, increasing the number of stings aided successful oviposition of *L. testaceipes*, particularly in fourth instars *A. craccivora* (Figs. 2 and 3).

The effects of energy and host age were significant for the number of stings and oviposition of the wasp on *A. craccivora* (Tables 1 and 2), while mating proved of minor importance for the number of stings (Table 1). However, mating significantly led to an adjustment of the oviposition of the wasp (Tables 2 and 3). The interaction between mating, energy and host age was significant for the number of stings of *L. testaceipes* on *A. craccivora* (Table 1). The binary logistic regression provided a good description of the effect of mating, energy and host age on the oviposition of *L. testaceipes* (Tables 2 and 3). These factors were significantly different from zero for the response investigated (Tables 2 and 3), showing that the three variables affected the oviposition of *L. testaceipes*. In addition, there were interactions between energy and the two other factors (mating and host age) (Table 2).

Discussion

A variety of factors determine aphids' defense against their parasitoids. Aphid cornicle secretion has different functions (Goff and Nault 1974). It may serve as either a kairomonal cue (Grasswitz and Paine 1992; Battaglia et al. 1993; Powell et al. 1998; Battaglia et al. 2000) or a defense stratagem (Nault and Phelan 1984; Rasekh et al. 2010; He et al. 2011). Compared to the second instars, fourth instars have rapid locomotion thanks to their developed body and legs. Increasing the speed of locomotion in fourth instars allowed the aphids to escape the wasp attack. Shaking was less common and was not observed in second instars in this study. Second instar aphids must engage in more costly defense behavior to escape from the wasp attack because, with their small bodies, shaking is not sufficient to stop the wasp from attacking. We observed that in some cases shaking was followed by kicking in fourth instar, in order to achieve an efficient defense mechanism. As a result, aphid defense behaviors were more frequently observed in fourth instars than second instars. Regardless of instar, we suggest that the aphid defense efficiency/cost increases as follows: shaking < kicking < walking away < cornicle secretion in *A. craccivora*.

Fourth instars were larger aphids and our data suggests that a low number of stings by the wasp would minimize the chance of successful oviposition in them due to their developed defense skills. Therefore, the persistent wasp stings for oviposition in larger aphids could be a response to expectations of enhanced fitness of future parasitoid progeny through ovipositing in the larger, high-value aphids (Kant et al. 2008; Barrette et al. 2009; Kant et al. 2012). We do, however, acknowledge that the experiments' no-choice condition may have affected the performance of the wasp, resulting in relatively high rates of oviposition in fourth instars. Nonetheless, we assume that the probability of oviposition may decline in the adult stage due to greater defense capability. In summary, the fact that *L. testaceipes* has the ability to parasitize both younger and older aphids has increased our understanding of the reason why this wasp has been so successful in establishing itself as an important biocontrol agent worldwide.

The present study demonstrated that energy increased the wasp's performance. Persad and Hoy (2003) indicated that water and honey are among the key food resources that lengthen the survival of *L. testaceipes*. Also, *Aphidius rhopalosiphi* and *Diaeretiella rapae* fitness significantly increased in terms of longevity and potential fecundity when supplied with carbohydrates (Tylianakis et al. 2004).

Our study shows that although mating is of minor importance for the number of stings of the wasp, it significantly involved a regulation of the oviposition. This could be in relation with the will of the wasp to adjust the sex ratio in its future progeny (Cloutier et al. 1991; Michaud and Mackauer 1995; Fauvergue et al. 2008). The interactions between energy and the two other factors (mating and host) were significant. This implies that we expect the oviposition rate of carbohydrate-supplied wasps to differ from that of carbohydrate-deprived ones with respect to the effects of mating and host age.

In conclusion, the combined effect of mating, energy and host age significantly influenced the number of stings and, therefore, the oviposition of the wasp, which provides evidence of a fairly complex mechanism mediating the preference behavior of *L. testaceipes*.

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Table 1 Effect of mating, energy and host age on the number of stings of *L. testaceipes* on *A. craccivora* prior to oviposition: ANOVA results.

Source	df	SS	MS	*F*	*P*
Mating	1	0.018	0.018	0.779	0.3776609
Energy	1	0.365	0.365	15.725	$7.541*10^{-5}$
Host	1	17.259	17.259	742.566	$< 2.2*10^{-16}$
Mating x Energy	1	0.274	0.274	11.789	0.0006058
Mating x Energy x Host	3	0.520	0.173	7.463	$5.674*10^{-5}$
Residuals	2392	55.595	0.023		

Table 2 Binary logistic regression of two aphid host stages of *A. craccivora* (fourth and second instars), two mating status of *L. testaceipes* (mated and virgin) and two energy status of the parasitoid (honey-fed and water-supplied) on aphids parasitized ($n = 2400$).

						95% confidence interval	
Predictor	Coefficient	SE coefficient	Z	P	Odds ratio[a]	Lower	Upper
Parasitized aphids							
Constant	-0.75005	0.10549	-7.11	0.000			
Mating (slope)	0.46486	0.11839	3.93	0.000	1.59	1.26	2.01
Energy (slope)	0.49829	0.14820	3.36	0.001	1.65	1.23	2.20
Host (slope)	0.355091	0.11835	3.00	0.003	1.43	1.13	1.80
Mating x Energy (slope)	-0.86481	0.17464	-4.95	0.000	0.42	0.30	0.59
Energy x Host (slope)	-1.17048	0.17557	-6.67	0.000	0.31	0.22	0.44

[a] Odds ratio means the estimated odds for parasitism to be successful/unsuccessful for fourth instar compared to second instar of *A. craccivora* attacked by mated females *L. testaceipes* compared to virgin and honey-fed female parasitoids compared to female parasitoids only supplied with water.

Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

Table 3 Results of goodness of fit tests related to the results of the binary logistic regression in Table 2.

Parameter of	Goodness of fit test	*Chi-square*	df	*P*
Table 2				
	Pearson	37.235	2	0.000
	Deviance	37.738	2	0.000

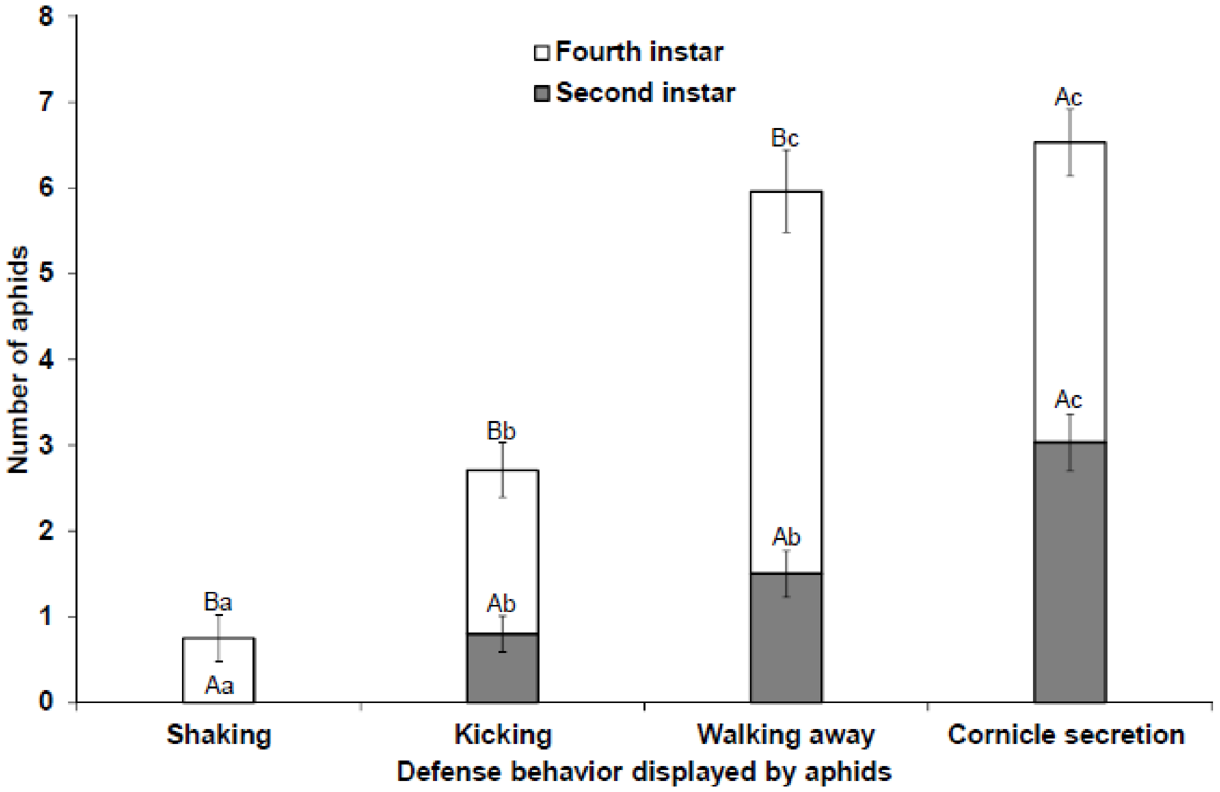
Figure captions

Fig. 1 Defense rate of second and fourth instars *A. craccivora* against *L. testaceipes*. Means with different capital letters are significantly different between second and fourth aphid instars at each defense behavior; and those with same small letters are not different among the four defense behaviors within each aphid instar; according to Tukey's tests at the 5% level. Error bars indicate one standard error of the means.

Fig. 2 Effect of mating (mated/virgin) and energy (honey/water) on the number of stings given by *L. testaceipes* to second and fourth instars *A. craccivora* prior to parasitoid oviposition. Values with same lowercase letters were not significantly different among treatments, according to the Tukey's tests at the 5% level. Error bars represent one standard error of the means.

Fig. 3 Effect of mating (mated/virgin) and energy (honey/water) on the parasitism rate of *L. testaceipes* on second and fourth instars of *A. craccivora*. Analyzed, using the binary logistic regression (Table 2). Values with different lowercase letters were significantly different among treatments, according to the Fisher's exact tests with Bonferroni correction at the 5% level. Error bars indicate one standard error of the means.

Fig. 1



Fourth instar
Second instar
Number of aphids
0
1
2
3
4
5
6
7
8
Ba
Aa
Bb
Ab
Bc
Ab
Ac
Ac
Shaking
Kicking
Walking away
Cornicle secretion
Defense behavior displayed by aphids

Fig. 2

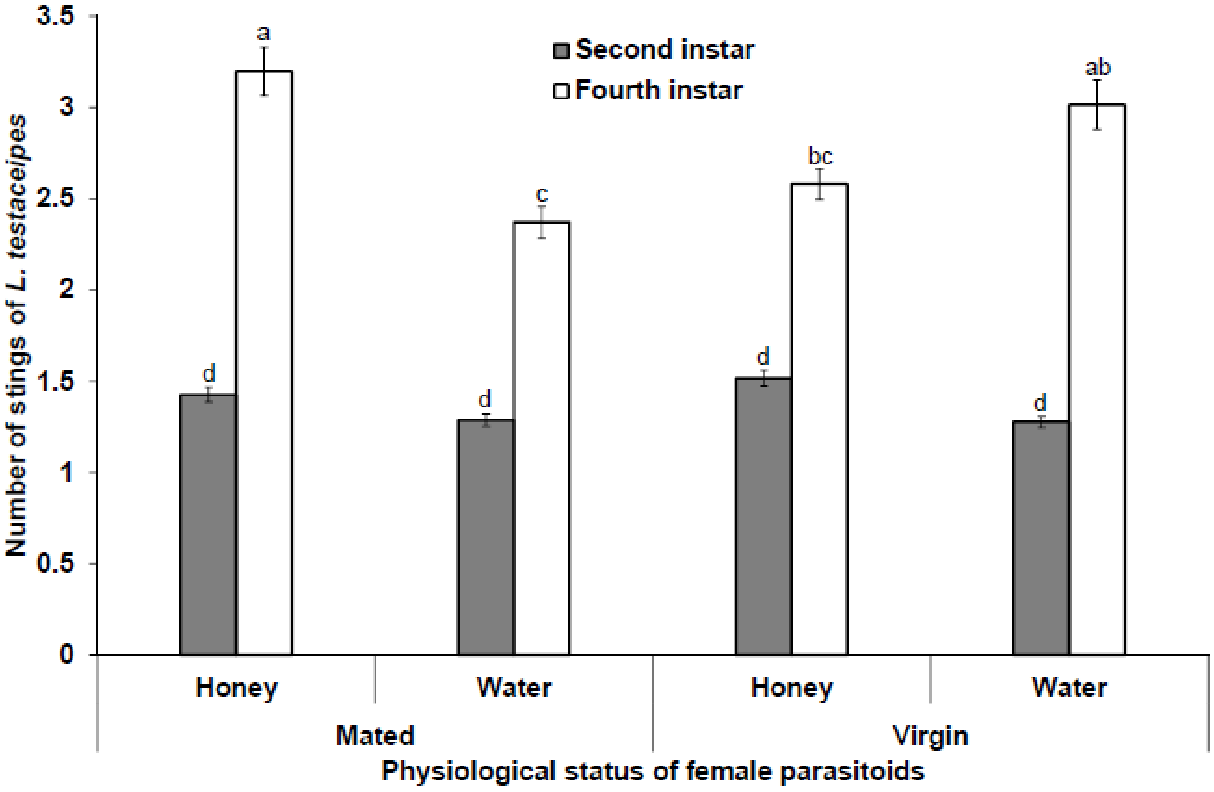
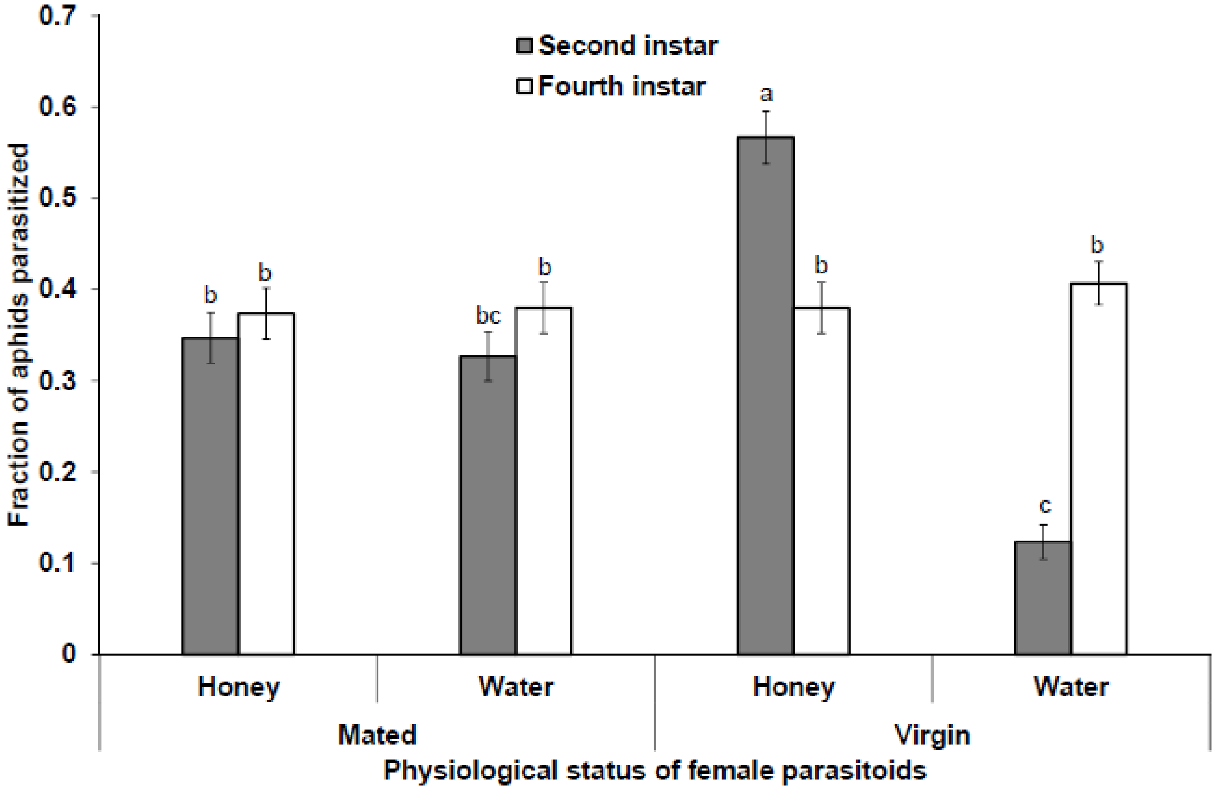

