Blaaspootjies, aanvaar asseblief my opregte verskoning.

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Ecology and biocontrol potential of the South African flower bugs *Orius thripoborus* and *Orius naivashae*

by

ir. Jochem Bonte

Thesis submitted in the fulfillment of the requirements for the Degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation:

Ecologie en bestrijdingspotentieel van de Zuid-Afrikaanse bloemenwantsen *Orius thripoborus* en *Orius naivashae*

Please refer to this work as follows:

Bonte, J. 2016. Ecology and biocontrol potential of the South African flower bugs *Orius thripoborus* and *Orius naivashae*. PhD thesis, Ghent University, Ghent, Belgium.

Front and backcover artwork: Thaïs Heijerick

ISBN-number: 978-90-5989-863-9

The research was conducted at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium and partly at the South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, South Africa.

Woord vooraf

De laatste vraag die ik me stel tijdens het schrijven van dit doctoraat is de eerste die je in dit werk terugvindt. Hoe vat je zeven fijne werkjaren samen in een dankwoord. Zeven jaren, de uiterste houdbaarheid van een assistent: duizenden regen- en spoelwormen werden gedissecteerd, veel collega's zijn oud-collega's en vrienden geworden, en talrijke wantsen zijn voor mij tot het uiterste gegaan. Maar ik ging dus wat fantastische mensen bedanken.

Patrick, net zoals vele/alle doctoraatsstudenten die hun proefschrift onder jouw deskundige begeleiding hebben behaald, zal ik uit hetzelfde arsenaal van lovende woorden putten om je te bedanken. Niet omdat ik me daar verplicht toe voel, maar uit oprechtheid. Mijn bewondering gaat - onder andere - uit naar de manier waarop je omging met mijn vaak cryptische vragen en dito teksten: met veel geduld, humor, gevoel voor realisme, en indien nodig zalvend. Merci voor het 'opwaarderen' van al mijn schrijfsels, en dat in een buitenaards tempo. Verder bedank ik je graag om je reisvoorraad paracetamol aan mij af te staan toen een Chileens virus mij had te pakken, je contacten met de 'Mossad' aan te spreken om één van de wantsenkweken eindelijk terug in Zuid-Afrika te krijgen, en om een fijne mens te zijn.

Luc en Patrick, bedankt om mijn kandidaatstelling als assistent te ondersteunen en me in alle vrijheid assistent te laten zijn. Jullie waren toffe bazen!

De leden van de examencommissie, voor de tijd die ze vrijmaakten om dit werk zorgvuldig na te lezen en aanbevelingen te maken ter aansterking van dit werk.

Des, thank you for making the South African part of this research possible by offering me good guidance during my stay at SASRI. And for the beers we had together. Cheers!

En dan de rest van het te-bedanken-personen-lijstje (maar daarom zeker niet minder):

- Mijn 'peetvaderen' (Nick en Maarten): ze leerden mij dat doctoreren ook wel dikke fun is
- De partner in PhD-crime (Dominiek): voor alle hulp, zever, zangstondes en om altijd in het labo te zijn (voor mij?)
- 'Supervrouw' Leen: voor alles (maar wat is dat dan? De vele hulp, de babbels, de moeite …)!
- Mijn ondertussen vrijgevochten 'slaven' (Ben, Dominiek, Madelena, Lien, Anaïs en Jonas): zonder jullie had ik nooit zoveel data kunnen verzamelen. En het was nog eens plezant ook
- Het assistentenpaleis met hun bewoners (Bjorn, Wannes, Astrid en Niels): bedankt voor de leuke jaren in dit lokaal met zijn eigen ecosysteem en speciale geur. Speciale dank aan Astrid voor de oppeppende babbels

- ‘Patrick’s special lab/weekend/holiday crew’ (Maarten, Nick, Bjorn, Joachim, Leen, Veronic, Brecht, Thijs, Sara, Dominiek, Tung, Veerle, Vincent, Thomas S., Hongsheng en Jiaqin): voor de vele hulp en de hilarische momenten die soms gepaard gingen met veel lawaai (sorry Patrick)
- Thijs: voor de labokwisvragen, de prachtige poëzie, het geleuter en het beetje verlies …
- Joachim: voor de zanglessen sopraan en om mijn nieuwe baas te willen zijn
- Tung, Jiaqin and Hongsheng: for bringing culture and creative English in the lab. Tung, I miss you so much that we have decided to visit you next Summer
- Vincent: for making the French language even harder for me
- Rik: om een sympathieke, wandelende plant- en tuingids te zijn
- Didier: ik heb je al genoeg bewierookt toen je op pensioen ging
- Bea: omdat ik je niet vergeten ben
- Paloma, Fermin and Marta (en ook een beetje Olivier): for the Spanish vibes
- Nicky, Sabina, Peter, Wim en Astrid: sorry dat ik, ondanks jullie gewaardeerde hulp, het *Wolbachia*-misterie niet heb kunnen oplossen
- De ‘lunch buddies’ (Astrid, Rik, Leen, Dominiek, Dorien, Kaat, Anneliezen, Laurens, Freja, Benigna, Maxim, Anneleen, Peter, Wim, Wannes, Niels, Bjorn en vele anderen …): voor de lachsalvo’s die het verteringsproces bevorderden
- De ‘klasgenootjes’: voor de gezellige collectieve alcoholconsumptiemomenten
- Dmitry Musolin: for your help with the to-diapause-or-not-to-diapause-question
- The other SASRI staff members, especially Nelson and Mike, for the help during lab and field work. Bèla and Katya, for taking me to South African rhinos, supermarkets, beaches and the braai at Mike’s place
- De vrienden van de volley: om mij scherp te houden
- Mams, paps, zus, broer en Sara: voor de goede zorgen, altijd en overal … en het lekkere eten
- Marleen, Luc, Naomi, Bennert en Arno: voor de vele sandwiches en frietjes, de gezellige vakanties en de verbouwhulp
- Thaïs: om onze verbouwing en mijn referentielijst op orde te zetten, enthousiast te zijn over mijn doctoraat, komende zomer samen door Vietnam te willen reizen en voor zoveel liefde.

Bij deze wens ik Google Scholar nog te melden dat er vanaf heden minder gezocht zal worden naar wetenschappelijke publicaties rond het hybride model van Toyota (Prius), dat slechts één letter/typfout verschilt van het onderwerp van dit doctoraat (en de P staat echt veel te dicht bij de O).

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List of abbreviations

ANOVA:	analysis of variance
ARS:	Analytical Research Systems Inc., Gainesville, Florida
DD:	degree-days
df:	degrees of freedom
EPPO:	European and Mediterranean Plant Protection Organization
F:	F-ratio; the statistic used in an ANOVA to test the hypothesis that the effects are real
G:	generation (e.g., G0 = founder population, G1 = first generation, …)
IPM:	Integrated Pest Management
K:	degree-day requirements (DD)
L:D:	light-dark cycle expressed in hours (e.g., 16:8 indicating 16h photophase and 8h scotophase
LT:	lethal time (days)
n:	number of sampled individuals
P:	significance of a statistical test
RH:	relative humidity (%)
r_m:	intrinsic rate of increase (females / female / day)
R^2:	coefficient of determination
SASRI:	South African Research Institute
SASA:	South African Sugar Association
SCP:	supercooling point (°C)

SE: standard error

SEM: standard error of mean

SIT: sterile insect technique

t_0: lower development threshold (°C)

Chapter 1

General introduction, objectives and thesis outline

1.1 General introduction

For many years, applying chemical pesticides has proven to be a reliable and cost-effective method for the control of pests and diseases in agriculture. However, the use of those chemicals also caused several problems in crop production, including the development of pesticide resistance in the pest insects, thereby drastically decreasing their efficiency (Mallet 1989; Georghiou 1990). Moreover, the use of broad-spectrum pesticides can eliminate natural enemies and other beneficial organisms, giving rise to secondary pest outbreaks. Furthermore, residues of pesticides in the food, the contamination of the soil and groundwater, and the reduction of biodiversity induced a growing public awareness regarding the risks associated with the use of chemical pesticides (Culliney et al. 1992; Pimentel et al. 1992; Danielopol et al. 2003; Gibbs et al. 2009; Cock et al. 2010). These growing concerns regarding chemical pest control led to the development of integrated pest management (IPM), which takes both economic as well as ecological aspects into account.

Biological control, one of the key strategies of IPM, encompasses the use of parasites, predators and pathogens for the regulation of host (pest) densities (De Bach & Schlinger 1964; Van Driesche & Bellows 1996; van Lenteren et al. 1997). There are three main techniques for biological control: classical, augmentative and conservation biological control. In classical biological control a natural enemy is collected in the area of origin of an exotic pest and introduced in the new region where the pest has become established. The aim is permanent establishment of the exotic natural enemy and long-term pest control. Conservation biological control involves taking various measures to enhance the abundance or activity of natural enemies of pests in the field. These measures include manipulation of the crop microclimate, creation of overwintering refuges, increasing the availability of alternative hosts and prey, and providing essential food resources such as pollen and nectar producing flowers (Bale et al. 2008). In augmentative biological control, mass-reared natural enemies are being released with the purpose of providing pest suppression in the short term (by inundation, with an immediate effect by the released individuals) or in the longer term (by (seasonal) inoculation, with an effect over a number of generations through in –field reproduction by the released individuals) (van Lenteren & Woets 1988; Van Driesche & Bellows 1996; De Clercq 2002). Since the 1970's dozens of arthropods were successfully commercialised and used in augmentative biological control programmes against

many economically important pests. At present, over 170 arthropod species are commercially available worldwide for augmentation programmes targeting a wide array of mite and insect pests (Cock et al. 2010). Within the Anthocoridae family, several species of the genus *Orius* are considered to be important beneficial insects in various agrosystems (Barber 1936; Carayon 1961; Kelton 1963; Oku & Kobayashi 1966; Alauzet et al. 1994). Currently, seven *Orius* species are commercially used in different parts of the world (van Lenteren 2012).

Sugarcane is one of South Africa's most important crops. The South African industry produces an estimated average of 2.2 million tons of sugar per season (South African Sugar Association 2015). As for many crops, sugarcane is susceptible to a range of pests, with *Fulmekiola serrata* Kobus (Thysanoptera: Thripidae), the sugarcane thrips, being one of the major emerging pests in the young cane stage. *Fulmekiola serrata* is native to Asia, but was recorded infesting sugarcane in mainland Africa for the first time in 2004 (Way et al. 2006b). Observations in South Africa suggest that damage could result in 10 to 20% reduction in sugarcane tonnage (Sallam 2009).

The South African Sugarcane Research Institute (SASRI), a division of the South African Sugar Association (SASA), is a renowned agricultural research institute at the forefront of a thriving sugar industry. The institute is situated in Mount Edgecombe in the eastern province KwaZulu-Natal, the centre of the South African sugarcane industry. Research at SASRI is clustered within four multidisciplinary programmes, with Crop Protection being one of them. The key objective of this programme is to minimise the effects of pest diseases and weeds on crop production. One of the projects within this Crop Protection programme concerns the crop losses caused by the sugarcane thrips (South African Sugar Association 2015).

Because of their cryptic lifestyle and fast development of resistance to pesticides, thrips are difficult to control. Therefore, the availability of an effective indigenous biological control agent could provide local growers with an alternative pest management strategy. To find these potential native biological control agents in South Africa, field surveys were done in 2008 and 2009 by three Master thesis students from Ghent University in association with the SASRI (Maes 2009; Cottenie 2010; Vangansbeke 2010). These surveys were performed in and around sugarcane fields in the South African provinces

Mpumalanga and KwaZulu-Natal, mainly resulting in observations of species from the Anthocoridae family, preying on individuals of *F. serrata* and other thrips species. The most abundant natural enemies of thrips were *Orius tantillus* (Motschulsky), *Orius naivashae* (Poppius) and *Orius thripoborus* (Hesse). Since *O. tantillus* is a well-studied species and its potential as a biological control agent against various insect pests in the Indo-Pacific region has been demonstrated (Mituda & Calilung 1989; Nakashima & Hirose 1997a, b; Nagai et al. 1998; Nakashima & Hirose 1999; Venkatesan et al. 2008; Gupta & Ballal 2009), only *O. naivashae* and *O. thripoborus* were considered in this dissertation. Colonies of the two latter species were established at the Laboratory of Agrozoology of Ghent University, Belgium, with the aim to determine whether *O. naivashae* and *O. thripoborus* can be effective natural enemies against *F. serrata,* but also against other thrips species and other arthropod pests for use in augmentative biological control programmes in both open field crops and protected cultivation in southern Africa. Further, the role of *O. naivashae* and *O. thripoborus* in conservation biological control was also considered as conservation measures may support natural populations of both species and can improve the performance of augmentatively released populations.

Successful biological control depends on a comprehensive understanding of the biology and ecology of the pest and natural enemy complex, and of the environments into which they will be released (Bale et al. 2008). In this dissertation, aspects of the autecology of *O. naivashae* and *O. thripoborus* were investigated, focusing on their interaction with their prey resources and with climatic conditions, including temperature and photoperiod. The natural enemies should be able to develop, reproduce and disperse in the climatic conditions under which they are to be used (van Lenteren and Woets 1988; Bale et al. 2008). Dormancy also may play a major role in the ability to produce and use these predators in (augmentative) biocontrol programmes (Coll & Ruberson 1998).

In augmentative biological control programmes, cheap and reliable mass production yielding high-quality natural enemies is a prerequisite for cost-effective pest control (Leppla & King 1997). The use of factitious foods or artificial diets can enhance mechanisation of rearing procedures and thus lower production costs. In commercial insectaries, *Orius* bugs are mainly reared on eggs of the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). This constitutes an effective but expensive factitious (i.e. unnatural) food, and therefore the search for a cheaper and

more easily available alternative food is still ongoing. Practice has shown that polyphagous predators, such as *Orius* spp., can be highly effective in augmentation biological control programmes (van Lenteren & Woets 1988; Albajes & Alomar 1999; Symondson et al. 2002). The fact that polyphagous predators are easily reared on unnatural foods and can be used against different pest species makes them attractive for commercialisation. By feeding on plants, omnivorous predators have less difficulty in maintaining their populations at low prey densities. As a result, they can sometimes be introduced in the crop before the target pest is present, thus preventing the buildup of pest populations before economic damage is done. On the other hand, facultative plant feeding by predatory arthropods exceptionally causes crop damage (De Clercq 2002).

Reliability of augmentative biological control programmes depends on the quality of the beneficial insects produced, which is largely determined by the quality of the diet used to rear them (Chaudhury 2009). The ultimate test for quality of predatory insects is the assessment of their field efficiency measured as the rate of predation and pest suppression. However, besides being expensive and time-consuming, the complexity of a field setting may obscure the actual cause for the failure or success of a natural enemy release. Therefore, the first assessment of the quality of an in-vitro- or in-vivo-produced beneficial will preferably be done in a laboratory setting (Grenier & De Clercq 2003).

1.2 Objectives and thesis outline

The overall objective of this thesis research was to elucidate the biology and biocontrol potential of the indigenous predatory bugs *O. thripoborus* and *O. naivashae* against (thrips) pests in South Africa. Firstly, field observations were performed to achieve a better understanding of some aspects of the ecology of the common anthocorids in South African cropping systems. Next, in the laboratory, the biology and predatory performance of both *Orius* species was studied, as well as aspects of its mass rearing. The objectives can be translated into the following research questions:

- What is the prevalence of *O. naivashae*, *O. thripoborus* and other anthocorids in and around sugarcane fields in South Africa?
- What is the effect of abiotic conditions (temperature and photoperiod) on the development and reproduction of *O. naivashae* and *O. thripoborus*?

- How do *O. naivashae* and *O. thripoborus* survive South African winter conditions and are they sensitive to diapause?
- How does feeding on plant materials affect the development and reproduction of *O. naivashae* and *O. thripoborus*?
- Can factitious prey support the development and reproduction of *O. naivashae* and *O. thripoborus*?
- What is the predation capacity, development and reproduction of *O. naivashae* and *O. thripoborus* on different prey types?

The research questions are addressed in several chapters: **Chapter 2** provides an overview of the literature on *Orius* bugs in general and on *O. naivashae* and *O. thripoborus* more in specific. In **Chapter 3** the occurrence of anthocorids in and around South African agricultural ecosystems is described. In **Chapter 4** development and reproduction of *O. naivashae* and *O. thripoborus* are studied at several temperatures and thermal requirements are estimated. Cold tolerance traits and the effect of photoperiod on diapause incidence are assessed in **Chapter 5**. **Chapter 6** focuses on the predation capacity, development and reproduction of both anthocorids on various prey. In **Chapter 7** the effects of a moisture source on development of *O. naivashae* and *O. thripoborus*, and of natural and factitious foods on their development and reproduction are investigated; a quick dissection method as quality control tool for predicting their reproductive potential is evaluated. The search for alternative factitious prey supporting the mass production of both anthocorids was continued in **Chapter 8**. The final chapter (**Chapter 9**) presents a general discussion of the findings of this study and provides further research perspectives.

Chapter 2

A literature review: *Orius* species as biocontrol agents

2.1 Taxonomy

The taxonomic classification of the two studied *Orius* species, *Orius thripoborus* and *Orius naivashae*, is as follows:

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Order	Hemiptera
Suborder	Heteroptera
Subgroup	Geocorisae
Infraorder	Cimicomorpha
Superfamily	Cimicoidea
Family	Anthocoridae Fieber, 1836
Subfamily	Anthocorinae Reuter, 1984
Tribe	Oriini Carayon, 1958
Genus	*Orius* Wolff, 1811
Subgenus	*Orius sensu stricto* Wolff, 1811
Species	***thripoborus* Hesse, 1940**
Subgenus	*Dimorphella* Reuter, 1884
Species	***naivashae* Poppius, 1920**

The classification within the Anthocoridae family is still in a state of flux (Schuh & Štys 1991). In this work we adopted the system suggested by Carayon (1972) and Péricart (1972), assented by Cassis and Gros (1995) and by Lattin (1999). This classification recognises the single family Anthocoridae, with three subfamilies: Anthocorinae, Lasiochilinae and Lyctocorinae. This system differs slightly from that proposed by Schuh and Slater (1995), who accorded these subfamilies family status. The Oriini, as described by Carayon (1972), is a tribe of Anthocoridae within the subfamily Anthocorinae. The group consists of 17 genera (Carayon 1972) of which the largest is *Orius* Wolff (Postle et al. 2001). The genus *Orius* contains about 75 described species distributed throughout the world (Péricart 1972; Lattin

1999). In Africa it comprises more than 38 described species, but only 60 to 70% of the actual African fauna of *Orius* is known (Hernández & Stonedahl 1999).

The genus *Orius* can be divided into different subgenera, depending on the place of origin of the species. For the classification of Palearctic and African species Carayon (1972) and Péricart (1972) used the classification of Wagner (1952). Latter author divided the genus *Orius* into four subgenera: *Orius sensu stricto*, *Heterorius* Wagner, *Microtraechelia* Blöte and *Dimorphella* Reuter. This classification cannot be applied to the taxa of the Western Hemisphere and the Asian species (Herring 1966). For the Asian species, Yasunaga (1993) created three new subgenera: *Paraorius*, *Xylorius* and *Trichorius*. The value of these and other classifications of the genus *Orius* cannot be adequately assessed without more comprehensive studies of the world species (Hernández & Stonedahl 1999).

2.2 Distribution

The genus *Orius* contains more than 70 species distributed throughout the world (Péricart 1972) and has a strong representation in the Oriental, Ethiopian, Palearctic and Neotropical regions, but is relatively poor represented in the Nearctic (Horton 2008). *Orius thripoborus* is known from Kenya (van den Berg & Cock 1995; van den Berg et al. 1997), South Africa (Hesse 1940; van Hamburg & Guest 1997) and St. Helena (Carayon 1976), but is probably more widely distributed in intervening parts of southern Africa (Hernández & Stonedahl 1999). *Orius naivashae* is only known from Kenya (Hernández & Stonedahl 1999).

2.3 Morphology

2.3.1 Egg

Freshly laid eggs of all *Orius* species are about 0.4 mm long and 0.13 mm in diameter. They are initially colourless, but get a milky-white colour after several hours. Eggs are imbedded in the host plant tissue (= endophytic oviposition) and are sometimes laid in small clusters, but usually they are deposited singly. The insertion of the eggs in plant tissue may protect them not only from dessication, but from predation as well (Groenteman et al. 2006). The petioles are usually selected for oviposition, but the female might lay its eggs in the plant stem or in the main veins at the lower side of the leaf (**Figure 2.1**). Only the top of the eggs is visible as they are inserted nearly perpendicular to the surface of the

plant; hatched eggs are easier to spot as the operculum (egg cap) is clearly visible (Askari & Stern 1972a; Malais & Ravensberg 2002). The structure of the opercular surface varies between species (Sands 1957). If the eggs are not fully embedded in the plant tissue the red eyes and yellowish-orange body become visible through the chorion, with the timing of this phenomenon being dependent on the temperature (Isenhour & Yeargan 1981a). If the plant tissue becomes dry before the eggs hatch, the embryos are usually killed (Hagen et al. 1999).

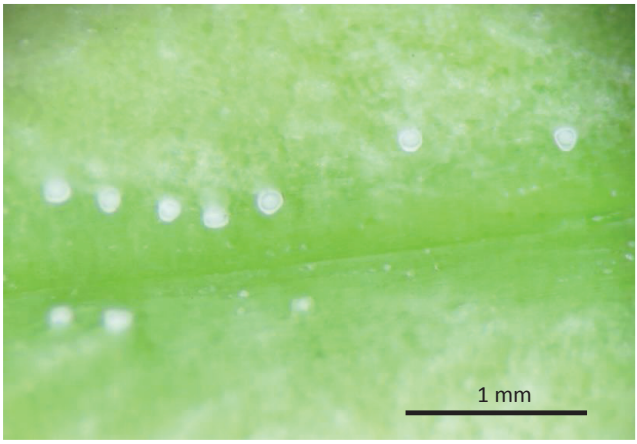

Figure 2.1 Orius eggs imbedded in plant tissue (photo: A. Van de Walle)

2.3.2 Nymph

All *Orius* species have five nymphal stages (instars). Nymphs emerging from the egg are glossy and colourless but turn yellow after several hours. Second and third instars can be yellow, orange or brownish, depending on the observed species and stage (**Figure 2.2**). In the last two instars the brown colour is more pronounced and nymphs start to look more like adults. In all stages the characteristic red eyes of the nymphs are clearly visible. Ocelli are not present in nymphs. Wing formation starts in the second instar but the wing pods are only externally distinguishable in the last instar (**Figure 2.2**) (Askari & Stern 1972a; Isenhour & Yeargan 1981a; Malais & Ravensberg 2002).

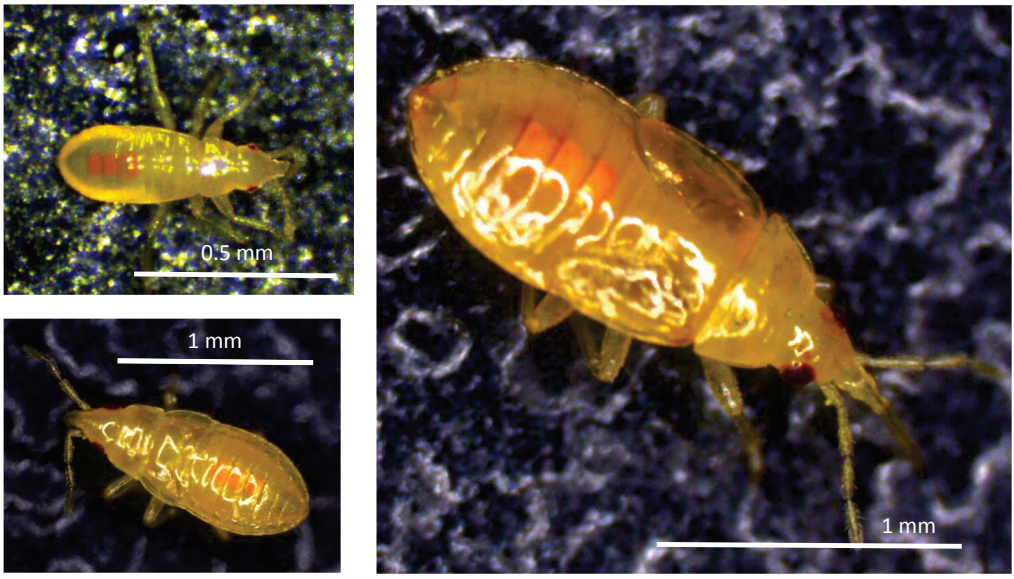

Figure 2.2 Nymphal stages of Orius species: N2 (upper left), N4 (lower left) and N5 (right) (photos: author)

2.3.3 Adult

Shortly after the final moult, adults are pale and soft. After some hours their cuticle hardens and they obtain their characteristic colouration: from brown to blackish, with pale grey-white to brown areas on the wings. The colouring of male and female adults is identical. The wings are deployed about one hour after moulting (Askari & Stern 1972a; Malais & Ravensberg 2002). In general, *O. thripoborus* adults are smaller than those of *O. naivashae*, and female adults are slightly larger than males. The size of female adults varies between 1.8 and 2.1 mm for *O. naivashae* and between 1.86 and 1.98 mm for *O. thripoborus*. Males lengths are between 1.67 and 1.95 mm, and between 1.61 and 1.86 mm for *O. naivashae* and *O. thripoborus*, respectively (Hernández & Stonedahl 1999). The general outer morphological structures of *Orius* species are illustrated in **Figure 2.3**.