Second instar
Fourth instar
Number of stings of *L. testaceipes*
3.5
3
2.5
2
1.5
1
0.5
0
a
c
bc
ab
d
d
d
d
Honey
Water
Honey
Water
Mated
Virgin
Physiological status of female parasitoids

Fig. 3


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Fraction of aphids parasitized
■ Second instar
□ Fourth instar
b
b
bc
b
a
b
c
b
Honey
Water
Honey
Water
Mated
Virgin
Physiological status of female parasitoids

Paper IV

Intraguild predation of an alien aphid parasitoid *Lysiphlebus testaceipes* by three indigenous predators

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Abstract

Recent laboratory studies have shown that the newly discovered, alien but dominant aphid parasitoid species, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae), is one of the key natural enemies of aphids in vegetable agroecosystems in Benin. However, intraguild predation may affect the potential of the wasp to control aphids in the new environment. Therefore, a study was carried out in the laboratory to measure the intraguild predation capability of three common indigenous aphid predators *Cheilomenes propinqua* (Mulsant), *Cheilomenes sulphurea* (Olivier) (Coleoptera: Coccinellidae) and *Ischiodon aegyptius* (Wiedemann) (Diptera: Syrphidae) over *L. testaceipes*. Our results proved that *C. propinqua*, *C. sulphurea* and *I. aegyptius* are potential competitors to *L. testaceipes*, because they exhibited high predation rates on unparasitized *A. gossypii*. We demonstrated that *C. propinqua* and *C. sulphurea* are omnivorous intraguild predators of *L. testaceipes*, while the data showed that *I. aegyptius* did not attack *A. gossypii* mummies parasitized by *L. testaceipes*. The results indicate that the intraguild interactions between *L. testaceipes* and the native predators are likely to play important roles in determining the community structure, and they also act as regulators of both the aphid populations and the alien parasitoid populations.

Keywords *Lysiphlebus testaceipes*, *Aphis gossypii*, *Cheilomenes propinqua*, *Cheilomenes sulphurea*, *Ischiodon aegyptius*, alien parasitoid, indigenous predators, intraguild predation

Introduction

Recent studies have shown that the newly discovered, alien but dominant aphid parasitoid species, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae), is one of the key natural enemies of aphids in vegetable agroecosystems in Benin (Sæthre et al. 2011). Follow-up studies in the laboratory have shown that the species willingly attacks two of the most important pestiferous aphid host species on vegetables in the country, *Aphis gossypii* Glover and *Aphis craccivora* Koch (Homoptera: Aphididae) (Tepa-Yotto et al. 2013). However, the effect of intraguild predation (IGP) on the potential of the wasp to provide efficient control of aphids in the new environment is unknown.

The ladybirds *Cheilomenes propinqua* (Mulsant), *Cheilomenes sulphurea* (Olivier) (Coleoptera: Coccinellidae) and the hoverfly *Ischiodon aegyptius* (Wiedemann) (Diptera: Syrphidae) were recorded as dominant and indigenous aphid predators on vegetables in Benin (Sæthre et al. 2011). All of these natural enemies along with the parasitoid *L. testaceipes* were frequently observed attacking the aphid *A. gossypii* in the fields. However, to date the interactions between the alien aphid parasitoid *L. testaceipes* and the three native predatory species have not been studied.

The parasitoid *L. testaceipes* is a native nearctic species and a typical species of North America (Starý 1970). To date *L. testaceipes* has been reported in six African countries, namely Kenya (Starý et al. 1985; Greathead 2003), South Africa (Starý et al. 1985), Burundi (Autrique et al. 1989), Tunisia (Ben Halima-Kamel 2011), Algeria (Laamari et al. 2010) and Benin (Sæthre et al. 2011). In these six African countries the species was introduced for the biological control, specifically of *Schizaphis graminum* (Rondani) in both Kenya and South Africa, and of *Aphis fabae* Scop. (Homoptera: Aphididae) in Burundi. *Lysiphlebus testaceipes* successfully established itself only in Burundi (Autrique et al. 1989). The parasitoid spread in Algeria and Tunisia naturally, likely after being air-borne over the Gibraltar strait from the Mediterranean coastal areas of Europe (Laamari et al. 2012). The origin of *L. testaceipes* in Benin is unknown.

Both *C. propinqua* and *C. sulphurea* are native to the Afrotropical Region. Both ladybird species have very large distribution areas, ranging from the Cape Provinces of South Africa north-eastwards towards Ethiopia, and skirting the Congo Basin, westwards on towards West Africa including Nigeria (R. Stals, pers. comm.) and Benin (Sæthre et al. 2011).

The hoverfly *I. aegyptius,* which has aphidophagous larvae, is also widespread in Africa. The distribution of this species extends far into the offshore islands of Madagascar, the Seychelles, the Canary Islands and Madeira. It is also present in parts of the Middle East and the eastern Mediterranean. In tropical Africa, *I. aegyptius* is one of the commonest aphid predator species among the Syrphidae (N. Wyatt, pers. comm.).

Intraguild predation has been increasingly emphasized as significant interspecific interactions of key importance regulating populations and determining community structure of the guild formed by aphids (Meyhöfer and Hindayana 2000; Pell et al. 2008; Meisner et al. 2011; Chacón et al. 2012; Traugott et al. 2012). Therefore, this question has become a major topic of investigation in the field of biological control (Chacón and Heimpel 2010) and conservation ecology (Müller and Brodeur 2002; Alhmedi et al. 2010). Royer et al. (2008) defined intraguild predation interaction as 'the killing and eating of species that compete for a common resource'. More specifically, this interaction includes competition for aphid-prey as food resource, not only coincidental, but also omnivorous intraguild predation as important trophic relationships (Hemptinne et al. 2012). In the guild where aphid is the prey, coincidental intraguild predation refers to the killing of parasitoid larvae by eating living parasitized aphids, whereas omnivorous intraguild predation which is asymmetrical between parasitoids and predators addresses the damage by the predators to parasitoid pupae inside mummified aphids (Pell et al. 2008).

The overall objective of this study was to carry out a laboratory investigation with the aim to better understand and quantify trophic interactions that may occur in the field with respect to intraguild predation of *C. propinqua*, *C. sulphurea* and *I. aegyptius* on *L. testaceipes*. More specifically, our goal was to (i) estimate the predation rates of the larvae of the two ladybirds and the hoverfly on unparasitized *A. gossypii*; and (ii) estimate the predation risk of the aphid predators on *A. gossypii*-reared mummies of *L. testaceipes*.

Material and methods

Collection and rearing of aphid predators

A laboratory culture of the aphid species *A. gossypii* had already been established with a view to feeding the predators. *Aphis gossypii* was reared on sweet pepper, *Capsicum annuum* L., in insect rearing cages (46 x 45 x 43 cm).

The aphid predators *C. propinqua*, *C. sulphurea* and *I. aegyptius* including larvae, pupae and adults were collected during January-April 2010 in vegetable producers' fields in southern Benin and taken to the laboratory. The larvae were then carefully reared to adulthood. The different instar larvae were fed daily ad libitum with the aphid *A. gossypii* in Petri dishes until they pupated. The pupae were conditioned in the Petri dishes until adults emerged. Each individual instar of the predators including the coccinellids at their adult stage required a large number of aphids and a fairly long time to complete development, which makes rearing them a challenging procedure.

Twenty adults of both sexes of each predator species were allowed to copulate in small plastic boxes (15 x 12 x 10 cm) and the females were released in the insect rearing cages to lay eggs. Four *A. gossypii*-infested potted plants of sweet pepper were arranged per cage. Adult flies of *I. aegyptius* were fed with a 10% honey solution. Two days after the adults were released in the cages, we collected eggs from plant organs and the cage walls using a fine brush and a magnifying glass. This was needed especially for the smaller hoverfly eggs. A high density of aphids on plants increased the number of eggs laid by the females. The eggs collected from the cages were incubated for two days on a sweet pepper leaf section in Petri dishes and emerging larvae were reared inside these Petri dishes. The hoverfly develops through three larvae instars and the coccinellids have four larval stages. The production of molt exuviae was the indication of larvae growth to the next developmental stage. These larvae were used in the experiments. The rearing was performed under laboratory conditions at 26 ± 1°C temperature and 12 hours photophase.

Production of aphid mummies

A laboratory culture of *L. testaceipes* was already established, having been reared on *A. gossypii* on sweet pepper. At the same time as the predators were reared, simultaneous production of *A. gossypii*-mummies of *L. testaceipes* for use in experiments was carried out. Third instars *A. gossypii* were exposed to parasitism by *L. testaceipes*. Mummification occurred on average within five days after parasitization. Mummies less than two days old were used to study the predation risk of *C. propinqua*, *C. sulphurea* and *I. aegyptius* on *L. testaceipes*.

Estimation of predation potential on unparasitized aphids

The larvae of *C. propinqua*, *C. sulphurea* and *I. aegyptius* were used to estimate their potential to eat unparasitized third instars *A. gossypii*. The predators' larvae were isolated and starved for 12 hours before use to standardise their hunger level. Each larval instar of each predator species was provided with one hundred aphids in a Petri dish (5.5 cm diameter) in the laboratory at 26 ± 1°C temperature and 12 hours photoperiod. The number of aphids consumed by each larva was established by counting the number of left-over aphids within 24 hours.

Estimation of predation risk on mummies

The predation of mummies was studied as a measurement of the intraguild interaction between *L. testaceipes* and three aphid predators. Fifty mummies of *A. gossypii* less than two days old, and previously parasitized by *L. testaceipes*, were exposed to 12-hour-starved larvae of *C. propinqua*, *C. sulphurea* and *I. aegyptius* for a period of 24 hours in Petri dishes (5.5 cm diameter) in the laboratory at 26 ± 1°C temperature and 12 hours photophase. The predation risk of each instar-larva of the predatory species on *L. testaceipes* mummies was then measured as their ability to chew the mummies and thereby damage the parasitoid pupa inside the mummies. At the end of the experiment, the remaining mummies were examined with the aid of a stereomicroscope at 40x magnification for evidence of attack by the predators.

Statistical analysis

The data was log-transformed before analysis to meet the assumptions of normality and equal variance. Transformed data was then analyzed using a linear analysis of variance model (ANOVA type II sum of squares). The Tukey's post hoc tests at the 5% level were used to test for significant differences among groups, followed by pairwise comparisons (R statistical software; R Core Team, 2012). The predation rates on both unparasitized and mummified aphids were compared at each larva instar within each predator species using a one-way ANOVA followed by pairwise comparisons.

Results

Predation potential on unparasitized aphids

We found that all larval instars of *C. propinqua*, *C. sulphurea* and *I. aegyptius* preyed on unparasitized third instar of *A. gossypii* (Table 1; Fig. 1). The voracity of the three predators increased with older instars. Daily consumption of unparasitized aphids by first instars of the predators was 9.58 ± 0.32; 12.03 ± 0.51 and 17.40 ± 0.95 for *C. propinqua*, *C. sulphurea* and *I. aegyptius*, respectively. The consumption rate increased 7.99 and 7.38 times for fourth instars *C. propinqua* and *C. sulphurea*, respectively; and 5.00 times for third instar *I. aegyptius*. This gives a total consumption of 152.94; 178.88; and 154.43 aphids throughout the larval stages of *C. propinqua*, *C. sulphurea* and *I. aegyptius*, respectively. The aphidophagous larvae of *I. aegyptius* were the most voracious when compared to their corresponding instars of the ladybirds *C. propinqua* and *C. sulphurea* (Table 1; Fig. 1).

Predation risk on mummies

The results showed that parasitism and mummification significantly reduced the suitability of *A. gossypii* for the three predators (Table 1; Fig. 2). Younger coccinellid instars i.e. first and second instars *C. propinqua* ($F_{3,230}$= 3445.8; $P< 2.2*10^{-16}$) and first instar *C. sulphurea* ($F_{3,182}$= 459.2; $P< 2.2*10^{-16}$) did not attack *A. gossypii* mummies of *L. testaceipes* (Table 1; Fig. 2). Fourth instar of *C. sulphurea* was the most damaging intraguild predator, which killed 46.06% of *L. testaceipes* pupae within 24 hours. The hoverfly *I. aegyptius* did not attack the aphid mummies at any of its larval stages (Table 1).