The head of *Orius* species (**Figure 2.3 (1)**) is short, black, and smooth (*O. thripoborus*) or coarsely punctate (*O. naivashae*). Whilst the tylus is surpassing the tip of the first antennal segment in *O. naivashae*, it is less strongly produced in *O. thripoborus*, but carries erect setae apically. Mouthparts are of the piercing-sucking type, in the form of a slender beak or labium (**Figure 2.3 (2)**). The labium or rostrum is short and reaches the anterior coxae. It has three visible segments and serves to house the

four piercing stylets (paired mandibles and paired maxillae), which collectively form the two channels through which digestive enzymes and the ingested food products are moved (Horton 2008).

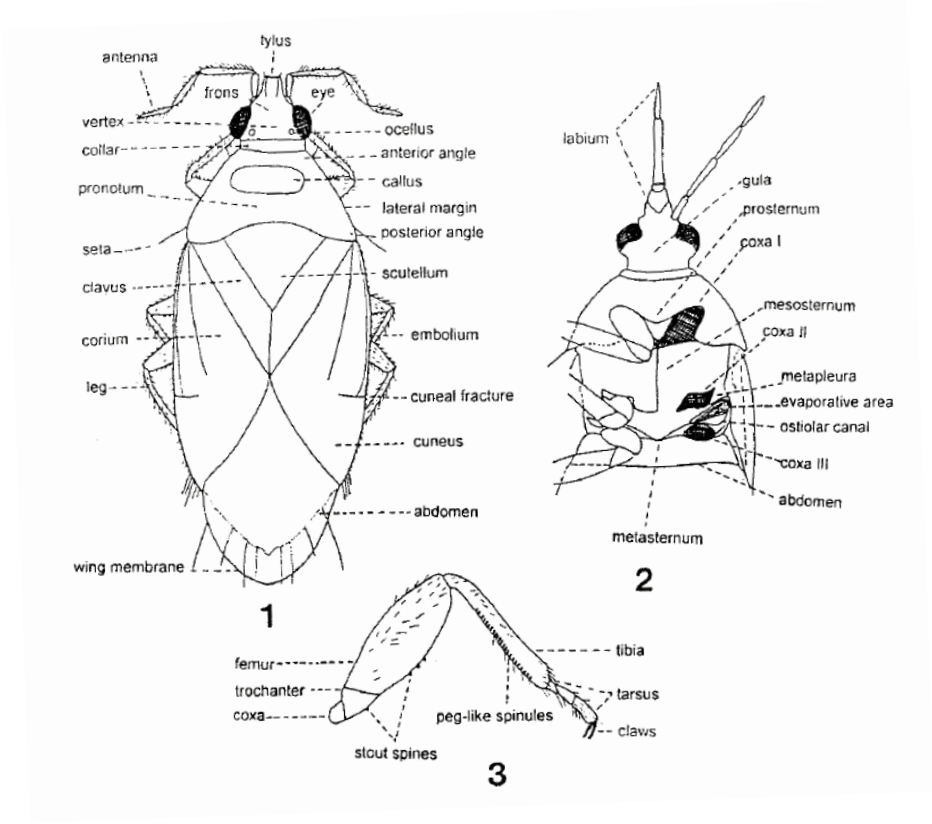

Figure 2.3 Morphological structures of Orius species: (1) dorsal view; (2) ventral view; (3) leg (Hernández & Stonedahl 1999)

Figure 2.4 Male adult of O. thripoborus (left); female adult of O. naivashae (right) (photo: A. Van de Walle)

The eyes are prominent and situated laterally on the head, occupying the entire height of the head in lateral view. Paired ocelli are present near the eyes (Horton 2008). The antennae have four segments and are inserted in front of the eyes. The trapezoidal pronotum (**Figures 2.3 (1) and 2.4**) of *O. naivashae* is black, evenly and coarsely punctured, without long setae at its anterior angles. For *O. thripoborus*, the pronotum is dark brown or black, smooth and shining, with the anterior angles each bearing a long bristle-like seta. The lateral margin of the pronotum has a distinct carina (ridge-like elevation) towards the anterior angle. The callosities of the pronotum (calli) of *O. naivashae* are flat or weakly convex with fine punctures, separated by a deeply punctate area. The calli of *O. thripoborus* are prominent and confluent, and depressed behind. The hemelytra (fore wings) are macropterous (long-winged) and mostly yellow (more pale for *O. thripoborus* (Carayon 1961)), with the membrane of the fore wing (apex of clavus and cuneus) of *O. naivashae* being smoky-brown (**Figure 2.4**). For *O. thripoborus*, the cuneus, and sometimes also the apex of the clavus, is dark brown or black, the membrane is hyaline and smoky apically, and the hemelytra have short yellow setae and longer brown setae at the apex of the cuneus. The abdomen consists of nine segments, but only eight are easily visible. The males have segments VI to VIII strongly asymmetrical. The female ovipositor is well developed and symmetrical (**Figure 2.5**). The last two segments contain the paragenital system, which is the most important character for species identification (Ribaut 1923). *Orius* males have a single spiral-shaped paramere, distinguished by a strongly curved flagellum without teeth or processes on the lame in *O. thripoborus* (**Figure 2.6**). This left paramere of *O. naivashae* males is characterised by a thick, lamelliform process, reaching the tip of the lame (**Figure 2.6**). The female copulatory tube opens ventrally on the intersegmental membrane and is composed of a basal part (basal segment) and a distal portion (apical tube). For *O. thripoborus* females, the basal segment is long and curved, with the apical tube being fairly short (**Figure 2.7**). *Orius naivashae* females are distinguished by a more elongated basal segment of the copulatory tube, separated from the intersegmental membrane with a short but distinct apical tube (**Figure 2.7**) (Hernández & Stonedahl 1999).The metathoracic scent efferent system is located ventrally on the metapleura (**Figure 2.3 (2)**). The sculpturing of the evaporative area is very useful in the separation of subgenera and closely related species. The peritreme of the methathoric scent system is very narrow for both *O. naivashae* and *O. thripoborus*, but has a narrow, smooth, shiny region anteriorly in the latter species (**Figure 2.8**). The metasternum is triangular (**Figure 2.3 (3)**). The legs

(**Figures 2.3 (3) and 2.4**) are linear, with three to five stout, dark spines on the ventral aspect of the profemora of *O. naivashae* males. Except for the yellow protibia, legs of the latter species are dark brown, with the profemora extensively darkened. Pro- and mesofemora of *O. thripoborus* are uniformly pale yellow (Hernández & Stonedahl 1999).

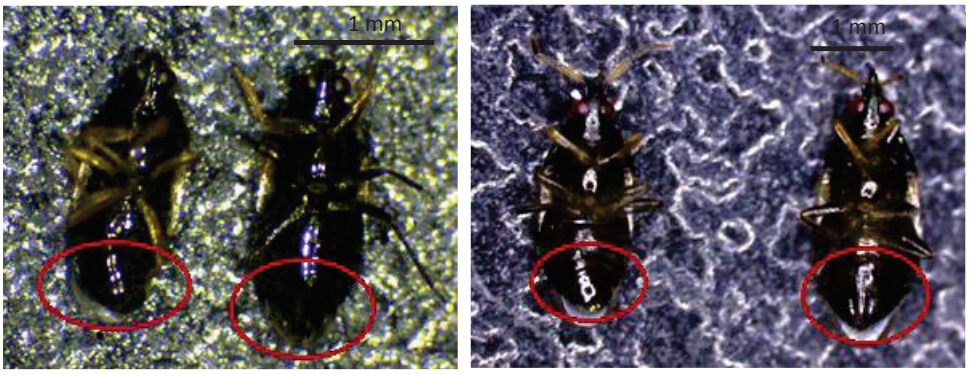

Figure 2.5 Ventral side of male (left) and female (right) adults of O. thripoborus (photo on the left) and O. naivashae (photo on the right). The (a)symmetry of the last abdominal segments is used for sex determination (photos: author)

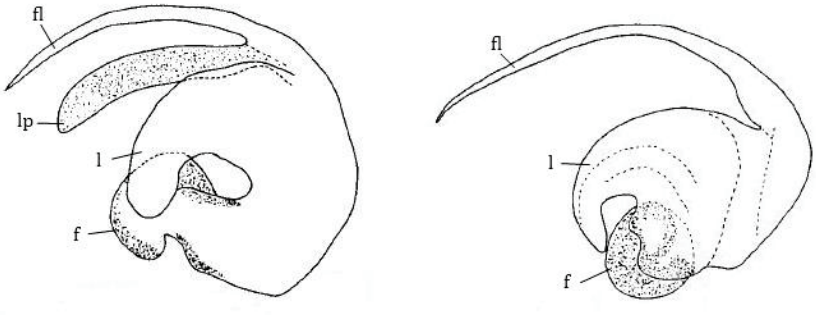

Figure 2.6 Left paramere of male genitalia of O. naivashae (left) and O. thripoborus (right) (f = foot, l = lame, lp = lamilliform process, fl = flagellum) (Hernández & Stonedahl 1999)

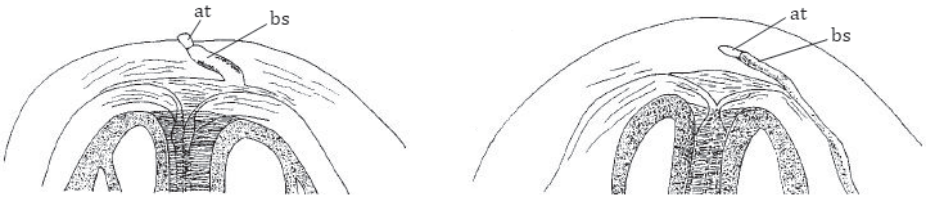

Figure 2.7 Female paragenital system of O. naivashae (left) and O. thripoborus (right) (at = apical tube, bs = basal segment) (Hernández & Stonedahl 1999)

Figure 2.8 Metathoracic scent efferent system of O. thripoborus (Hernández & Stonedahl 1999)

2.4 Ecology

2.4.1 Life cycle

Orius species are hemimetabolous insects with seven stages of development: an egg stage, five nymphal instars, and an adult stage (Malais & Ravensberg 2002) (**Figure 2.9**). Hemimetabolous insects undergo an incomplete metamorphosis, and the nymphs resemble the adults morphologically except in lacking wings, ocelli and reproductive structures (Horton 2008). In their natural habitat *Orius* species are multivoltine and the number of generations developing annually varies from two to eight. This number is usually determined by the required sum of effective temperatures and thus depends on the geographic zone or latitude (Horton 2008; Saulich & Musolin 2009). The availability of food may also influence the number of generations (Saulich & Musolin 2009). Most *Orius* species from temperate and colder climate regions overwinter as adults in dry and protected places (**see 2.4.4**).