Discussion

The results show that *A. gossypii* has a high rate of attack by *C. propinqua* and *C. sulphurea,* as previously reported for a wide range of coccinellids (Lee and Kang 2004; Işikber 2005; Yu et al. 2005; Bayoumy 2011; Chenaux et al. 2011). This also resembles the consumption capability of the coccinellid *Cheilomenes lunata* (Fabricius) on the cowpea aphid *Aphis craccivora* Koch; fourth instar of the former having daily feeding rates of 85.3 ± 6.89; 83.8 ± 7.21; 60.4 ± 5.43; 54.9 ± 5.63; 28.6 ± 3.47 of first, second, third, fourth and adult stages, respectively, of the later (Ofuya 1995). *Aphis gossypii* is also frequently attacked by *I. aegyptius*. Our data is comparable with earlier reports on the potential of syrphids to eat aphids (Tenhumberg and Poehling 1995; Pineda et al. 2007; Nelson et al. 2012). However, the high voracity of *C. propinqua*, *C. sulphurea* and *I. aegyptius* on unparasitized third instar *A. gossypii* proves that they are potential competitors to *L. testaceipes* in the field.

Asymmetrical omnivorous intraguild predation of *A. gossypii* mummies parasitized by *L. testaceipes* was observed with *C. propinqua*, *C. sulphurea*, but not *I. aegyptius*. Similarly, Almohamad et al. (2008) found that second and third instars of the hoverfly *Episyrphus balteatus* DeGeer did not consume mummies of *Acyrthosiphon pisum* Harris parasitized by *Aphidius ervi* Haliday. The fact that the aphidophagous hoverfly larvae did not attack aphid mummies may have a mechanical explanation as the mouthparts of the syrphid have dissimilarities when compared to those of coccinellids. However, the exact explanation remains a subject for further investigation. In contrast, the predation on mummies by the ladybirds was observed and the rate of predation increased with older instars. Younger instars of the ladybirds (first and second instars *C. propinqua* and first instar *C. sulphurea*) did not attack aphid mummies, which may be a function of their stage of development as they were both small and had small mandibles compared to older instars. However, instars' size varied according to species, which is supported by the capability of second instars *C. sulphurea* to attack the mummies compared to the smaller second instars *C. propinqua.* Our data supports the conclusions of Royer et al. (2008), who showed that *Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Méneville first instars did not attack *Schizaphis graminum* Rondani mummies parasitized by *L. testaceipes*.

Mummification appears to protect the prey from predation (Xue et al. 2012) and there is some data showing that mummies have a negative impact on the development of intraguild predators (Takizawa et al. 2000; Bilu and Coll 2009). In contrast, Takizawa et al. (2000) reported that the consumption of *A. craccivora* mummies parasitized by *Aphidius colemani* Viereck did not negatively affect the survival of *Harmonia axyridis* (Pallas) and *Propylea japonica* Thunberg (Coleoptera: Coccinellidae). On the other hand, coincidental intraguild predation did not affect the oviposition behaviour of the syrphid *E. balteatus* (Almohamad et al. 2008) nor reduce survival or increase developmental time of *C. septempunctata*, *H. axyridis* and *P. japonica* (Takizawa et al. 2000).

Coincidental and omnivorous intraguild predation can be important sources of mortality for *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphidiidae) (Meyhöfer and Hindayana 2000) and *L. testaceipes* (Colfer and Rosenheim 2001). However, the overall ecosystem service with regard to the natural control of aphids is enhanced when both parasitoids and predators are present (Colfer and Rosenheim 2001; Snyder and Ives 2003; Kavallieratos et al. 2004). Similarly, a number of studies suggest that intraguild predation does not necessarily disrupt biological control by parasitoids (Meyhöfer and Klug 2002; Rutledge et al. 2004; Janssen et al. 2006; Straub et al. 2008; Xue et al. 2012). Nonetheless, in analysing behavioural responses as consequences of intraguild interactions regulation in the fields, it is well known that the oviposition behaviour of intraguild preys, including aphid parasitoids, may be adversely affected by the presence of top predatory larvae, in other words the intraguild preys seek to avoid ovipositing at sites where intraguild predators are abundant (Seagraves 2009), although some exceptions have been observed (Jazzar et al. 2008). Moreover, there is some data to show that predator' oviposition declines with the presence of aphid mummies (Pineda et al. 2007) or conspecific and heterospecific larval tracks (Almohamad et al. 2010; Dixon and Kindlmann 2012).

In conclusion, *C. propinqua*, *C. sulphurea* and *I. aegyptius* expressed high predation rates on unparasitized *A. gossypii* in the present study, proving that they are potential competitors to *L. testaceipes* under field conditions. We demonstrated that *C. propinqua* and *C. sulphurea* are omnivorous intraguild predators of *L. testaceipes*, while the data showed that *I. aegyptius* did not attack *A. gossypii* mummies parasitized by *L. testaceipes*. The results indicate that the intraguild interactions between *L. testaceipes* and the predators are likely to play important roles in determining the community structure, and they are also regulators of both the aphid populations and the alien parasitoid populations.

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Table 1 Comparative predation rates (means ± SE) of each instar of *C. propinqua*, *C. sulphurea* and *I. aegyptius* on both unparasitized and mummified third instars of *A. gossypii* parasitized by *L. testaceipes*. The numbers of replicates are given in the table (*n*).

IG-Predator instar	IG-Predator species											
	C. propinqua				*C. sulphurea*				*I. aegyptius*			
	n	Unparasitized	*n*	Mummified	*n*	Unparasitized	*n*	Mummified	*n*	Unparasitized	*n*	Mummified
1	81	9.58 ± 0.32a	68	0.00 ± 0.00b	73	12.03 ± 0.51a	55	0.00 ± 0.00b	57	17.40 ± 0.95a	62	0.00 ± 0.00b
2	69	23.87 ± 0.83a	61	0.00 ± 0.00b	65	28.54 ± 0.85a	48	4.90 ± 0.49b	54	50.07 ± 1.83a	49	0.00 ± 0.00b
3	63	42.97 ± 1.49a	56	11.96 ± 0.40b	61	49.46 ± 2.07a	43	15.88 ± 0.88b	45	86.96 ± 1.60a	41	0.00 ± 0.00b
4	58	76.52 ± 1.24a	49	18.59 ± 0.93b	59	88.85 ± 1.80a	40	23.03 ± 1.09b	-	-	-	-

Means followed by different lowercase letters are significantly different with regard to the predation rates of each predatory instar of each predator species between unparasitized and mummified aphids, according to the one-way ANOVA results followed by pairwise comparisons ($P< 0.0001$).

Figure captions

Fig. 1 Predation rates of *C. propinqua*, *C. sulphurea* and *Ischiodon aegyptius* on unparasitized third instars of *A. gossypii*. The initial number of prey offered to the predators was 100 aphids at each replicate. Within each predator species, means with different capital letter are significantly different between instars; means with different small letter are significantly different across predator species at each developmental stage; according to the Tukey's tests at the 5% level.

Fig. 2 Estimation of the predation risks of *C. propinqua* and *C. sulphurea* on mummies of *A. gossypii* previously parasitized at its third instar by *L. testaceipes*. A mummy was judged to be attacked when it was chewed and damaged by the predator. The initial number of prey exposed was 50 mummies at each replicate. Within each predator species, means with different capital letter are significantly different among instars; means with different small letter are significantly different across predator species at each developmental stage; according to the Tukey's tests at the 5% level.

Fig. 1

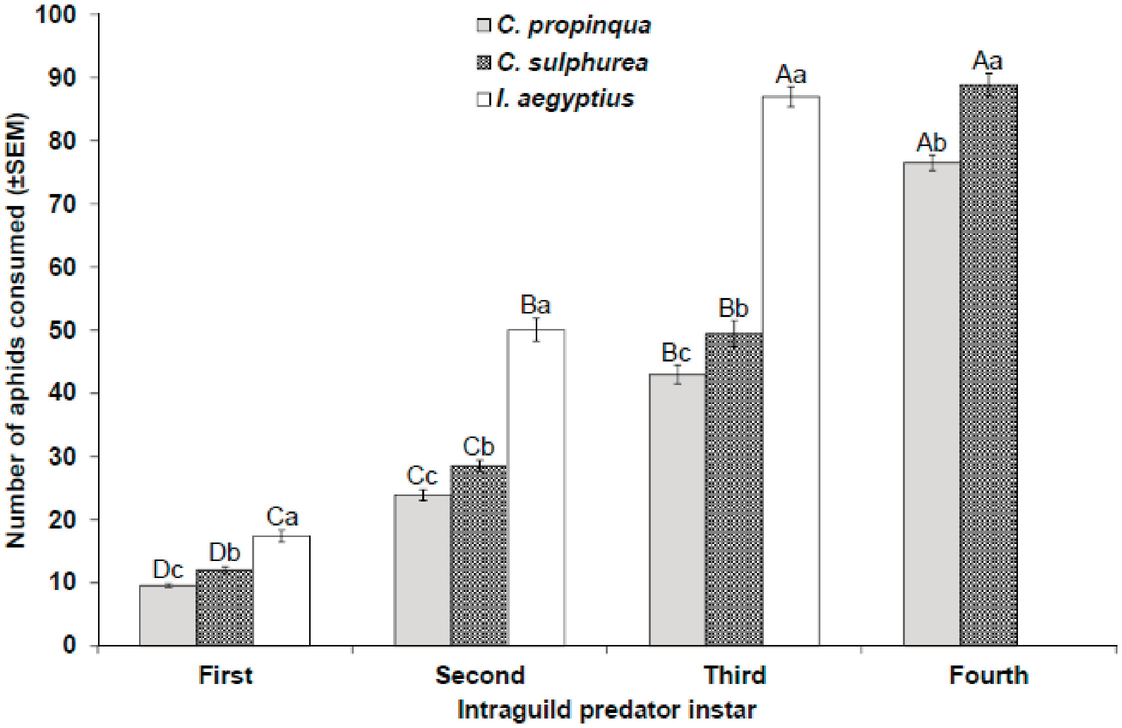
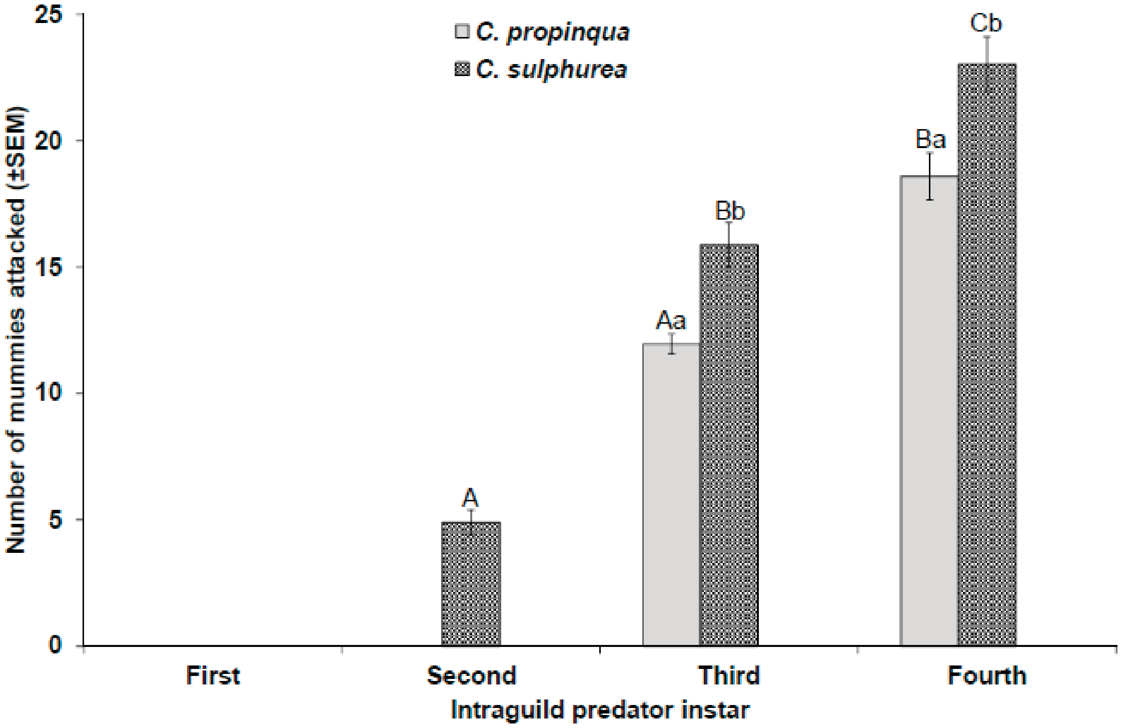

C. propinqua
C. sulphurea
I. aegyptius
Number of aphids consumed (±SEM)
100
90
80
70
60
50
40
30
20
10
0
Dc
Db
Ca
Cc
Cb
Ba
Bc
Bb
Aa
Ab
Aa
First
Second
Third
Fourth
Intraguild predator instar

Fig. 2



C. propinqua
C. sulphurea
Number of mummies attacked (±SEM)
0
5
10
15
20
25
A
Aa
Bb
Ba
Cb
First
Second
Third
Fourth
Intraguild predator instar

Paper V

Responses of *Lysiphlebus testaceipes* (Cresson) to varying densities of *Aphis gossypii* Glover: laboratory and field investigations including effect of indigenous predators

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Running title: Prey-density dependence of aphidophagous arthropods

Abstract

The objective of the present work was to examine, under laboratory and field conditions, the responses of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) to different host densities of *Aphis gossypii* Glover (Homoptera: Aphididae), a serious pest in vegetable agroecosystems in Benin. We also analyzed the numerical responses of key indigenous predatory species of *A. gossypii* in the field. The laboratory trials revealed a type II curve functional response of *L. testaceipes* on *A. gossypii* at 26 ± 1ºC during 24 hours exposure time. In field trials, we found that despite attacks by the native hyperparasitoid *Syrphophagus africanus* (Gahan) (Hymenoptera: Encyrtidae), the wasp was effective in suppressing aphids. Besides, three aphid predators exhibited positive numerical responses in the field. The data demonstrates that *L. testaceipes* is a good candidate for biological control of aphids in vegetable systems in Benin. The sustainable establishment of this alien species in the new environment may be partly regulated by the effect of potential indigenous intraguild predators such as *Cheilomenes sulphurea* (Olivier). However, our results support the possible coexistence of both the alien parasitoid *L. testaceipes* and the indigenous predators recorded.