2.4.2 Development

Development of *Orius* species is affected by several biotic and abiotic factors such as temperature, photoperiod, food source (**see 2.4.7**), and to a lesser degree host plant (**see 2.4.5**) and relative humidity.

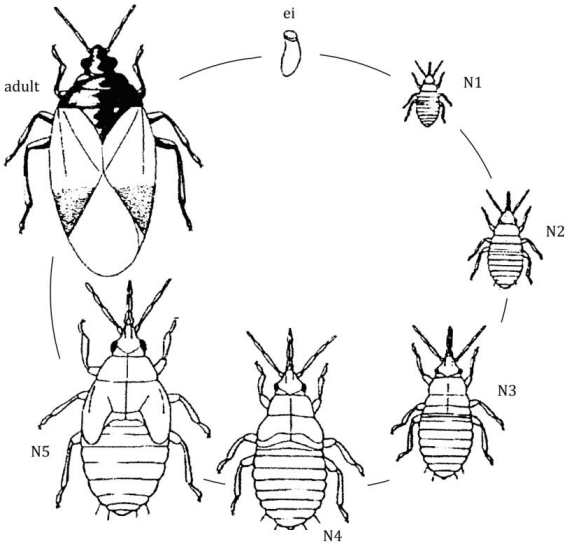

Figure 2.9 Life cycle of Orius species (after Malais & Ravensberg 2002)

Seasonal development of multivoltine species is chiefly controlled by daylength and temperature (Saulich & Musolin 2009). Temperature significantly affects both egg and nymphal development in *Orius* species. The duration of egg and nymphal development decreases significantly with each increase in temperature, but only within a range of moderate temperatures (Isenhour & Yeargan 1981a; Alauzet et al. 1994). The development of a species can only take place if the ambient temperature is above its lower development threshold. The latter parameter is defined as the temperature below which no measurable development occurs, based on a linear model (Campbell 1974). When exceeding the upper threshold temperature, developmental rate will decrease and development will eventually stop completely. The thermal requirement of a stage, expressed in degree-days (DD), is the amount of heat required to complete this stage (Peairs 1927). This need for

heat is thus a combination of temperature (with respect to the lower development threshold) and time. According to the linear degree-day model, the number of required degree-days will be constant at temperatures between the lower and the upper threshold temperature for a particular stage of development of a given species. However, there is a large variety of lower development thresholds and thermal requirements between and even within species due to environmental factors and cues such as food availability, geography and day length, and because of genetic differences (Musolin & Ito 2008; Saulich & Musolin 2009) (**Table 2.1**).

Table 2.1 Lower developmental thresholds (t_0) and degree-day requirements (K) for development of the immature stages of different Orius species

Species	Origin	t_0 (°C)		K (DD)		Prey	Reference
		egg	nymph	egg	nymph		
O. laevigatus	Europe	9.3	10.5	71.4	166.7	*E. kuehniella* eggs	Alauzet et al. 1994
O. insidiosus	USA	11.2	13.8	69.4	144.9	*Heliothis virescens* (F.) eggs	Isenhour & Yeargan 1981a
O. insidiosus	USA	8.8	10.7	66.5	240.4	*Trogoderma glabrum* (Herbst) eggs	Kingsley & Harrington 1981
O. insidiosus	Brazil	11.8	12.6	63.8	159.6	*E. kuehniella* eggs	Mendes et al. 2005
O. thyestes	Brazil	/	12.8	/	173.8	*E. kuehniella* eggs	Carvalho et al. 2005
O. sauteri	Japan	11.6	11.9	57.8	158.7	*Thrips palmi* Karny	Nagai 1993
O. sauteri	Japan	11.0	11.3	58.8	163.9	*Myzus persicae* (Sulzer)	Nakata 1995
O. sauteri	Japan	11.1	10.3	62.1	180.8	*Thrips palmi* Karny	Nagai & Yano 1999
O. strigicollis	Japan	11.5	10.8	57.5	162.7	*F. occidentalis*	Ohta 2001
O. tantillus	Japan	13.7	12.7	52.6	169.5	*Thrips palmi* Karny	Nakashima & Hirose 1997a
O. tristicolor	Japan	8.6	15.3	64.9	151.5	*Tetranychus pacificus* McGregor	Nakata 1995

Rates of nymphal growth and development in insects can be controlled not only by obvious environmental factors and cues such as temperature or food availability, but also by day length (Danks 1987; Saunders 2002). Photoperiodic responses reported in heteropteran species range from a slight retardation or acceleration of nymphal growth to prolonged nymphal diapause (Askari & Stern 1972b;

Saunders 1983; Kiritani 1985; Musolin & Saulich 1997, 1999; Tanaka et al. 2002; Saulich & Musolin 2007). In insects that overwinter in the adult stage in the temperate zone, acceleration of nymphal growth under late-season short-day conditions may ensure completion of nymphal development and synchronisation of adult emergence before the autumnal detoriation of environmental conditions (Ruberson et al. 1991; Musolin & Saulich 1997, 1999; Musolin & Ito 2008). The effect of day length on the duration of the nymphal period in *Orius* was studied in only a limited number of species. In *Orius majusculus* (Reuter) from the Netherlands and *Orius strigicollis* (Poppius) from central Japan, short days significantly accelerated the growth of nymphs at 18°C, although the trend was not always consistent (van den Meiracker 1994; Musolin et al. 2004; Cho et al. 2005). In *Orius insidiosus* (Say) from Arkansas, USA, short-day conditions accelerated the growth of nymphs at 20°C (Ruberson et al. 1991), but the trend was somewhat reversed, though not consistent, in nymphs of the same species from Georgia, USA, at 18°C (van den Meiracker 1994). Latter example shows that the response to photoperiod in *Orius* nymphs depends on the geographic origin of the population.

2.4.3 Reproduction

When *Orius* adults emerge they immediately start to mate (Tawfik & Ata 1973). The average duration of successful copulation in *Orius laevigatus* (Fieber) is 4.9 min, and the minimum time required for successful copulation is 1.75 min (Leon-Beck & Coll 2009). The left paramere of males (**see Figure 2.6**) is modified into an organ that serves to penetrate the body wall of the females during copulation. Males penetrate the female in the midventral abdominal copulatory site (between the seventh and eighth abdominal segment) and inject their sperm into the copulatory tube (**see Figure 2.7**). As insemination occurs outside of the reproductive tract and within the abdominal cavity, it is referred to as extragenital insemination. The sperm is collected in the spermatic pocket, which is a diverticulum of the anterior vaginal wall at the internal end of the copulatory tube. From the spermatic pocket, the sperm move to the ovaries through specialised conducting tissues, and fertilise the eggs within the vitellarium (Schuh & Slater 1995; Horton 2008).

Females will lay 2 to 3 eggs each day on average, starting from 2 to 3 days after mating, which is considered the preoviposition period needed for maturation of the eggs (Askari & Stern 1972a). The lifetime amount of eggs oviposited by *Orius* species reportedly ranges from 50 to 200 (Malais &

Ravensberg 2002). Besides temperature and food, the type of plant used as an oviposition substrate also affects the reproductive potential of *Orius* species (**see 2.4.6**).

The role of males in *Orius* species was studied by Mendes et al. (2003), Leon-Beck and Coll (2009) and Bonte and De Clercq (2010c). All studies concluded that virgin females of *Orius* species do not lay eggs as ovarian development was limited or even non-existent (Ito & Nakata 1998b; Bonte & De Clercq 2010c). In females of *Orius pumillo* (Champion), vitellogenesis is a two-stage process: early vitellogenesis requires a nutritious adult diet, whereas full vitellogenesis and egg maturation also require mating (Shapiro & Shirk 2010). Leon-Beck and Coll (2009) concluded that females of *O. laevigatus* are monandrous: once females have mated, they avoid any additional mating. In contrast, males seem to be polygynous. Leon-Beck and Coll (2009) also reported that the female to mate first with a male deposits more eggs than the following females. Further, female longevity and oviposition of *O. laevigatus* were significantly lower when mated pairs remained together during oviposition, than when females were isolated from the males after mating (Leon-Beck & Coll 2009). Age and feeding status of male mates may affect the reproductive output of females. Females of *O. laevigatus* mated with young virgin males produced fewer offspring than those mated with older virgin males, when males were fed on a suboptimal artificial diet but did not when males were reared on the highly nutritious eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Bonte & De Clercq 2010c). For some *Orius* species, male adults emerge earlier than females, particularly at lower temperatures (Musolin et al. 2004; Musolin & Ito 2008). Faster development of males might have an ecological significance: at the time females emerge, males are ready to mate with them (Musolin & Ito 2008). This strategy is designed as temperature-regulated protandry (Boyd & Alverson 2004). Faster development of male nymphs might be related to observations that males are always smaller than females in *Orius* species (Nakata 1995).

The reproductive capacity of *Orius* females can be influenced by low temperatures due to induction of reproductive diapause (**see 2.4.4**) or a decrease in mating activity (van den Meiracker 1994; Nagai & Yano 1999). Further, low temperatures in *Orius* species extend longevities (Cho et al. 2005) and (pre)oviposition periods, reduce fecundity, upturn adult body sizes (Nakata 1995), and increase mortality during overwintering (Saulich & Musolin 2009). In contrast, high temperatures, at given day

length, accelerate the prey consumption (Gitonga et al. 2002), mobility, and foraging/oviposition activity of *Orius* species (Cocuzza et al. 1997a; Tuda & Shima 2002; Baniameri et al. 2005).

2.4.4 Dormancy

In general, the arrestment in development that enables living organisms to synchronise their life cycle with favourable environmental conditions and that avoids unfavourable conditions is called dormancy, which can occur during all seasons. Two types of dormancy are usually distinguished in insects: quiescence and diapause. Quiescence is a reversible state, characterised by a reduction in metabolism as a direct response to exposure to environmental extremes, such as temperature or humidity, and which ends immediately when favourable conditions resume. Quiescence, or possibly weak diapause, was found in southern strains of *O. laevigatus* from Italy (Tommasini & Nicoli 1995) and France (Rudolf et al. 1993).

Diapause is an active response of individuals resulting in a dynamic state of low metabolic activity for adaptation to seasonal cycles and enables insects to circumvent adverse conditions. Winter is most commonly avoided in temperate zones, but diapause is also used to circumvent hot, dry summers and periods of food shortage. Diapause is a developmental response that is expressed only during a specific developmental stage, which depends on the species of insect. If the diapause occurs in response to environmental cues it is referred to as 'facultative diapause', but if it occurs during each generation regardless of the environmental cues it receives, it is considered to be 'obligatory diapause'. Both facultative and obligatory diapause are common in *Orius* species (Kingsley & Harrington 1982; Ruberson et al. 1991; van den Meiracker 1994).

Many factors (biotic and abiotic) can function as the token stimulus to induce diapause. In fact, the insects can translate the token stimuli in neurohormonal changes which lead to diapause (Williams 1952). Often, the most common and reliable token stimulus is photoperiod, whether or not in interaction with temperature (Beck 1980; Saunders 1982; Tauber et al. 1986; Gullan & Cranston 1994). Several factors other than temperature and day length, such as moisture, population density, food, presence of males and changes in these factors, may influence the incidence of diapause in insects (Tauber et al. 1986; Danks 1987).

Most insects in the temperate zone, including *Orius* species, use day length as a token stimulus to enter seasonal dormancy, usually adult (= reproductive) diapause in *Orius* species (van den Meiracker 1994; Ruberson et al. 1998, 2000; Musolin & Ito 2008; Kobayashi & Osakabe 2009; Saulich & Musolin 2009), and to stabilise their seasonal cycles (Danilevsky 1965; Beck 1980; Tauber et al. 1986; Danks 1987). For insects which are sensitive to photoperiod, the 'critical photoperiod' is defined as the length of the day at which 50% of the sensitive stages of the insect will enter diapause (Tauber et al. 1986; Danks 1987). The critical photoperiod varies from species to species as well as within the same species for populations occurring at different geographical areas (Tauber et al. 1986; Danks 1987; Leather et al. 1993; Shimizu & Fujisaki 2006). Reproductive diapause was found in the nearctic species *O. insidiosus* (Iglinsky & Rainwater 1950; Kingsley & Harrington 1982; Ruberson et al. 1991; van den Meiracker 1994) and *Orius tristicolor* (White) (Anderson 1962; Askari & Stern 1972b; Gillespie & Quiring 1993; van den Meiracker 1994), as well as in the palearctic species *O. majusculus* (Fischer et al. 1992; van den Meiracker 1994). No diapause was observed in *Orius thyestes* Herring from the neotropical region (Carvalho et al. 2006), *Orius albidipennis* (Reuter) from the Canary Islands and *Orius tantillus* (Motschulsky) from India and Southeast Asia (Nakashima & Hirose 1997a, b). There is no published information on diapause responses in *O. naivashae* and *O. thripoborus.*

All multivoltine anthocorids of the temperate zone studied to date have photoperiodic responses of a long-day type: the females reproduce under long-day conditions, but enter diapause under short-day conditions. Reproductive diapause was induced when reared under varying lengths of short-day conditions in species with critical day lengths at 18°C, indicated as follows: *O. majusculus* between 14 and 16 h (van den Meiracker 1994), *O. strigicollis* between 12 and 14 h (Cho et al. 2005), *O. insidiosus* from Georgia between 11 and 12 h (van den Meiracker 1994), *O. insidiosus* from Arkansas between 12 and 13 h (Ruberson et al. 1991). Stack and Drummond (1997) showed that, in the majority of *O. insidiosus* individuals, extending photoperiod with supplemental blue light enhances reproduction and averts reproductive diapause over a range of temperature regimes.

Towards the south, the photoperiodic response gradually becomes weaker: some populations do not enter diapause even under short-day conditions, especially at higher temperatures (Horton 2008; Saulich & Musolin 2009). A latitudinal cline was found in the photoperiodic response controlling

reproductive diapause in *Orius nagaii* Yasunaga, *Orius sauteri* (Poppius), *Orius minutus* (L.), *O. tantillus* and *O. strigicollis* from Japan: the lower the latitude, the lower the diapause incidence and the shorter the critical day length (Shimizu & Kawasaki 2001).

For *Orius* species in which reproductive diapause is induced under short-day conditions, the preoviposition period is inversely related to day length (Ruberson et al. 1991). In non-diapausing *Orius* females, the preoviposition period is rather constant at different photoperiods and at a given temperature (18°C) (Nakashima & Hirose 1997b).

Sensitivity to diapause-inducing photoperiods typically occurs in late instars, sometimes extending into the adult stage (Ruberson et al. 1998). In short-day sensitive *Orius* species, like *O. insidiosus* (Ruberson et al. 2000) and *O. strigicollis* (Cho et al. 2005), the sensitive stages seem to be the last (fourth and fifth) instars and early adult stage, though some variations in response were recorded. Overwintering in the adult stage may provide the greatest flexibility for location of, and movement within overwintering sites, as well as for movement towards food and reproductive resources when dormancy is completed (Tommasini & van Lenteren 2003). *Orius* species are reported to overwinter in the grasses in field borders (*O. insidiosus*; Elkassabany et al. 1996), in leaf litter (*O. tristicolor*; Anderson 1962), on the ground of crop fields (*Orius sauteri* (Poppius); Yasunaga 1993), or underneath the bark of trees (*O. sauteri*; Lee et al. 1992). In temperate areas, males usually copulate in autumn and die before or during winter (van den Meiracker 1994; Ito & Nakata 1998a; Ruberson et al. 1998; Shimizu & Kawasaki 2001; Musolin et al. 2004; Kobayashi & Osakabe 2009). Diapausing females contain no mature eggs and have a hypertrophic fat body (Ruberson et al. 1991; van den Meiracker 1994; Ito & Nakata 1998a; Ruberson et al. 1998). The return of suitable environmental conditions leads to termination of diapause (Beck 1962), but at least in some anthocorids, exposure to low temperatures for at least a few weeks is required to terminate diapause (Saulich & Musolin 2009). For the small number of *Orius* species in which termination of diapause was studied, temperature was found to be the most important factor (Saulich & Musolin 2009).

Whereas temperature itself is not a reliable seasonal indicator, its effect upon photoperiodic response of diapause induction is significant in many insect species. The mode of action of temperature may differ even among related species (Musolin et al. 2004). Constant temperatures are mostly reported

to affect the photoperiodic response curve in two ways. First, temperature can modify the critical day length. Second, temperature can modify the degree to which an insect responds to photoperiod (Beck 1980; Saunders 1982; Tauber et al. 1986). Low temperature and/or decrease in temperature and day length enhance diapause induction in many insect species (Tauber et al. 1986; Danks 1987; Kohno 1998). Diapause in *O. albidipennis* from warm regions is mostly controlled by temperature, or is weak in individuals from these populations (Carnero et al. 1993; van den Meiracker 1994; Chyzik et al. 1995; Saulich & Musolin 2009). Increased temperature affects the photoperiodic response in *O. sauteri* but not in *O. minutus* (Kohno 1998; Musolin & Ito 2008b). However, for *O. insidiosus* and *O. strigicollis*, day length plays a dominant role in diapause induction: the photoperiodic response is thermostable at a moderate temperature range, but high temperatures (>28°C) strongly suppress induction of diapause (van den Meiracker 1994; Musolin et al. 2004).

The presence or absence of diapause in an important criterion to select an effective biocontrol agent, mainly when the pest can overwinter without undergoing diapause (Ito & Nakata 2000; Tommasini & van Lenteren 2003). In addition, the capacity to withstand periods with temperatures around or below zero can also be an asset for a candidate biological control agent confronted with cold winters. Insects can survive at low temperatures by either tolerating the formation of internal ice (freeze tolerance) or by freeze avoidance, which involves both physiological and biochemical mechanisms to avoid freezing of intracellular and extracellular body fluids (Bale 1996). At sub-zero temperatures, some freeze-avoiding insects migrate to warmer regions, though in most insects a behavioral response directs them to thermally-buffered overwintering sites. In addition, insects can invoke several cold-hardiness mechanisms, but, to date, this has hardly been studied in anthocorids (Denlinger & Lee 1998; Danks 2005). The only noteworthy fact is that freezing of the adult body might not be the cause of the overwintering mortality in males of *O. sauteri* and *O. minutus* as the supercooling point in adults reared under short days was quite low (<−20°C) in both sexes (Ito & Nakata 1998a).

2.4.5 Habitat and host plants

Orius are small predaceous insects that occur in a variety of habitats, but are mainly found on forbs and shrubs, especially in their flowers, as they also feed on pollen (Lattin 1999; Horton 2008; Malais & Ravensberg 2002). *Orius* species are thigmotactic, preferring the small crevices in these flowers

(Chambers et al. 1993; Lattin 1999). Shipp et al. (1992) determined intra-plant spatial patterns of *Orius insidiosus* (Say) and *O. tristicolor* populations in greenhouse pepper and found that both *Orius* adults and nymphs were aggregated in the top one-third of the plants. *Orius* species are typical agrobionts, being abundant in arable, horticultural and ornamental agro-ecosystems where they feed on various pest species and plant tissues (Bosco & Tavella 2008; Tommasini 2004; Perdikis et al. 2011). In agroecosystems, *Orius* has been observed in a wide range of pollen bearing field crops, greenhouse vegetables and ornamentals such as maize (*Zea mays* L.) (Mészáros et al. 1984; Albajes et al. 2011; Veres et al. 2012), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), alfalfa (*Medicago sativa* L.) (Bokina 2008), soybean (*Glycine max* (L.) Merr.) (Lundgren et al. 2009), faba bean (*Vicia faba* L.) (Atakan 2010), chili pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.) (Fathi 2009), cotton (*Gossypium* sp.) (Atakan 2006; Lucas & Rosenheim 2011) and strawberry (*Fragaria* sp.) (Bosco & Tavella 2008). A few species of *Orius* have been recovered from grape vine (*Vitis vinifera* L.), and apple and pear orchards, feeding on small insects, or were found breeding on hawthorn (*Crataegus monogyna* L.), alder (*Alnus sp.*) and poplar (*Populus* sp.) (Lattin 2000).

The southern African species *O. naivashae* and *O. thripoborus* are also associated with forbs and pollen producing crops. *Orius thripoborus* has been reported on a series of crops: avocado (*Persea americana* Mill.), citrus (*Citrus* sp.), peach (*Prunus persica* L.), cotton, sunflower (*Helianthus annuus* L.) and cassava (*Manihot esculenta* Crantz) (Hesse 1940; Hamburg & Guest 1997; Steyn et al. 2003). This species has been collected as well from the forbs *Commidendrum robustum* (Roxb.) DC. and *Aster glutinosus* (Less.) Kuntze, and from the acacia tree (*Acacia* sp.) (Hesse 1940; Hernández & Stonedahl 1999). The only published records of *O. naivashae* were made on cotton (*Gossypium arboretum* L.).

The (semi)-natural habitats surrounding an agroecosystem serve as reservoirs for *Orius* species (Kemp & Barret 1989; Ohno & Takemoto 1997). *Orius* species found in vineyards in California were more abundant within a 20 m distance from a forest or from a corridor of flowering plants (Nicholls et al. 2000). The abundance pattern of *Orius* species at a landscape scale is shown to be driven by resource patterns and availability of both semi-natural and cultivated areas, which may vary within the season and between years (Péricart 1972; Veres et al. 2012). Tolerance for weeds in agricultural fields and manipulation of vegetation near crops may favour *Orius* species populations (Scutareany et al. 1993).

Predators are expected to attain higher densities in more diverse habitats because a greater variety of prey is available at different times, a larger variety of microclimates (shade, moisture, and temperature levels) and microhabitats is present, and more pollen and nectar food sources are available. Besides, diverse habitats can harbour more prey refuges and overwintering sites, resulting in persistence of prey and predator populations (Coll 1998; Landis et al. 2000; Lundgren & Fergen 2006). Moreover, increasing plant diversity allows predators to optimise their fitness by exploiting various plant-based resources such as nutrition and oviposition sites (Lundgren et al. 2008).

2.4.6 Role of plant material

Many predatory heteropterans benefit from plant material at some time in their life cycle. The importance of plant materials is four-fold: 1) plants may serve as oviposition substrates; 2) plant material can be a source of nutrients and water for the beneficial insect (**see 2.4.7.2**); 3) plants may obstruct the predator in its behavior; and 4) plant material provides hiding places for insects (Coll 1998).

Most *Orius* species display clear preferences for certain plant species as oviposition sites (Lundgren & Fergen 2006). When selecting an oviposition site, omnivores are expected to respond to both prey availability and, even more strongly, to plant traits that affect both females and their offspring (Groenteman et al. 2006; Lundgren et al. 2008). *Orius* bugs are characterised by endophytic oviposition: their females insert their eggs into the plant tissue using an ovipositor. Such females prefer plants as ovipositional hosts that have the thinnest external tissues. When the plant has been chosen they will start searching for spots with low trichome densities and epidermis thickness (Lundgren & Fergen 2006; Lundgren et al. 2009).

A number of plant morphological features are known to affect the behaviour and fitness of insects. Trichomes (hair-like appendages) impede insect movement and constitute a physical and sometimes chemical barrier to insect feeding (Lundgren et al. 2008). For example, the high trichome density lowers the foraging speed of *O. insidiosus* on tomato (Coll & Ridgeway 1995; Coll et al. 1997; Coll 1998). Plants, as host plants for their prey species, may also indirectly influence survival and oviposition of the predators. The presence of plant allelochemicals in prey may inhibit feeding on that prey, lower the predator's fitness or even induce mortality in a predator (Orr & Boethel 1986; Coll 1998).

Plant material also has the function of providing natural shelter to the insects. Taking away hiding spots could lead to greater stress, resulting in energy loss and affecting the overall fitness of the insect in its environment (Coll 1998).

2.4.7 Feeding ecology

Predatory bugs of the genus *Orius* (Hemiptera: Anthocoridae) display trophic omnivory (also called zoophytophagy) and are characterised by piercing and sucking mouthparts, with which they feed on a wide array of arthropod prey as well as on plant materials such as pollen and plant juices (Carayon & Steffan 1959; Salas-Aguilar & Ehler 1977; Coll 1998; Cohen 2000). Most *Orius* species, if inadvertently exposed to human skin, will probe and 'bite', especially if perspiration is present (Henry 1988).