Keywords: *Lysiphlebus testaceipes*, alien parasitoid, *Aphis gossypii*, functional and numerical responses, field investigation, *Syrphophagus africanus*, *Cheilomenes propinqua*, *Cheilomenes sulphurea*, *Ischiodon aegyptius*, indigenous hyperparasitoid and predators

Introduction

The polyphagous *Aphis gossypii* Glover (Homoptera: Aphididae) is a serious pest in vegetable agroecosystems in Benin. The recent discovery of *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae, Aphidiinae) (Sæthre et al. 2011) therefore provides new alternatives for integrated pest management (IPM), particularly for biological control of the aphid pest species. However, the same study identified several common predator and hyperparasitoid species on aphids in the vegetable fields, which may result in intraguild reactions.

The measurement of the functional and numerical responses is commonly performed to reveal the potential of a candidate species as a biocontrol agent. It is broadly recognized that prey density is an obvious predictor for the response of a natural enemy (Chiou et al. 2004). The functional response of *L. testaceipes* has previously been investigated under laboratory conditions on *A. gossypii* (Bazzocchi and Burgio 2001) and *Schizaphis graminum* Rondani (Jones et al. 2003). This adds to the extensive number of studies that investigate the functional responses of aphid parasitoids to changing host densities. However, the response of the wasp in open fields continues to be poorly documented, due to the fact that such studies not only require considerable efforts but are also complex and technically challenging, and therefore difficult to conduct. In attempts to address the functional response of aphidiids in the fields, there have been some manipulations with the aid of field cages (Snyder and Ives 2003), but no adequate open field investigation successfully reports on such data. In contrast to the small number of studies on aphid parasitoids, the demographic and aggregative numerical responses have been extensively documented for aphid predators in the field (Frazer and Raworth 1985; Evans and Youssef 1992; Agarwala and Bardhanroy 1999; Monsrud and Toft 1999; Omkar and Pervez 2004).

Under field conditions, a variety of biotic and abiotic factors will influence the response of a natural enemy. The aphid parasitoid *L. testaceipes* is a new species in West Africa, and its performance as a biological control agent in its new geographical area requires further investigation. Therefore, the goal of the present work was to assess the potential of this wasp species as a biocontrol agent against aphids in its new environment. More specifically, the study aimed to examine the responses of *L. testaceipes* to different densities of *A. gossypii* in both laboratory and field conditions. In addition, we measured the numerical responses of other aphidophagous indigenous predators encountered in the field and analyzed the combined effect of the alien aphid parasitoid along with the predators.

Materials and Methods

Laboratory experiment

Lysiphlebus testaceipes was obtained from an already established laboratory culture using the aphid host *A. gossypii* on sweet pepper *Capsicum annuum* L. at the International Institute of Tropical Agriculture (IITA-Benin). The wasps' response to five different aphid densities (10; 25; 50; 100; and 150 *A. gossypii*) was examined at 26 ± 1ºC in Petri dishes (8.5 cm diameter) during contact periods of 24 hours. In previous studies, third instar-nymphs of *A. gossypii* had proven higher instar suitability for *L. testaceipes* (Tepa-

Yotto et al., unpublished data). In this experiment, the third instar was therefore used in order to standardize host age. The parasitism of *L. testaceipes* was recorded by dissecting and counting the number of parasitoid larvae in the hosts, two days after parasitization. Sweet pepper leaf sections were used as vegetal substrate. The wasps used in this study were less than 24 hours old, mated and without any previous oviposition experience.

Field experiments

Field trials were conducted during the period April-July 2011 on the IITA-Benin station to analyze the parasitism of *L. testaceipes* on four different sweet pepper plots initially infested with different levels of density of *A. gossypii*. The trials were repeated from December 2011 to March 2012. Prior field diagnoses revealed that *L. testaceipes* was established in the study area (Sæthre et al., 2011). In addition, pre-sampling was performed in and around the selected fields, also with a view to ensuring the presence of *L. testaceipes* in the area. Pre-sampling confirmed the presence of the parasitoid in a cowpea field infested with *Aphis craccivora* Koch.

Study area

The study area is located in the humid forest zone in the southern coastal area of Benin (6º 24 latitude north and 2º 24 longitude east). The climate is characterized by a bimodal-rain regime, with on average 1250 mm rainfall per year. The dry seasons roughly comprise 6 months, December-March and August-September. During field trials, the temperature and relative humidity fluctuated between 22.9-33.1ºC and 61.5-96.8%; and 21.8-33.7ºC and 48.1-97.1% in April-July 2011 and December 2011-March 2012, respectively. The light-dark regime was approximately 12:12. The monthly rainfall ranged between 49.4-110.3mm and 0-33mm in April-July 2011 and December 2011-March 2012, respectively. Sandy ferralitic soil typically dominates on the experimental site.

Experimental procedure for field trials

Nurseries of sweet pepper plants were established in a screenhouse and healthy four-week old seedlings were later transplanted into the experimental beds in the field. Four blocks were included in the design at distances from each other of around 50-80m. Each block consisted of four experimental beds (6m x 1.2m). The beds were 15m apart and enriched with organic fertilizer in order to promote healthy crop growth. The seedlings were transplanted with a spacing of 40cm between plants and between rows, giving a total of 45 plants of sweet pepper per bed. The experiment was a randomized complete block design.

Nets were used to cover the newly transplanted plants in order to protect them from any infestations or attacks of pests. Four weeks after transplanting, the nets were removed and each of the beds in a block was infested with an initial density of *A. gossypii*: 0 (control); 10; 50; and 100 aphids, randomly assigned to the four experimental beds per block. The aphids were placed on fresh leaves (in the afternoon) to enable rapid establishment. On each bed, the sweet pepper plants were infested with *A. gossypii* from the culture already established in the screenhouse in accordance with the procedure outlined above.

Sampling and observations in the field

Sampling and observations were done in the field at five-day intervals in order to quantify naturally occurring aphid parasitoids. On each sampling date, ten leaves (five from the upper level of the plants and five from the lower level) were randomly sampled and observed from each experimental bed. These leaves were also brought to the laboratory for further observation. All aphid mummies sampled in the field were isolated in individual vials for hatching in the laboratory. Emerging adult parasitoids and hyperparasitoids were stored in 70% alcohol and later identified to species level. Non-mummified aphids were kept on fresh leaves to check for further mummification and those which mummified were handled as described above, and included in the data set. All empty mummies that hatched in the field or later in the laboratory, were observed under a stereomicroscope at 40x magnification. The shape of the exit hole of the primary parasitoid is always different from the hole made by hyperparasitoids; therefore, empty mummies collected in the field could be sorted into these two categories (Hofsvang et al., unpublished data).

Aphid predator species were also identified and counted. After having been recorded, predators at their different stages were removed from the patches. Immature stages were then reared to adulthood for purposes of identification confirmation.

Data analysis

For the functional response experiment in the laboratory, the response (y-values) was coded into two classes: 0 (when $y = 0$) and 1 (when $y \geq 1$); where y was the number of *L. testaceipes* larvae found in aphid hosts after dissection. Although superparasitism (when

$2 \leq y \leq 4$) was rather more frequently observed at low aphid density (10 aphids prey density), it was rare in general. Binary logistic regression with a logit link function was used to test for the effect of aphid density as a continuous explanatory variable on *L. testaceipes* parasitism (MINITAB 2011).

For field experiments, the data was $\log_{10}(y + 1)$ transformed before analysis to meet the assumptions of normality and equal variance. A linear mixed effects model analysis of variance (ANOVA type III sum of squares) was performed on the transformed data where the initial aphid density (treatment factor) and sampling date were considered as fixed factors and block as a random factor. The Tukey's post hoc tests at the 5% level were used to test for significant differences among treatment means, followed by pairwise comparisons (R statistical software; R Core Team 2012). The Restricted Maximum Likelihood (REML) was used as a more robust method to cope with negative variance components estimates (R Core Team 2012).

Results

Laboratory experiment

Laboratory investigation established that the number of aphids parasitized by *L. testaceipes* increased with increasing host density; while the percentage of aphids attacked decreased with increasing host density (Figs. 1a and 1b). Therefore, the functional response of *L. testaceipes* during 24 hours exposure time exhibited a type II curve (Figs. 1a and 1b). The binary logistic regression provided a good description of the effect of *A. gossypii* density as a continuous explanatory variable on the parasitism by *L.*

testaceipes (Tables 1 and 2). This variable was significantly different from zero for the response investigated (Tables 1 and 2), showing that it affected the parasitism of *L. testaceipes*.

Field experiments

A total of 3053 and 4751 mummies were sampled in 2011 and 2012 respectively. The field trials revealed that *L. testaceipes* was the only species of primary parasitoids on *A. gossypii* at this location. The parasitoid was attacked by one species of hyperparasitoid *Syrphophagus africanus* (Gahan) (Hymenoptera: Encyrtidae). Three predatory species of aphids were recorded; *Cheilomenes propinqua* (Mulsant), *Cheilomenes sulphurea* (Olivier) (Coleoptera: Coccinellidae) and *Ischiodon aegyptius* (Wiedemann) (Diptera: Syrphidae).

The results showed that the densities of higher trophic level arthropods were affected by both initial aphid density and sampling date (Tables 3, 4, 5 and 6). However, initial aphid density was not significant for *C. propinqua* in 2011 ($F_{3,1584}$= 0.15; P= 0.9298) or for *I. aegyptius* in 2012 ($F_{3,1584}$= 1.10; P= 0.3464). Field occurrence of the hymenopteran species, *L. testaceipes* and *S. africanus* displayed unimodal curves; while those of the predators *C. propinqua*, *C. sulphurea* and *I. aegyptius* were bimodal in both 2011 and 2012 (Figs. 2, 3, 4, 5 and 6). In the year 2011, the occurrence of these arthropods grew steadily (Figs. 7a, 7b, 7c and 7d). In this year, the first peaks appeared 25-30 days after infestation, followed by the second peak numbers of predators 30-40 days after infestation. In addition, *L. testaceipes* was higher in numbers compared to other arthropods (Tables 5 and 6).

The year 2012 was characterized by early occurrence of aphid natural enemies including the hyperparasitoid (Figs. 8a, 8b, 8c and 8d). Throughout the experiments, there were significant differences between *L. testaceipes* and its hyperparasitoid *S. africanus*, except in 2012 for the initial aphid densities ten, fifty and the control (Table 6). Having said that, there was no significant difference among aphid predators, apart from the fact that Fig. 8a exhibited a marked resurgence of the aphid predator *C. sulphurea* in 2012 on the control ($F_{4,1947}$= 2.28; P= 0.0585) at 30 days after infestation ($F_{9,1947}$= 7.52; P= 0.0001). During the three months' experimental period, natural infestation of *A. gossypii* occurred on the control plants about mid-way through the experiment, and it is also likely that some additional infestation occurred in the other three treatments.

Discussion

Functional and numerical responses

Our data reveals a type II curve functional response of *L. testaceipes* on *A. gossypii* during the exposure period of 24 hours in the laboratory (Figs. 1a and 1b). The type II functional response of parasitoids is common in biological control (Fernández-Arhex and Corley 2003), although type III functional response is also observed in *Aphidius colemani* Viereck (Aphidiidae) on *Myzus persicae* (Sulzer) and in *Aphelinus asychis* Walker (Aphelinidae) on both *M. persicae* and *Macrosiphum euphorbiae* (Thomas) (Byeon et al. 2011). Earlier findings showed that *L. testaceipes* exhibited type II and type III functional responses on *A. gossypii* at 20 and 28ºC, respectively, during observation periods of 30 minutes (Bazzocchi and Burgio 2001).

The shapes of the response curves are known to be functions of the period during which hosts and parasitoids are exposed to each other (Hofsvang and Hågvar 1983) and the temperature (Jones et al. 2003; Zamani et al. 2006). Also, Byeon et al. (2011) argued that the difference in functional response types among studies may be partly attributed to the range of host densities, or to the searching area available to the parasitoid. However, the type II response, as found by Zamani et al. (2006) for both *A. colemani* and *Aphidius matricariae* Haliday on *A. gossypii*, assumes that while the rate of parasitization rises, it does so at a gradually declining rate (Brown and Rothery 1993 cited by Byeon et al. 2011). Our data also establishes this pattern with *L. testaceipes* on *A. gossypii* in the laboratory at 26 ± 1ºC during exposure period of 24 hours, confirming the type II functional response (Figs. 1a and 1b). Snyder and Ives (2003) proposed that if a parasitoid were to show a strong type II functional response, interaction with predators would likely be synergistic, and both the parasitoid and predators would act additively with respect to regulation of the aphids. Alternatively, Bazzocchi and Burgio (2001) argued that a sigmoid relationship is considered to be the most important regulating factor in the population dynamics of the pest and natural enemy, thus suggesting that the enemy has the ability to control its prey without responding numerically, within certain limits of prey density.

Based on the data presented here, it is difficult to conclude which types of functional response were displayed by *L. testaceipes* in the field. However, the field investigations provided estimates of the aphid parasitoid' and predators' numerical responses. Despite the fact that a disruptive sampling technique was used in the field, data analysis showed that the densities of the natural enemies had strong positive correlation with aphid densities.

Seasonal effects in 2011 and 2012

Field experiments were conducted during the rainy (2011) and dry season (2012). In contrast to 2011, the significant effect of prey density on *C. propinqua* density in 2012 suggests an aggregative numerical response by the ladybird, although it is difficult to distinguish the two types of numerical responses (demographic or aggregative). The hoverfly *I. aegyptius* did not respond positively in numerical terms in 2012, which makes temporal variation a more plausible explanation than any seasonal effect. However, our demonstration that the response of the majority of the aphids' natural enemies to prey density was significant in numerical terms is comparable with the findings of Ofuya and Akingbohungbe (1988) for *Cheilomenes lunata* (Fabricius) on *A. craccivora*.