2.4.7.1 Animal prey

Orius species are predators of various small, soft-bodied arthropods such as thrips, aphids (Hemiptera: Aphididae), whiteflies (Hemiptera: Aleyrodidae), mites (Arachnida: Acari), young lepidopterous larvae and small arthropod eggs (Barber 1936; Péricart 1972; Cranshaw et al. 1996; van Lenteren et al. 1997; Lattin 1999; Malais & Ravensberg 2002).

Orius bugs are quick and active predators (Montserrat et al. 2004) that occur predominately on the lower leaf side, and are reported to mainly attack their prey upon encounter (Shields & Watson 1980; Malais & Ravensberg 2002; Yano et al. 2005). They do appear to have the ability to locate their prey by means of olfactory and sense cues and to a lesser extent through visual cues (Carvalho et al. 2011). The antennae of *Orius* species play an important role in the detection of prey movement, though the predators perceive the prey only at 0.5 to 1 cm distance. After encountering prey, *Orius* species initiate area-restricted search (Shipp et al. 1992). However, Teerling et al. (1993) discovered that, in the absence of prey capture, the alarm pheromone of *Franklinella occidentalis* (Pergande) nymphs is used as a prey-finding kairomone by *O. tristicolor* to initiate area-restricted search. The searching efficiency of a predator is affected by plant species, both directly and indirectly, by the effect of the plant on prey density and distribution (Coll et al. 1997). Furthermore, search rate decreases with gut fullness (Sabelis 1992).

Despite *Orius* species being polyphagous, they show a preference for thrips (Salas-Aguilar & Ehler 1977; Riudavets 1995; Malais & Ravensberg 2002; Baez et al. 2004; Kakimoto et al. 2006; Arnò et al.

2008; Xu & Enkegaard 2009). This can be an inherent prey preference or a preference driven by overlapping habitats (Cloutier & Johnson 1993; Hansen et al. 2003). Prey preference is not necessarily based on nutritional factors (Thompson & Hagen 1999). Also the vulnerability, type, density, size, and mobility of the prey may influence the prey selection of *Orius* species (Evans 1996; Eubanks & Denno 2000; Mendes et al. 2002; Malais & Ravensberg 2002; Baez et al. 2004; Reitz et al. 2006). Furthermore, Venzon et al. (2002) suggested that *O. laevigatus* selects prey based on patch productivity, i.e., the number of eggs produced on a patch per bug per day, rather than on prey quality.

Once the prey is located, the stylets make contact and the front pair of the legs keep the prey under control (Cocuzza et al. 1997a; Malais & Ravensberg 2002). The stylets pierce through the prey's cuticle and by way of extracorporeal digestion solid prey materials are liquefied into slurries. This feeding method is termed 'solid-to-liquid feeding'. This implies that digestive enzymes are injected into the prey and together with specialised mechanical actions of the stylets the solid nutrients from the prey's carcass are predigested (type I extra-oral digestion/nonrefluxers). The digestive enzymes that are mixed with the food externally are recaptured and ingested along with the food. This allows digestion to continue in the gut and allows the predators to reduce the loss of proteins (Cohen 2004). This extra-oral digestion allows them to utilise the high-nutrient prey tissues besides hemolymph and predisposes them to attack relatively large prey (Cohen 1990, 1995, 2000). After feeding, *Orius* species clean their mouthparts with the forelegs and appear to rest; they are relatively inactive for short periods (Askari & Stern 1972a).

Handling times in *Orius* are relatively short. Isenhour and Yeargan (1981b) found average feeding times of hungry females of *O. insidiosus* on adult thrips ranging from 9 to 19 min, whereas capture took only a few seconds and pursuing rarely occurred. *Orius* predator bugs often consume their prey only partially, and sometimes they do not even feed on killed prey (Askari & Stern 1972a; Isenhour & Yeargan 1981c).

2.4.7.2 Plant foods

Orius bugs are known to be facultatively phytophagous or omnivorous (Coll 1996, 1998; Lattin 1999; Lundgren 2009, 2011). They probe the plant in the first place to fulfill their need for water, as in many cases water extracted from prey is insufficient to meet the predators' needs. Besides, the acquired

water may be essential for the process of prey feeding (Gillespie & McGregor 2000). In addition, *Orius* can extract supplementary nutrients from plants (Coll 1998; Coll & Guershon 2002; Lundgren 2009, 2011). The American species *O. insidiosus* has been noted to feed on xylem and mesophyll contents, allowing the bugs to ingest water, small amounts of sugars, starches and amino acids from the plant (Armer et al. 1998). Likewise, Lundgren et al. (2008) showed that neonate *O. insidiosus* are able to use plant tissues for nutrition in their early developmental stages and that the bugs not only feed on xylem but also on the more nutritious phloem allowing them to survive solely on plant materials for several days. There is a higher ability to utilise plant material in young nymphs and females than in older nymphs and males (Coll 1996, 1998). Groenteman et al. (2006) showed that a high nitrogen level in leaves stimulates development and survival in first instars of *O. albidipennis*. On the other hand, Coll (1996) asserted that it is likely that protein-poor plant diets impede egg maturation by the predator (Coll 1996). *Orius* species do not produce damage when piercing plant tissue (Malais & Ravensberg 2002).

Species of *Orius* occur commonly in the flowers of herbaceous vegetation and in other plant parts that offer nectar (Barber 1936; van den Meiracker & Ramakers 1991; Coll 1998), and are known to supplement their diets with pollen (Cocuzza et al. 1997b; Horton 2008). Pollen does not only serve as a source of nutrients but also of moisture, as they are able to take up water from a humid environment into their interior due to capillary effects (Diehl et al. 2001). Most pollen are known for their high levels of proteins, amino acids, starch, lipids and some minor nutrients such as vitamins and minerals (Patt et al. 2003; Lundgren & Wiedenmann 2004; Lundgren 2009). Besides interspecific differences in the nutritional value of pollen, particularly in amino acid and lipid content, intraspecific variability (e.g., hybrids) can also have a significant influence on the biological performance of pollen feeding insects (Richards & Schmidt 1996a; Lundgren 2009). Pollen may even show defensive properties against larcenous pollinivores. The deterring structure and appendages, lower nutrient levels and toxic phytochemicals of the pollen grains may deter facultatively pollinivorous natural enemies from consuming them (Lundgren 2009).

There is considerable variation in the performance of *Orius* species on pollen. Fauvel (1974) concluded that pollen is not an important food for *Orius vicinus* Ribaut and that it served primarily to attract the

bugs towards the flowers in which they can find prey. In contrast, Carayon and Steffan (1959) stated that pollen of the host plants is the main diet for *Orius pallidicornis* Reuter and may be used as an alternative food source. The omnivorous feeding strategy of *Orius* species may allow them to perform better in terms of development and reproduction or survive periods of prey scarcity (Kiman & Yeargan 1985; Cocuzza et al. 1997b), but it can also entail certain risks. For instance, when a crop is treated with systemic pesticides, the toxins may harm the predators when they ingest the contaminated plant sap or pollen (Ridgeway et al. 1967; Horton 2008).

2.5 Biological control

2.5.1 Use in biocontrol

Anthocorid bugs are often considered effective biological control agents based on their high efficiency of prey seeking, ability to concentrate in the areas of the highest density of the potential prey, and potential for rapid population growth (Hodgson & Aveling 1988). Among the Anthocoridae, many species of the genus *Orius* are economically important beneficial insects in various agroecosystems (Barber 1936; Carayon 1961; Kelton 1963; Oku & Kobayashi 1966; Alauzet et al. 1994). Seven *Orius* species are currently commercially used of which *O. insidiosus* and *O. laevigatus* are being produced on a very large scale with production figures of hundred thousands to millions of individuals per week (van Lenteren 2012).

In many agricultural and horticultural crops *Orius* species are important natural enemies of a variety of pest species such as thrips, whiteflies, aphids, lepidopterous larvae, and mites (Hernández & Stonedahl 1999), but they have mainly gained attention for their capability of controlling the western flower thrips, *F. occidentalis* (Cocuzza et al. 1997a), and the onion thrips, *Thrips tabaci* Lindeman.

There are many advantages of using *Orius* species as biological control agents (Applied Bio-nomics 2015; Biobest 2015). *Orius* species are omnivores and are able to feed on plant material, which enables their preventive use in pollen producing crops (**see 2.4.7.2**). These predators feed on all life stages of thrips and often kill more prey than needed to survive (**see 2.4.7.1**). *Orius* species can locate prey efficiently and have a small size, allowing them to prey on thrips in cryptic locations (Dennill 1992). Further, *Orius* predators can easily be combined with other biological control agents such as

Macrolophus pygmaeus (Rambur) (Jakobsen et al. 2004; Messelink & Janssen 2014) and several predatory mites (e.g., Gillespie & Quiring 1992; Madadi et al. 2009; Chow et al. 2010).

A disadvantage of using *Orius* species as biological control agents is that several species are prone to diapause when day length shortens (**see 2.4.4**), so supplemental lighting may be needed when used in protected cultivation early in the season. Cannibalism is a recurring problem between adults or between adults and nymphs (**see 2.5.2.4**). *Orius* species are also affected by systemic insecticides or pesticides with long residual action, such as abamectin, teflubenzuron, diflubenzuron, imidacloprid and spinosad (Delbeke et al. 1997; Studebaker & Kring 2003; Biondi et al. 2012; Biobest 2015). Although a good dispersal ability is often considered a beneficial trait contributing to the success of a biological control agent (van Lenteren & Woets 1988), the successful augmentation of natural enemies at the same time requires that the released individuals remain and reproduce in the target field (Wang et al. 2001). The dispersal capacities of members of the genus *Orius* are moderate: they have functional wings, although their small size and limited flight capabilities make them more dependent on passive dispersal via upper air currents for long range movements. Local dispersal of *Orius* adults is commonly accomplished by diurnal, short, low-level flights, as compared to other anthocorids (Southwood 1960; Lattin 1999). Dispersal ability of the predators is also relevant for their role in conservation biological control and determines their capacity to move in and out of crops from and to conservation areas and landscape elements. To our knowledge, however, little or no studies have addressed this for *Orius* spp.

Orius species can be released preventively at a 5,000 to 10,000 *Orius*/ha (or 0.5 to 1 *Orius*/m²) density in pollen-bearing protected crops such as sweet pepper, gerbera (*Gerbera* sp.), strawberry and egg plant. In several greenhouse vegetable and ornamental crops, *Orius* can also be released curatively at 5 to 10 *Orius*/m² near local pest outbreaks (Biobest 2015). Although *Orius* species are routinely applied, with overall good success, in augmentative biological control programmes in protected cultivation (e.g., van den Meiracker & Ramakers 1991; Riudavets 1995; Cranshaw et al. 1996; van Lenteren et al. 1997; Ohta 2001), the number of success stories in open-field cropping systems remains limited (Frescata & Mexia 1996; Funderburk et al. 2000; Wang et al. 2001).

Commercially available *Orius* predatory bugs are offered in plastic bottles, containing 500 to 2,000 adults and nymphs in dispersal carrier (buckwheat and/or vermiculite). The material can be dispersed

from the bottle directly on the plant or applied by distribution boxes that are hung on the plants. It is important that the dispersal carrier remains at its introduction site for a few days so that the predators get a chance to mate and spread throughout the plant or crop. Four to six weeks are required after release of *Orius* bugs before thrips populations decline markedly (Applied Bio-nomics 2015; BioBee 2015; Biobest 2015; Koppert 2015).

In South Africa, Steyn et al. (1993) reported *O. thripoborus* as a natural enemy of two species of thrips damaging avocado, *Heliothrips haemorrhoidalis* (Bouché) and *Selenothrips rubrocinctus* (Giard). Dennil (1992) concluded that this species might be a useful biological control agent against both thrips species in the eastern Transvaal. Also in South Africa, Hesse (1940) found *O. thripoborus* feeding on the citrus thrips *Scirtothrips aurantii* Faure, not only occurring on citrus fruits, but also on leaves of peach and acacia. Further, *O. thripoborus* was seen preying on eggs of *H. armigera* in South African cotton fields (Hamburg & Guest 1997) and on sunflower (*H. annuus*). This predator is also associated with the cassava green mite *Mononychellus tanajoa* (Bonder) (Tetranychidae) on cassava (*Manihot esculenta*). *Orius naivashae* is only known from Kenya, where it was observed on cotton preying on *Helicoverpa armigera* (Hübner) (Hernández & Stonedahl 1999). Up to present, however, there is no commercial use of *O. thripoborus* or *O. naivashae* in southern Africa.

2.5.2 Artifical rearing systems

For reasons of confidentiality, there is little published information on commercial mass-rearing systems for predatory heteropterans (De Clercq et al. 2013). van Lenteren and Tommasini (2003) presented a mass production scheme for *Orius* bugs, based on *E. kuehniella* eggs as food and bean pods as oviposition substrate and moisture source (**Figure 2.10**).

Several papers have described systems for medium- to large-scale production of economically important predatory heteropterans. Major factors that were indicated to determine the success of a rearing system were climate, food quality and quantity, living and oviposition substrate, type of container, and rearing density/cannibalism (**see 2.5.2.4**). According to Mackauer (1976) and van Lenteren (1991), the provision of some variation in rearing conditions (food, microclimate, space) may enhance fitness and minimise selection during laboratory propagation of biological control agents (Bueno & van Lenteren 2012).

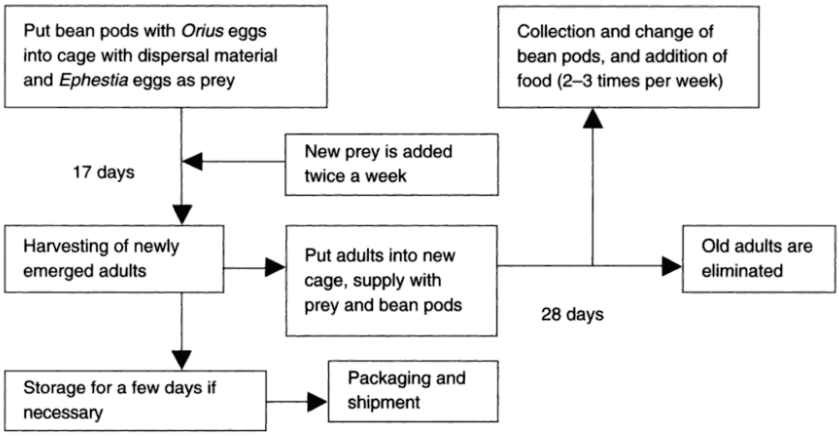

Figure 2.10 Production scheme for Orius species (van Lenteren & Tommasini 2003)

Some natural enemies are produced in a rearing system in which they are fed on their natural prey or host. The natural prey or host is, in turn, reared on its natural host plant. Thus, this rearing method is essentially a tritrophic system. Maintaining three trophic levels is highly labour intensive, requires plenty of space and is therefore expensive. Eliminating the use of plants for the prey could yield a first reduction of the rearing costs of a predator. Implementing factitious (or unnatural) prey in the rearing system often eliminates the use of plant material and thus decreases production costs (**see 2.5.2.1**). The next level of progression in cost-effective rearing of predators may involve the utilisation of an artificial diet that obviates the use of prey (**see 2.5.2.2**). Finally, in order to realise a complete artificial rearing system, the use of plant materials should be minimised (**see 2.5.2.3**).

2.5.2.1 Factitious foods

Factitious prey are comprised of organisms that are not normally attacked by the predator, mostly because they do not occur in its natural habitat, but do sustain its development in a laboratory environment (De Clercq 2008). Factitious prey may be offered fresh, but in many cases, they are frozen, irradiated, or lyophilized for improved storage or use in predator cultures (Riddick 2009).

The use of factitious foods for the production of arthropod predators and parasitoids may allow some degree of mechanisation of rearing procedures and lead to a significant reduction of operation costs,

which in turn may make augmentation biological controls more competitive with chemical controls (van Lenteren & Tommasini 2003; De Clercq 2008). As many heteropteran predators used in augmentative biological control programmes (e.g., *Orius* species) are highly polyphagous, they are amenable to rearing on factitious prey (Riddick 2009).

The most frequently used and most successful group of factitious foods are eggs of Lepidoptera. Eggs of the Mediterranean flour moth *E. kuehniella* (**Figure 2.11**), the Indian meal moth *Plodia interpunctella* (Hübner), the rice moth *Corcyra cephalonica* (Stainton), and the Angoumois grain moth *Sitotroga cerealella* (Olivier) yielded satisfactory to excellent results when offered as a food to insect predators, including predatory bugs (De Clercq et al. 2013). Since the 1990s, *E. kuehniella* eggs have become the standard food for the production of *Orius* species (Schmidt et al. 1995; van den Meiracker 1999; Tommasini et al. 2004; Kakimoto et al. 2005; Bueno et al. 2006; Bonte & De Clercq 2008; Venkatesan et al. 2008). The nutritional value of *E. kuehniella* eggs for *Orius* species exceeds that of pollen or other alternative diets (Bonte & De Clercq 2010a). The developmental and reproductive rates of *O. laevigatus* have even been reported to be higher on eggs *of E. kuehniella* than on certain natural prey (Cocuzza et al. 1997b; Bonte & De Clercq 2008).

Figure 2.11 Adult of O. naivashae feeding on E. kuehniella eggs (photo: author)

The continuous use of lepidopteran eggs as a factitious food in mass-rearing systems does have some drawbacks, the most important of which is their high cost. Although the moths are easily produced on

inexpensive foods, there are substantial monetary investments for the mechanisation of rearing procedures, for climate management, and for the health care of the workers. This has led to high market prices for *E. kuehniella* eggs, which are currently about US500$ per kilogram (De Clercq 2008; De Clercq et al. 2013).

As a result, a search began to replace *E. kuehniella* eggs with other, cheaper factitious foods. Brine shrimps of the genus *Artemia* naturally occur in lakes or pools with high salinities and are routinely used as a feed in aquaculture (Lavens & Sorgeloos 2000). Hydrated, decapsulated *Artemia franciscana* cysts (diapausing eggs) were found to be nutritionally sufficient to sustain development and reproduction of *Orius* species and other predators (Hongo & Obayashi 1997; Arijs & De Clercq 2001b; Castañé et al. 2006). Depending on their quality, these cysts can be an order of magnitude cheaper than *E. kuehniella* eggs (Arijs & De Clercq 2001b). However, prolonged rearing on cysts as a sole food has been associated with fitness losses in *Orius* bugs (De Clercq et al. 2005). Therefore, dry *Artemia* cysts are currently used only in part of the life cycle or in a mixture with lepidopteran eggs in the production process of different predatory heteropterans, including *Orius* bugs (Bonte & De Clercq 2008; De Clercq et al. 2013).

Another potential source of factitious foods for predatory heteropterans produced by the billions in mass-rearing facilities for sterile insect techniques is fruit flies (Tephritidae). Takara and Nishida (1981) reared *O. insidiosus* on a diet of of eggs of the oriental fruit fly, *Dacys* (*Bactrocera*) *dorsalis* (Hendel), with similar developmental and reproductive performance as compared to a natural diet. Further, eggs of the medfly, *Ceratitis capitata* (Wiedemann), have been used to produce the mirid predators *Cyrtorhinus lividipennis* Reuter (Liquido & Nishida 1985) and *M. pygmaeus* (Nannini et al. 2009) and were considered to have potential for predators of other heteropteran families.

2.5.2.2 Artificial diets

Artificial diets for insects have traditionally been classified as holidic (chemically defined), meridic (most components are chemically known), or oligidic (mainly composed of crude organic materials) (Dougherty 1959). As the distinction between these three categories is not always clear, Grenier and De Clercq (2003) proposed a classification system that separates artificial diets for insect natural enemies on whether they contain insect components (e.g., tissues, hemolymph, cells, protein, amino acids) or not. Artificial diets containing insect components are useful when predators require certain

growth factors, feeding stimulants and other chemical cues that are typically found in arthropod prey (De Clercq 2008; Riddick 2009)

A nutritionally adequate artificial diet should contain the basic nutrients (proteins or amino acids, lipids, carbohydrates) in appropriate proportions, and water. In addition, some specific minor components may be needed as growth factors, like sterols, vitamins, minerals and nucleic acids (Cohen 2004; De Clercq 2008). Besides, artificial diets can also contain stabilisers, preservatives and sometimes fillers or bulk agents and certain token stimuli. Token stimuli are components that evoke feeding but have no known function in the insect's metabolism (Cohen 2004).

The mode of presentation of an artificial diet is important in determining its acceptance by a predator (Grenier et al. 1994; Cohen 2004). Important issues in diet presentation include phagostimulants, texture, liquid or semi-solid state of the ingredients, and methods of containment (Ferkovich et al. 2007).

Ferkovich and Shapiro (2004) suspected that eggs of the Indian meal moth, *P. interpunctella*, and of *E. kuehniella* contain a specific nutritional factor that stimulates egg production in *O. insidiosus*. Adding soluble proteins from these lepidopteran eggs or adding an embryonic cell line of *P. interpunctella* to an artificial diet (based on soy protein acid hydrolysate and chicken egg yolk) was reported to enhance fecundity of the predator (Ferkovich & Shapiro 2004, 2005, 2007).

Using a deletion and addition approach, Arijs and De Clercq (2001a) found that egg yolk and beef liver were important components, whereas ground beef, ascorbic acid and sucrose were minor components in an artificial diet that supported the development of *O. laevigatus*. The artificial diet was packaged in Parafilm. Further, Bonte and De Clercq (2008) tested four artificial diets, wrapped in Parafilm, and concluded that a diet based on liver and ground beef resulted in better developmental and overall fitness of *O. laevigatus* than egg yolk based meridic diets. Still, the developmental and reproductive performance of *O. laevigatus* reared on eggs of *E. kuehniella* was superior to that of those reared on artifical diets (Bonte & De Clercq 2008, 2010a). Tan et al. (2013) developed a microencapsulated artificial diet for the rearing of *O. sauteri*. The ingredients included egg yolk, whole-pupa homogenate of the Tussah silk moth *Antheraea paphia* (L.), honey, sucrose, rapeseed (*Brassica napus* L.) pollen and

sinkaline (choline chloride). A complex coacervation method was used to make the artificial diet microencapsules (Tan et al. 2010).

2.5.2.3 Plant materials

Pods of green bean (*Phaseolus vulgaris* L.) are routinely used as a moisture source and oviposition substrate, in addition to insect prey, in cultures of *Orius* bugs (**see 2.5.2**). As compared to free water, bean pods can have positive, neutral, or negative effects on the development and/or reproduction of *Orius* species (Naranjo & Gibson 1996; Coll 1998; Bonte & De Clercq 2010a, 2011). Under some conditions, *Orius* species can complete development when provided with only bean pod sections (Salas-Aguilar & Ehler 1977; Richards & Schmidt 1996a).

Supplementing a diet of *E. kuehniella* eggs with pollen increased the fecundity in *O. albidipennis* and *O. insidiosus*, but not in *O. laevigatus* (Richards & Schmidt 1996a; Cocuzza et al. 1997b). Many *Orius* species can survive on pollen alone, however the resulting developmental and reproductive rates are very poor (Salas-Aguilar & Ehler 1977; Kiman & Yeargan 1985; Cocuzza et al. 1997b; Vacante et al. 1997; Venkatesan et al. 2008; Bonte & De Clercq 2010a, 2011). Adding pollen to the diet of *Orius* species may not only affect reproduction and survival, but also their behaviour. Supplementing a diet of thrips with pollen did not enhance egg production by *O. laevigatus*, but surprisingly increased predation rates on thrips larvae (Hulshof & Linnamäki 2002). Further, the use of pollen in insect cultures has some practical drawbacks as its quality tends to detoriate quickly, and, particularly when offered fresh, it is prone to fungal contamination (De Clercq et al. 2013).