During the rainy season (2011) rainfall and lower temperatures combined to slow down population growth of both aphids and natural enemies, thus delaying the enemies' attraction to the plants. In addition, heavy tropical rain can wash aphids off the host plants, thus reducing population growth, compared to the dry season (2012). In the dry season, temperatures are generally higher (excepting December-January, which is the Harmattan season). The elevated temperatures favor rapid aphid population growth. However, the fall in recorded aphid natural enemies towards the end of experiments in 2011 may be a result of the decrease in aphid populations, which is, in turn, probably due to the lesser plant sap quality of old plants. We also suggest that because of the presence in larger numbers of aphids in the surrounding ecosystem in 2012 relative to 2011, the natural enemies were also abundant, thus explaining the early occurrence of the natural enemies on experimental plants in 2012. However, the period of the trial in 2012, which is the longer dry season, was unsuitable for the survival of these natural enemies (Donaldson et al. 2007) by mid-late season. This is supported by the fall in their numbers roughly 20 days after aphid infestation.

Effect of the primary parasitoid, the hyperparasitoid and aphid predators

The current field results show that *L. testaceipes* population grows more quickly than predators' populations. The average life-time fecundity of *L. testaceipes* has previously been calculated in the laboratory (on *A. gossypii*) to be 180.0 eggs at 25ºC (van Steenis 1994). The total development time of *L. testaceipes* from oviposition to emergence commonly ranges between 9.5-11.1 days at 25ºC on a variety of aphid host species (Hight et al. 1972; van Steenis 1994; Silva et al. 2008). Fernandes et al. (1998) established that *L. testaceipes* can maintain *S. graminum* densities below the economic threshold, if introduced early enough in relation to greenbug population dynamics, in the context of augmentative biological control. Rutledge et al. (2004) and our findings both support that the presence of *L. testaceipes* is more effective with regard to aphid suppression than a scenario with no *L. testaceipes*.

The data revealed that *L. testaceipes* was attacked by the indigenous hyperparasitoid *S. africanus* in the field. Our observations confirm those of Sæthre et al. (2011) and Hofsvang et al. (unpublished data). Both listed a number of native hyperparasitoids (of *L. testaceipes*) including *S. africanus*. In addition, our findings support the data of Ganyo et al. (2012) that the indigenous hyperparasitoid *S. africanus* could parasitize both living aphids (*A. craccivora/L. testaceipes*) and aphid mummies (*A. craccivora/L. testaceipes*), preferring newly formed mummies in the laboratory. However, to date the extent to which hyperparasitism disrupts biological control of aphids using their parasitoids in the fields is not well determined (Morris et al. 2001; Acheampong et al. 2012). In addition, the dominating presence of *L. testaceipes* in the field demonstrated in the present study permits the possibility of using the wasp in an IPM strategy on vegetables.

Our field investigation established that *L. testaceipes* and the aphid predators *C. propinqua*, *C. sulphurea* and *I. aegyptius* could be found simultaneously on *A. gossypii*. The daily predation rate on unparasitized third instar *A. gossypii* was estimated to 76.52 ± 1.24; 88.85 ± 1.80 and 86.96 ± 1.60 for *C. propinqua* (fourth instar), *C. sulphurea* (fourth instar) and *I. aegyptius* (third instar), respectively, in recent laboratory experiments at 26ºC (Tepa-Yotto et al., unpublished data). In addition, the current results show that *C. sulphurea* reached a predominant position 25-40 days after infestation, particularly in 2012. In contrast to other predators, this ladybird adapted and survived in 2012 despite a higher mummification rate, thus changing the composition of its diet (Figs. 4, 5 and 6). On the other hand, the presence of mummies on sweet pepper plants may also explain the further decrease of the aphid predators' populations. Earlier reports support similar conclusion. For example, *Vicia faba* L. plants infested with *Acyrthosiphon pisum* Harris mummies parasitized by *Aphidius ervi* Haliday were less attractive for oviposition of the hoverfly *Episyrphus balteatus* DeGeer (Almohamad et al. 2008). Likewise, *E. balteatus* laid fewer eggs in *M. persicae* colonies with mummies parasitized by *A. colemani* (Pineda et al. 2007).

In analyzing the biotic factors interacting with the parasitoid, the decline in the number of the wasp after predator populations reaching their peak, may also suggest the avoidance of further oviposition by the wasp in the field. Our conclusion that the presence or abundance of predators reduces parasitoid oviposition is supported by earlier findings (Takizawa et al. 2000). For instance, more mummies of *Acyrthosiphon pisum* (Harris) parasitized by *A. ervi* were found on *V. faba* plants without tracks of the intraguild predator *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Meisner et al. 2011). Similarly, the parasitoid *A. colemani* had a higher patch-leaving tendency from sweet pepper *C. annuum* plants infested with *M. persicae* when the predator *Macrolophus caliginosus* (Wagner) (Hemiptera: Miridae) was present (Martinou et al. 2009). On the other hand, recent laboratory studies (Tepa-Yotto et al., unpublished data) showed that omnivorous predation on *A. gossypii* mummies parasitized by *L. testaceipes* occurs by the two coccinellids *C. propinqua* and *C. sulphurea*. However, our data confirms the possible coexistence of *L. testaceipes* and the predators. Moreover, although coccinellid beetle predation may explain the mortality at immature stages of *L. testaceipes*, this does not necessarily lead to any disruption of biological control (Colfer and Rosenheim 2001; Costamagna et al. 2007). This is also consistent with the conclusions of Snyder and Ives (2003) and those of Bilu and Coll (2007); both support the positive combined effect of aphid parasitoids and predators, despite the fact that intraguild predation of parasitoids was observed.

In summary, our data indicate an adjustment of both alien aphid primary parasitoid oviposition and indigenous predators' oviposition in the field, as a result of intraguild interactions.

Conclusion

We demonstrated that *L. testaceipes* is a good candidate for biological control of aphids on vegetables in Benin. The sustainable establishment of this alien species in the new environment may to some extent be regulated by several indigenous intraguild predators, such as *C. sulphurea*. However, our field data supports the possible coexistence of both *L. testaceipes* and the indigenous predators recorded, thereby enhancing the impact of biological control.

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Table 1 Binary logistic regression of density of *A. gossypii* (10; 25; 50; 100; and 150 aphids) on parasitism by *L. testaceipes* (n = 1516).

						95% confidence interval	
Predictor	Coefficient	SE coefficient	Z	P	Odds ratio[a]	Lower	Upper
Constant	0.722127	0.123452	5.85	0.000			
Density	-0.006075	0.001101	-5.52	0.000	0.99	0.99	1.00

[a] Odds ratio means the estimated odds for parasitism to be successful/unsuccessful for each increase of host density.

Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, and the data were pooled.

Table 2 Results of goodness of fit tests related to the results of the binary logistic regression in Table 1.

Goodness of fit test	*Chi-square*	df	*P*
Pearson	2.55986	3	0.465
Deviance	2.52822	3	0.470

Table 3 ANOVA results (unrestricted models) describing data presented in Figs. 2-6.

Source	df	MS	F	P-values
Fig. 2 *L. testaceipes* 2011				
Aphid density	3	0.88	8.89	0.0001
Sampling date	9	1.69	17.13	0.0001
Block	3	0.10	1.00	0.3915
Residuals	1584	0.10	-	-
Fig. 2 *L. testaceipes* 2012				
Aphid density	3	2.54	30.94	0.0001
Sampling date	9	4.26	51.88	0.0001
Block	3	0.32	3.94	0.0081
Aphid density x Sampling date	27	0.73	8.90	0.0001
Residuals	1557	0.08	-	-
Fig. 3 *S. africanus* 2012				
Aphid density	3	1.39	26.01	0.0001
Sampling date	9	2.59	48.64	0.0001
Block	3	0.23	4.30	0.0050
Aphid density x Sampling date	27	0.44	8.34	0.0001
Residuals	1557	0.05	-	-

Table 3 (*continued*).

Source	df	MS	*F*	*P*-values
Fig. 4 *C. propinqua* 2011				
Aphid density	3	0.001	0.15	0.9298
Sampling date	9	0.017	2.63	0.0051
Block	3	0.008	1.26	0.2857
Residuals	1584	0.007	-	-
Fig. 4 *C. propinqua* 2012				
Aphid density	3	0.05	3.82	0.0097
Sampling date	9	0.10	8.32	0.0001
Block	3	0.02	1.40	0.2420
Residuals	1584	0.01	-	-
Fig. 5 *C. sulphurea* 2012				
Aphid density	3	0.07	3.11	0.0255
Sampling date	9	0.26	10.86	0.0001
Block	3	0.09	3.81	0.0097
Aphid density x Sampling date	27	0.04	1.74	0.0105
Residuals	1557	0.02	-	-

Table 3 (*continued*).

Source	df	MS	*F*	*P*-values
		Fig. 6 *I. aegyptius* 2011		
Aphid density	3	0.015	3.25	0.0211
Sampling date	9	0.015	3.20	0.0008
Block	3	0.008	1.66	0.1743
Residuals	1584	0.005	-	-
		Fig. 6 *I. aegyptius* 2012		
Aphid density	3	0.008	1.10	0.3464
Sampling date	9	0.056	8.21	0.0001
Block	3	0.009	1.15	0.2899
Residuals	1584	0.007	-	-

Table 4 ANOVA results (REML) describing data presented in Figs. 3 and 5.

Source	df	*Chi-square*	*P*-values
Fig. 3 *S. africanus* 2011			
Aphid density	3	56.509	$3.27*10^{-12}$
Sampling date	9	109.164	$2.20*10^{-16}$
Aphid density x Sampling date	27	120.268	$9.75*10^{-14}$
Fig. 5 *C. sulphurea* 2011			
Aphid density	3	26.115	$9.02*10^{-6}$
Sampling date	9	49.666	$1.25*10^{-7}$

Table 5 ANOVA results (REML) describing data presented in Figs. 7a; 7b and 7d.

Source	df	*Chi-square*	*P*-values
Fig. 7a Aphid parasitoid, hyperparasitoid and predators 2011			
Species[a]	4	43.382	$8.62*10^{-9}$
Sampling date	9	39.631	$8.86*10^{-6}$
Species x Sampling date	36	94.279	$4.08*10^{-7}$
Fig. 7b Aphid parasitoid, hyperparasitoid and predators 2011			
Species[a]	4	82.072	$2.20*10^{-16}$
Sampling date	9	56.940	$5.19*10^{-9}$
Species x Sampling date	36	97.556	$1.39*10^{-7}$
Fig. 7d Aphid parasitoid, hyperparasitoid and predators 2011			
Species[a]	4	93.700	$2.20*10^{-16}$
Sampling date	9	114.240	$2.20*10^{-16}$
Species x Sampling date	36	122.920	$2.00*10^{-11}$

[a] *L. testaceipes*, *S. africanus*, *C. propinqua*, *C. sulphurea*, *I. aegyptius*.

Table 6 ANOVA results (unrestricted models) describing data presented in Figs. 7c and 8.

Source	df	MS	*F*	*P*-values
Fig. 7c Aphid parasitoid, hyperparasitoid and predators 2011				
Species[a]	4	0.63	16.89	0.0001
Sampling date	9	0.35	9.49	0.0001
Block	3	0.04	1.14	0.3302
Species x Sampling date	36	0.12	3.25	0.0001
Residuals	1947	0.04	-	-
Fig. 8a Aphid parasitoid, hyperparasitoid and predators 2012				
Species[a]	4	0.021	2.28	0.0585
Sampling date	9	0.068	7.52	0.0001
Block	3	0.038	4.25	0.0053
Species x Sampling date	36	0.014	1.50	0.0282
Residuals	1947	0.009	-	-
Fig. 8b Aphid parasitoid, hyperparasitoid and predators 2012				
Species[a]	4	0.14	5.89	0.0001
Sampling date	9	0.47	19.83	0.0001
Block	3	0.10	4.31	0.0049
Species x Sampling date	36	0.11	4.48	0.0001
Residuals	1947	0.02	-	-

[a] *L. testaceipes*, *S. africanus*, *C. propinqua*, *C. sulphurea*, *I. aegyptius*.

Table 6 (*continued*).

Source	df	MS	*F*	*P*-values
Fig. 8c Aphid parasitoid, hyperparasitoid and predators 2012				
Species[a]	4	1.41	29.03	0.0001
Sampling date	9	1.73	35.72	0.0001
Block	3	0.10	2.05	0.1055
Species x Sampling date	36	0.39	7.99	0.0001
Residuals	1947	0.05	-	-
Fig. 8d Aphid parasitoid, hyperparasitoid and predators 2012				
Species[a]	4	2.48	40.32	0.0001
Sampling date	9	3.66	59.51	0.0001
Block	3	0.21	3.46	0.0159
Species x Sampling date	36	0.75	12.25	0.0001
Residuals	1947	0.06	-	-

[a] *L. testaceipes*, *S. africanus*, *C. propinqua*, *C. sulphurea*, *I. aegyptius*.

Figure captions

Fig. 1 Number (a) and percentage (b) of aphids parasitized by *L. testaceipes* describing the functional response of females of *L. testaceipes* to the density of third instar of *A. gossypii*, after 24-hour exposure period at 26 ± 1ºC. Analyzed using the binary logistic regression (Table 1). The dots represent observations with fitted regression lines. The numbers of replicates corresponding to the host densities, 10; 25; 50; 100; and 150 aphids were 9; 7; 6; 6; and 5, respectively.

Fig. 2 Field occurrence of *L. testaceipes* of mummies of *A. gossypii* on sweet pepper plants initially infested with varying densities of aphids (zero, ten, fifty and one hundred aphids) in the years 2011 and 2012.

Fig. 3 Field occurrence of *S. africanus* of mummies of *A. gossypii* on sweet pepper plants initially infested with varying densities of aphids (zero, ten, fifty and one hundred aphids) in the years 2011 and 2012.

Fig. 4 Field occurrence of *C. propinqua* (larvae, pupae and adults) on sweet pepper plants initially infested with varying densities of *A. gossypii* (zero, ten, fifty and one hundred aphids) in the years 2011 and 2012.

Fig. 5 Field occurrence of *C. sulphurea* (larvae, pupae and adults) on sweet pepper plants initially infested with varying densities of *A. gossypii* (zero, ten, fifty and one hundred aphids) in the years 2011 and 2012.

Fig. 6 Field occurrence of *I. aegyptius* (larvae and pupae) on sweet pepper plants initially infested with varying densities of *A. gossypii* (zero, ten, fifty and one hundred aphids) in the years 2011 and 2012.

Fig. 7 Field occurrence of aphid parasitoid, hyperparasitoid and predators on sweet pepper plants initially infested with varying densities of *A. gossypii* (zero (a), ten (b), fifty (c) and one hundred (d) aphids) in the year 2011.

Fig. 8 Field occurrence of aphid parasitoid, hyperparasitoid and predators on sweet pepper plants initially infested with varying densities of *A. gossypii* (zero (a), ten (b), fifty (c) and one hundred (d) aphids) in the year 2012.

Fig. 1

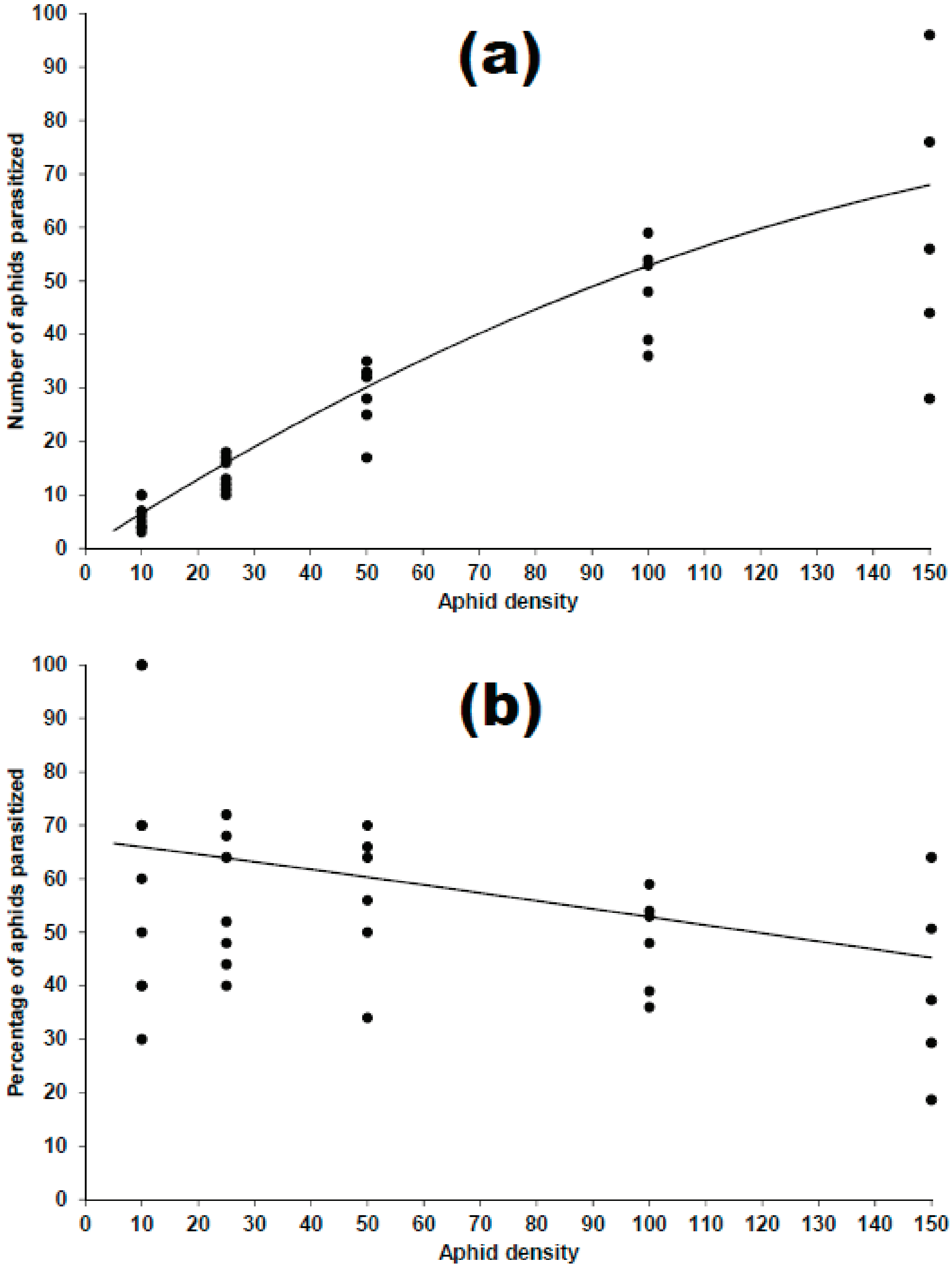

(a)
Number of aphids parasitized
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Aphid density
(b)
Percentage of aphids parasitized
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
Aphid density

Fig. 2

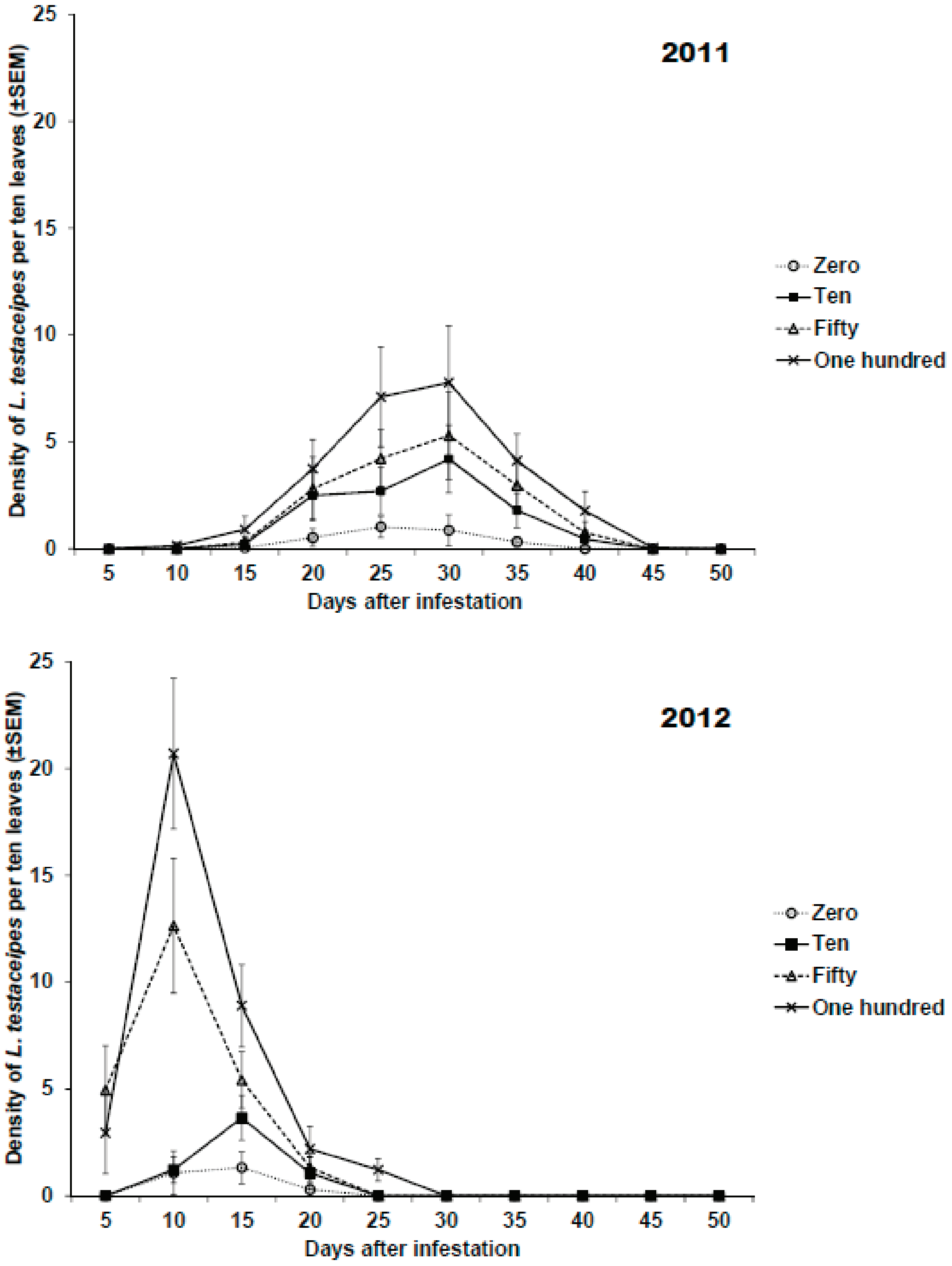

2011
Density of L. testaceipes per ten leaves (±SEM)
0
5
10
15
20
25
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred
2012
Density of L. testaceipes per ten leaves (±SEM)
0
5
10
15
20
25
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred

Fig. 3

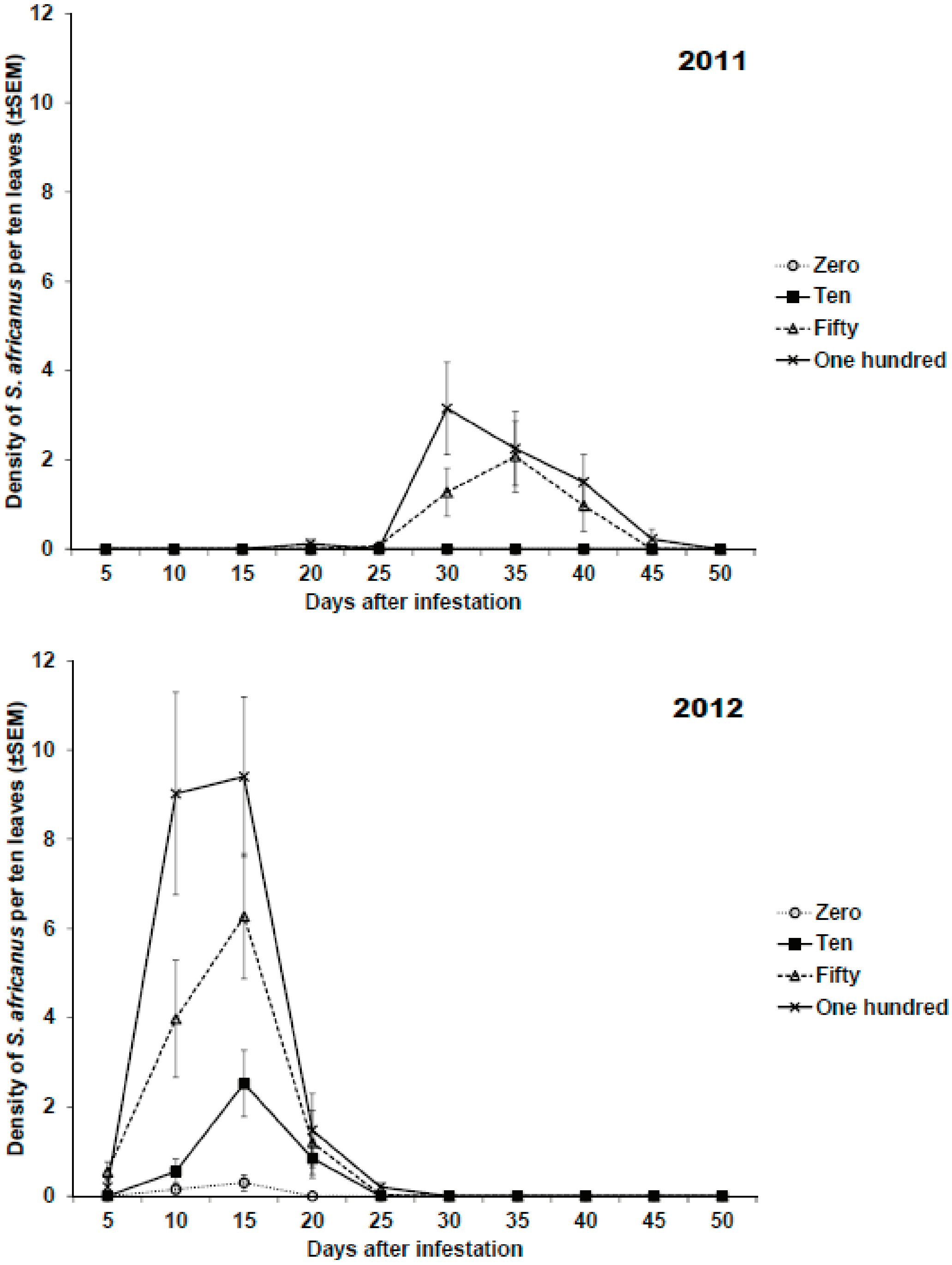

2011
Density of S. africanus per ten leaves (±SEM)
12
10
8
6
4
2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred
2012
Density of S. africanus per ten leaves (±SEM)
12
10
8
6
4
2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred

Fig. 4

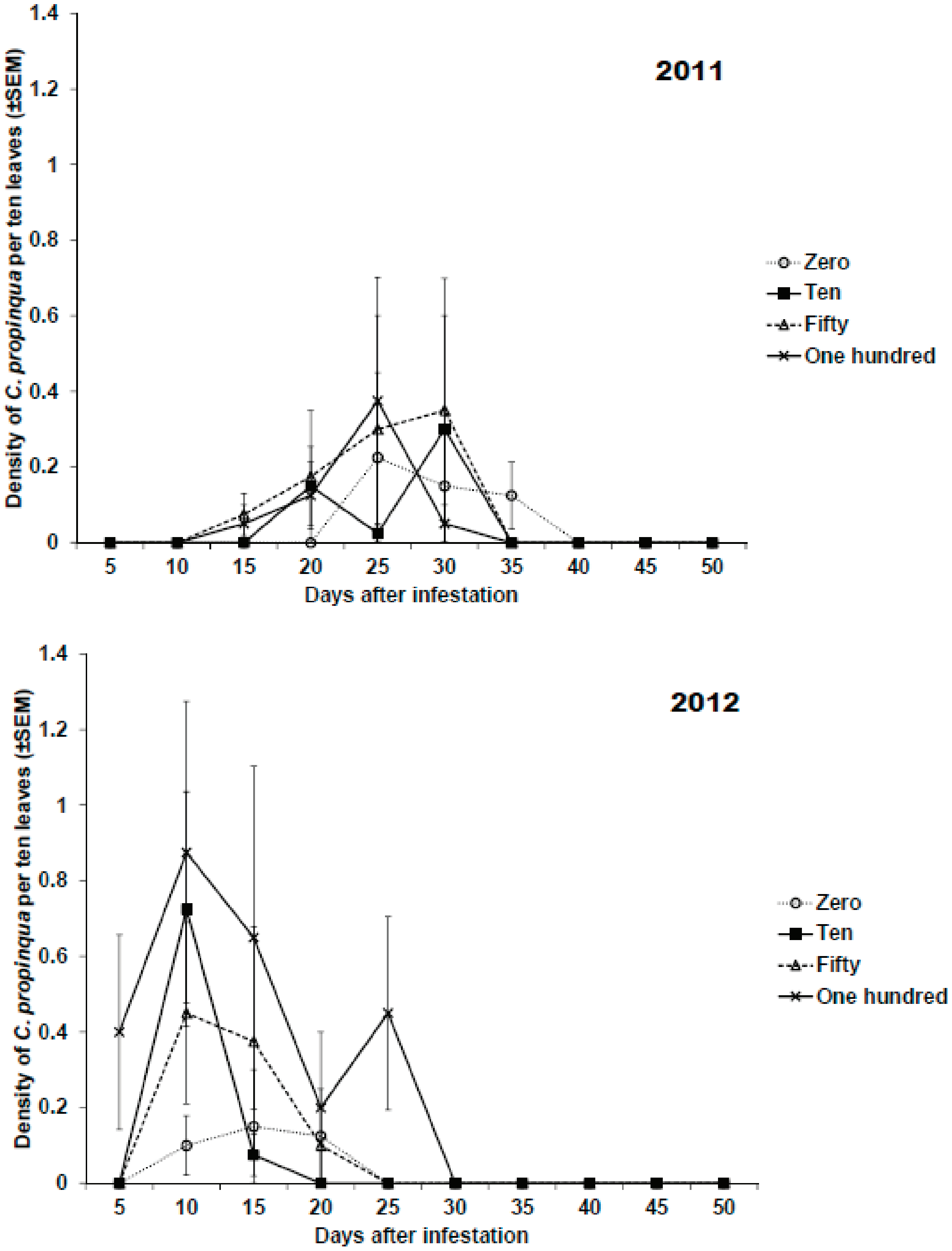

2011
Density of *C. propinqua* per ten leaves (±SEM)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred
2012
Density of *C. propinqua* per ten leaves (±SEM)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred

Fig. 5

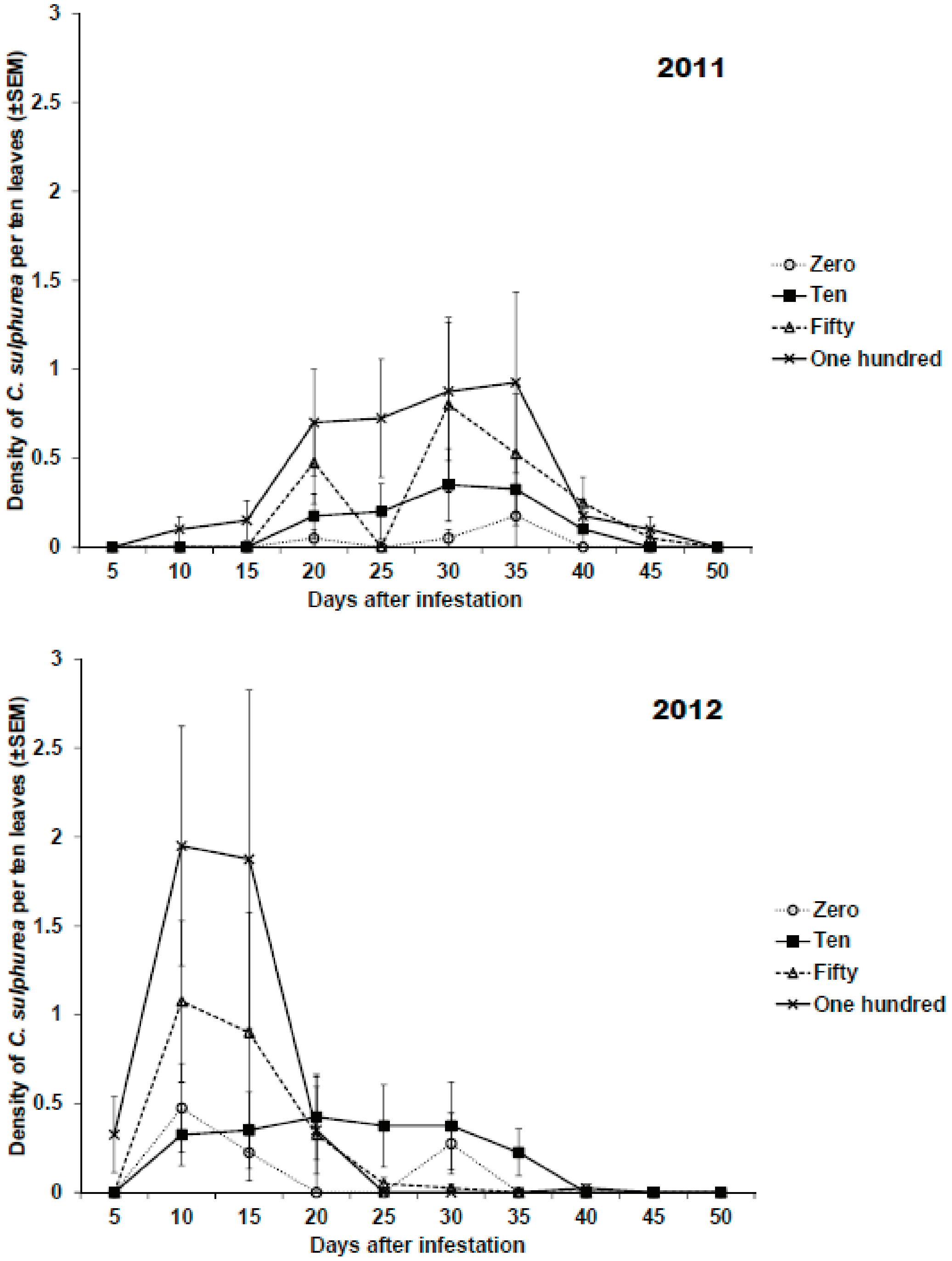
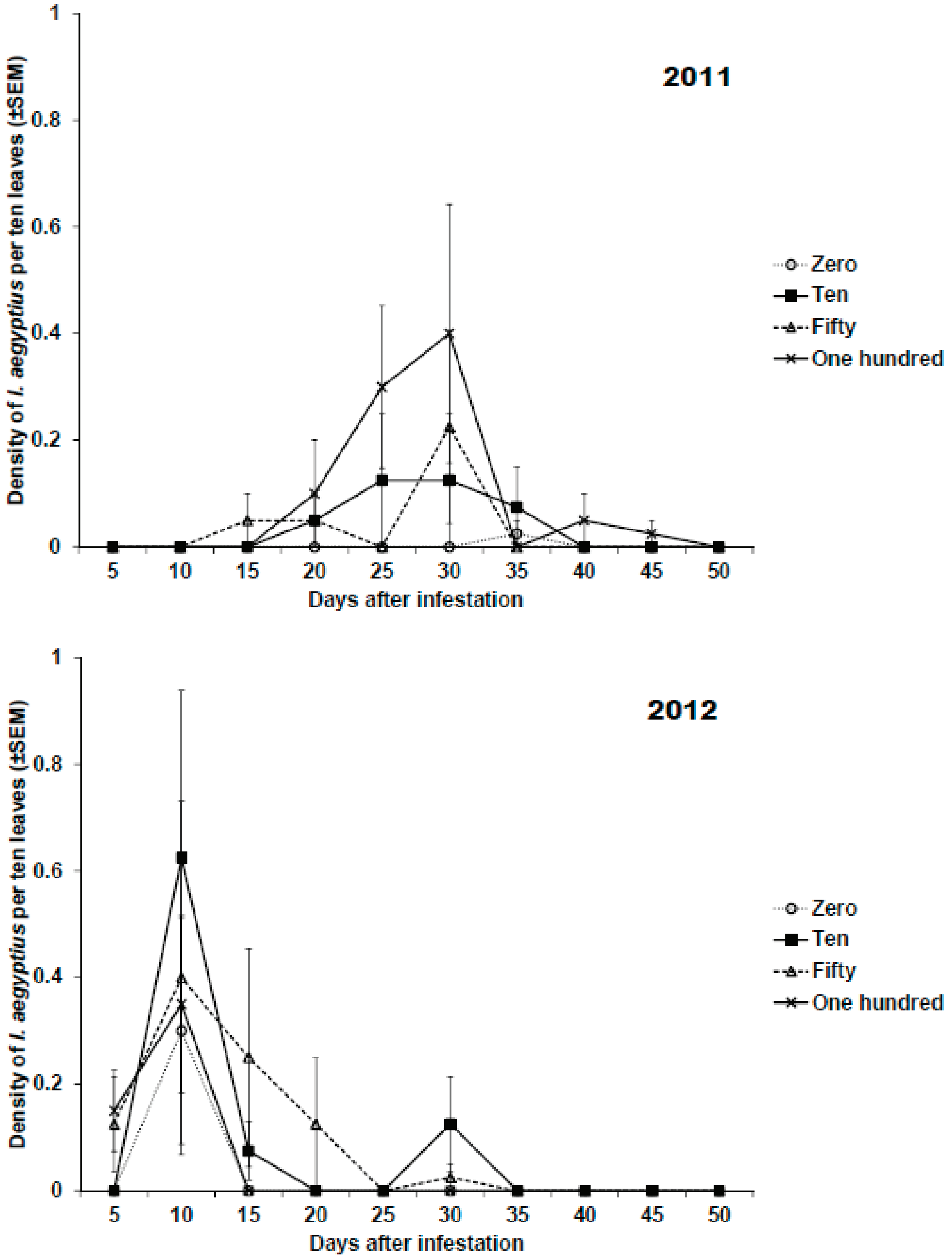
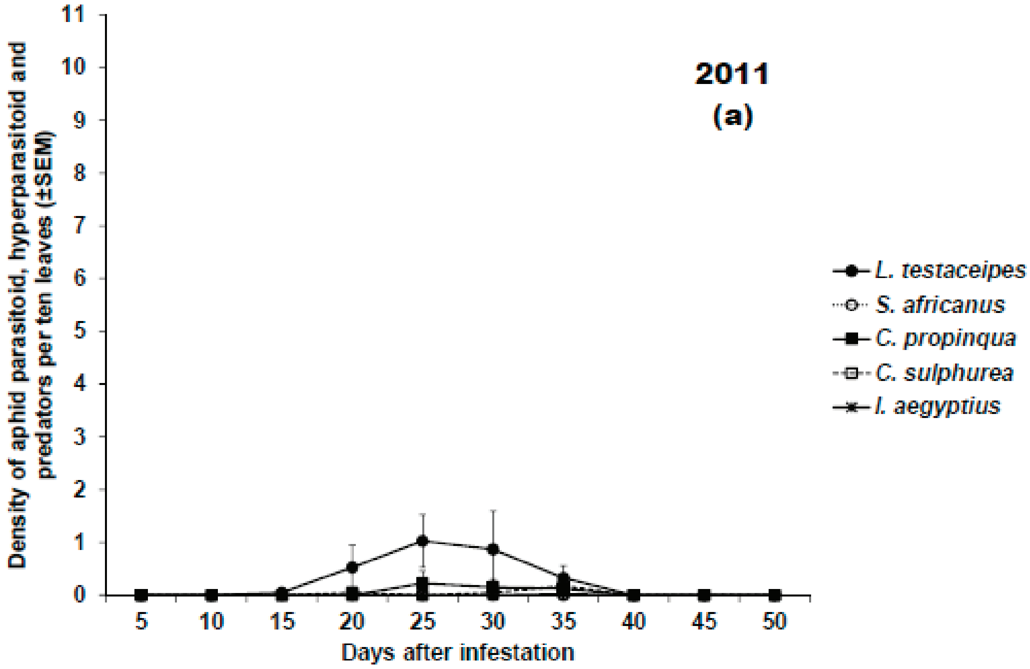

2011
Density of C. sulphurea per ten leaves (±SEM)
3
2.5
2
1.5
1
0.5
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred
2012
Density of C. sulphurea per ten leaves (±SEM)
Days after infestation
Zero
Ten
Fifty
One hundred

Fig. 6


2011
Density of *I. aegyptius* per ten leaves (±SEM)
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred
2012
Density of *I. aegyptius* per ten leaves (±SEM)
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
Zero
Ten
Fifty
One hundred

557 **Fig. 7**



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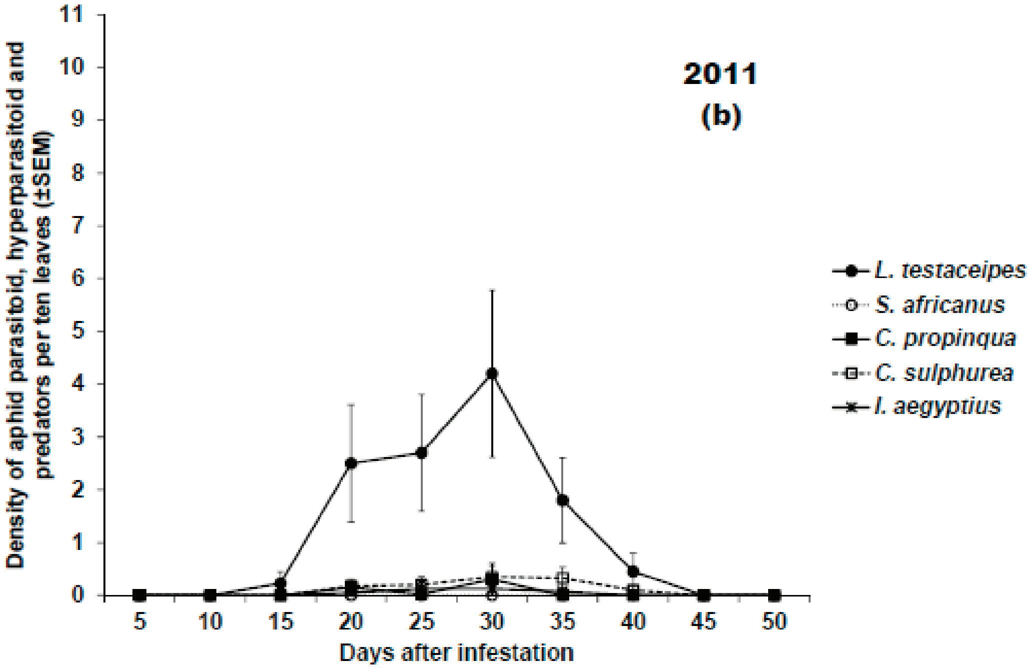

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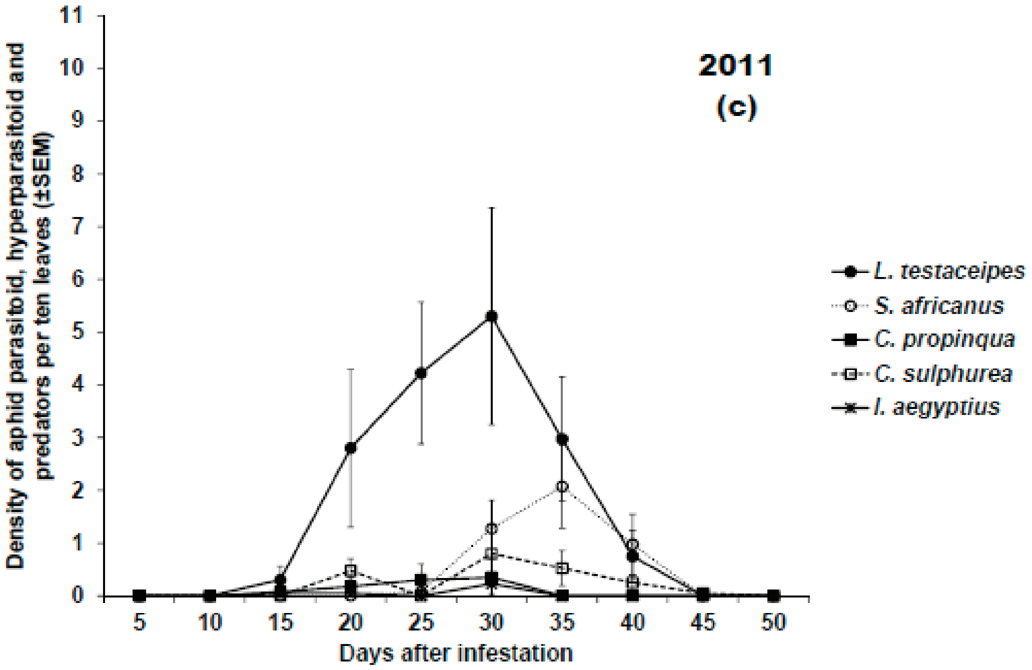

2011
(c)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
0
1
2
3
4
5
6
7
8
9
10
11
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius

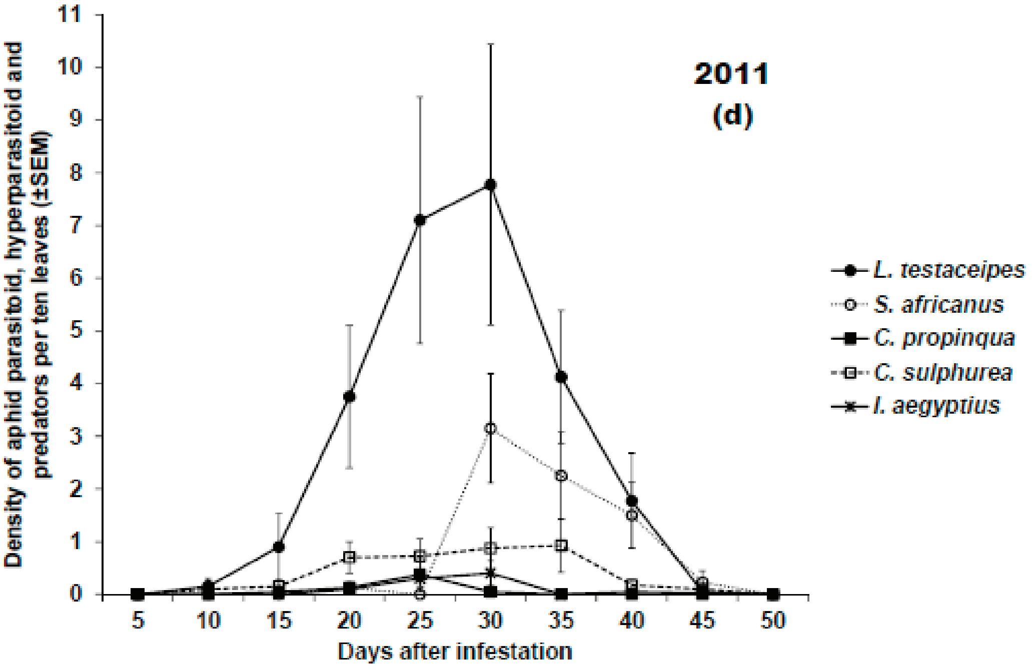
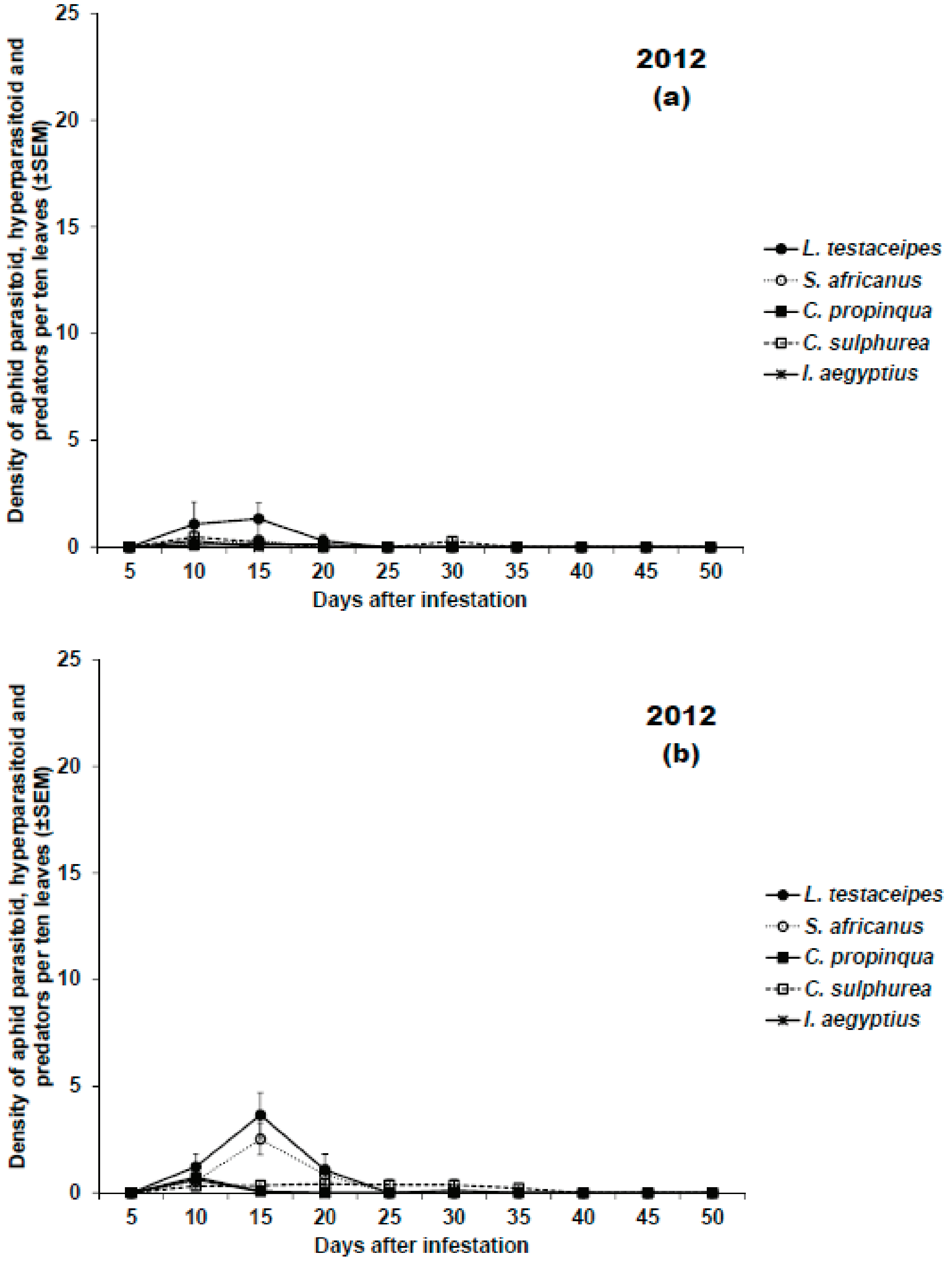
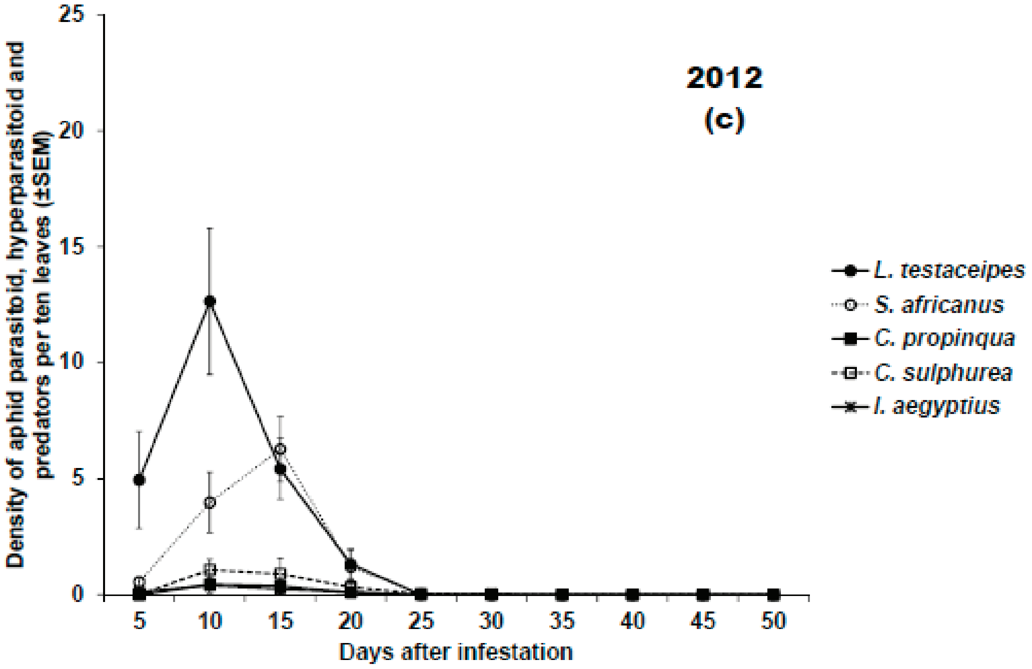
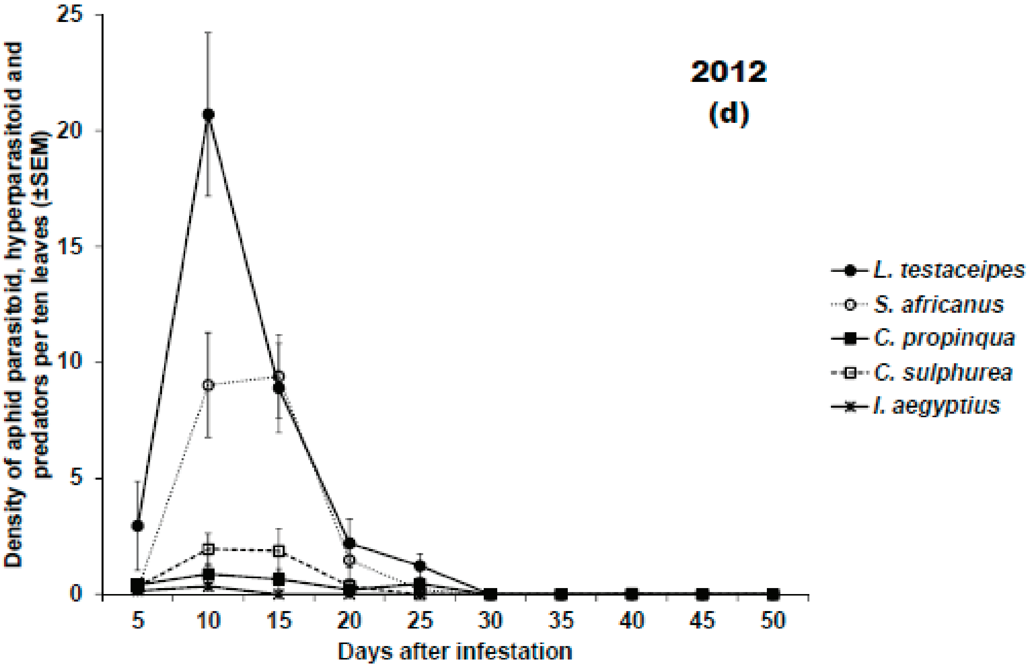

2011
(d)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
0
1
2
3
4
5
6
7
8
9
10
11
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius

Fig. 8


2012
(a)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
0
5
10
15
20
25
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius
2012
(b)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
0
5
10
15
20
25
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius


2012
(c)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
25
20
15
10
5
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius

568


2012
(d)
Density of aphid parasitoid, hyperparasitoid and predators per ten leaves (±SEM)
25
20
15
10
5
0
5
10
15
20
25
30
35
40
45
50
Days after infestation
L. testaceipes
S. africanus
C. propinqua
C. sulphurea
I. aegyptius

569

570