The list of plants or plant parts used as an oviposition substrate in *Orius* production systems is long: green bean pods, plant seedlings (e.g., cotton, soybean, and sharp pepper), sprouts (e.g., potato, soybean, alfalfa and broad bean), stems (e.g., green bean and geranium), leaves (e.g., geranium and ivy), and inflorescences (e.g., farmer's friend *Bidens pilosa* L.) (Isenhour & Yeargan 1982; Kiman & Yeargan 1985; Ruberson et al. 1991; van den Meiracker & Ramakers 1991; Alauzet et al. 1992; Chyzik et al. 1995; Richards & Schmidt 1996b; Cocuzza et al. 1997b; Vacante et al. 1997; Coll 1998; Ito 2007; Murai et al. 2001; Bonte & De Clercq 2010a). However, in most *Orius* mass rearing systems green bean is used as an oviposition medium (and moisture source). Nonetheless, this is not an optimal substrate because of its perishability, limited seasonal availability, and the risk of contaminating the colony with

pathogens or pesticide residues (Castañe & Zalom 1994; Murai et al. 2001). To overcome these disadvantages, artificial oviposition substrates could provide valuable alternatives for plant material. Some workers succeeded in developing an artificial oviposition substrate on which *Orius* bugs are able to produce viable eggs (Castañé & Zalom 1994; Shapiro & Ferkovich 2006; De Puysseleyr et al. 2014). However, none of these workers have succeeded in rearing these anthocorids without plants for several generations, or the substrates are highly impractical for being used in mass rearing systems.

Plant material is also commonly used for storage, shipping, or application of commercially reared predatory bugs (Coll 1998). For instance, buckwheat hull is used in cultures of *Orius* bugs (e.g., Thomas et al. 2012) and as a carrier (in a mixture with vermiculite) in commercial packaging of these predators.

2.5.2.4 Cannibalism

Many of the commercially available predators are generalists and exhibit cannibalism in laboratory cultures, especially when kept at high densities (van Lenteren & Tommasini 2003). Particularly in populations with overlapping life stages, cannibalism is not uncommon, and older or larger predators may look upon younger and smaller conspecifics (including eggs) as potential prey (Tommasini et al. 2002; Rudolf 2007; Bonte & De Clercq 2011; De Clercq et al. 2013). Cannibalism has been observed in several *Orius* species, both in the laboratory and the field (Askari & Stern 1972a; Nakata 1994; van den Meiracker 1999; Bueno & van Lenteren 2012).

Food quality and quantity affects cannibalistic behaviour by *Orius* species (Dong & Polis 1992; Malais & Ravensberg 2002). Studies of Leon-Beck and Coll (2007) and Tommasini et al. (2002) showed a reduction in the frequency of cannibalistic behaviour for *O. laevigatus* when offered increasing quantities of a nutritionally superior food consisting of *E. kuehniella* eggs or plant material such as pollen. These findings suggest that insects such as *O. laevigatus* can sustain themselves on plant material when prey are scarce without encountering the risks associated with cannibalism (Leon-Beck & Coll 2007). However, Bonte and De Clercq (2011) reported that providing ad libitum *E. kuehniella* eggs to *O. laevigatus* did not prevent cannibalistic behaviour at high nymphal densities. The higher attractiveness of the mobile conspecific food versus that of the immobile heterospecific (factitious) food was believed to be the reason for this behaviour in this and other species of predatory bugs (Grundy et al. 2000; Bonte & De Clercq 2011).

In order to prevent cannibalism in laboratory colonies, several measures can be taken. Shelter materials can be placed into the rearing cages, such as shredded paper (Chambers et al. 1993; Arijs & De Clercq 2001a), wax paper (Bonte & De Clercq 2010a, 2011), mesh sheets (Shimizu & Kawasaki 2001), rice grains (Ito & Nakata 1998b) and wheat grains (Ito 2007). Survival rates showed that oviposition substrates such as bean pods or lipophilic surfaces such as wax paper and plastic were more suitable for rearing *O. laevigatus* than household paper (Bonte & De Clercq 2010a). Moreover, nymphal population density should be kept low to prevent mortality caused by cannibalism, competition for food and space, and susceptibility to pathogens. In commercial facilities, initial densities of *O. laevigatus* can go up to 4.25 eggs/cm² (Bonte & De Clercq 2011).

2.5.3 Storage

Orius species can be stored for relatively short periods at low temperatures (e.g., 9 to 13°C), but a negative effect of the thermoperiodic condition on female fecundity and longevity may occur (Rudolf et al. 1993). An appropriate temperature for cold storage of *O. laevigatus* without quality loss appeared to be 10°C, and this for a maximum of 36 days (Kim et al. 2009). Bueno et al. (2014) found that *O. insidiosus* can be stored up to 10 days at 8°C without loss of quality.

Diapausing predators can be stored for significantly longer periods and with considerably less negative impact (e.g., reduced mortality) than is the case when non-diapausing predators are simply held at lower temperatures (Tiitanen 1988; Tauber et al. 1993). For example, half of the females of *O. sauteri* and *O. minutus* from Japan, in which diapause was induced under short days, survived for more than 100 days at 0°C (Ito & Nakata 1998a). Understanding the timing of photosensitive stages and the conditions required for diapause induction, maintenance, and termination allows for more efficient use of diapause in mass production and long-term storage of commercially produced predators (Ruberson et al. 2000; Musolin et al. 2004).

2.5.4 Quality assurance

Augmentative biological control has become a worldwide booming business. This success puts increasing pressure on the production capacities of the commercial insectaries, which may result in the production of lower quality natural enemies. In order to anticipate problems concerning the quality of the produced natural enemies quality assurance procedures are in place. The overall quality of a

beneficial organism can be defined as its ability to function as intended after release into the field (van Lenteren 2003). This implies that for every natural enemy quantifiable characteristics relevant for field release must be defined (Bigler 1989). Possible quality control parameters for arthropod predators are: morphological characters (body size, weight and abnormalities), developmental (duration and survival) and reproduction parameters (sex ratio and symbiont association, fecundity and longevity), biochemical composition (protein, lipid and carbohydrate content), behaviour and genetic traits. The establishment of relationships between certain parameters, e.g., between body size and fecundity or longevity, may help in simplifying quality control procedures. The ultimate quality criterion for a mass-produced natural enemy is its capacity to reduce pest populations, which can be evaluated by measuring the predation efficiency (Thompson & Hagen 1999; Grenier & De Clercq 2003; De Clercq 2008). Besides being expensive and time-consuming, the complexity of a field setting may obscure the actual cause for the failure or success of natural-enemy release. Therefore, the first assessment of the quality of an in-vitro- or in-vivo-produced beneficial will preferably be done in a laboratory setting (Grenier & De Clercq 2003). However, predation rates measured under unrealistic laboratory conditions should be extrapolated to the field situation with caution.

The physiological (morphology, development and reproduction) and behavioral (predation or parasitation rate, host localisation, walking and flying) characteristics of an insect can be influenced by genetic and non-genetic factors. The most important genetic factor in a rearing environment is selection. Selection pressure increases as the rearing becomes more artificial. The use of artificial diets may also lead to genetic bottleneck effects further decreasing the genetic variability. This could lead to a reduced capacity of the natural enemy to interact with its natural (target) prey or to adapt to nonstandard environmental conditions. A further approach to maintain populations of good quality during mass-rearing is to avoid the detrimental effects of inbreeding as much as possible during the first generations of a new captive population. When inbreeding is extreme, loss of fitness is observed, such as reduction in size, fertility and vigor (Manson et al. 1987; Grenier & De Clercq 2003). Inbreeding problems depend on species characteristics, but are strongly influenced by the genetic heterogeneity of the founder population. This heterogeneity largely depends on the number of founder individuals in the colony. Castañé et al. (2014) showed that 10 founder couples suffice for starting a *O. laevigatus* colony without loss in quality of its relevant biological characteristics. In practice, fitness reductions

are often prevented by regularly replacing laboratory populations with new, field-collected individuals (Hoekstra 2003).

Non-genetic parameters that can affect the characteristics of an insect include the physical conditions of rearing systems, the presence of pathogens and the quality of the food offered (Bigler 1989; Grenier & De Clercq 2003). *Wolbachia* is a well-known Rickettsiaceae that strongly interferes with 'normal' sex ratio determination in many arthropod species (Werren 1997; Stouthamer et al. 1999). For entomophages the main reported effects are cytoplasmic incompatibility and thelytokous parthenogenesis in Hymenoptera, and male killing in Coccinellidae. Cytoplasmic incompatibility was also reported in the Heteropteran species *M. pygmaeus* (Machtelinckx et al. 2009) and *O. strigicollis* (Watanabe et al. 2011). The antibiotics used in artificial diets for preventing bacterial contamination, mainly by gamma Proteobacteria, are not effective in removing endosymbiotic *Wolbachia* from infected strains (Stouthamer et al. 1990). In fact, penicillin and streptomycin are used for bacterial control and tetracyclin and rifampicin for *Wolbachia* elimination. Pure female lines with a *Wolbachia* infection could be an advantage for biological control, because female predators kill most prey. On the other hand, *Wolbachia* infections may modify the fecundities. The presence or absence of a symbiont could be a key quality criterion for some insect species (Grenier & De Clercq 2003; Machtelinckx et al. 2009).

For a number of arthropod biological control agents the International Organisation for Biological Control (IOBC) has developed quality control standards for commercially produced batches (van Lenteren et al. 2003). These standards are mainly focused on the number of living specimens in a container, their sex ratio, longevity and fecundity. In many synovigenic insects (i.e., insects in which egg production is more or less continuous during the lifetime of the female), however, determining lifetime fecundity is a tedious and time consuming activity. Callebaut et al. (2004) and Vandekerkhove et al. (2006) proposed a method to assess fecundity of the predatory mirid *M. pygmaeus* based on counting oocytes in dissected female adults. Likewise, Bonte and De Clercq (2008) found a strong correlation between oocyte counts, lifetime oviposition, and the number of eggs laid after 8 days by *O. laevigatus* females. This type of rapid dissection assay may thus be effective to reliably and economically assess the fitness of these predators.

Chapter 3

Orius species in the South-African sugarcane agro-ecosystem: their potential as biological control agents for *Fulmekiola serrata* Kobus and other sap sucking pests

3.1 Introduction

The sugarcane thrips, *Fulmekiola serrata* Kobus (Thysanoptera: Thripidae), is native to Asia and has spread to Madagascar, Mauritius, Réunion, Barbados, Guadeloupe, Trinidad, Venezuela and South Africa. It was possibly introduced into South Africa with planting material or by wind from Mauritius (Way et al. 2006b; Sallam 2009). Since its first record in December 2004, *F. serrata* has rapidly spread throughout South Africa's sugarcane industry (*Saccharum* spp. hybrids) (Leslie & Donaldson 2005; Way et al. 2006a; Way 2008). The species causes damage in young sugarcane (**Figure 3.1**), inhabiting rolled leaf spindles and curled margins of leaves, where it oviposits and feeds on the leaf epidermis and chlorophyll, thus reducing the plants photosynthetic ability (Sallam 2009). Because of their cryptic lifestyle and fast development of resistance to pesticides (Jensen 2000), thrips are notably difficult to control. Therefore, the availability of an effective indigenous natural enemy of *F. serrata,* living in the same cryptic habitat, could provide local growers with an alternative management strategy against this invasive pest. Such natural enemies may be even more valuable for biological control programs if they also attack other sugarcane pests such as aphids (Hemiptera: Aphididae), and thrips pests in other South African agricultural systems. Besides *F. serrata* in sugarcane, other economically important thrips pests in South Africa include the citrus thrips *Scirtothrips aurantii* Faure and two avocado related thrips species, *Heliothrips haemorrhoidalis* Bouché and *Selenothrips rubrocinctus* (Giard) (Hesse 1940; Dennil 1992; Way et al. 2006b). Further, EPPO (2012) reported the presence in South Africa of *Frankliniella occidentalis* (Pergande), a worldwide pest of a wide range of vegetable and ornamental crops.

By examining more than 50,000 spindles of 3 to 4 month old sugarcane from 2005 to 2007 in South Africa, Way (2008) found anthocorids (Hemiptera: Anthocoridae) to be amongst the most abundant predators inhabiting the same ecological niche as *F. serrata* does. These insects, also called flower bugs or minute pirate bugs, are common in many agricultural habitats and are typically amongst the most abundant predators in field-cropping systems (Hernández & Stonedahl 1999). Only 14 anthocorid species have been recorded from southern Africa to date, but the museums possess many unidentified specimens and it is highly likely that many more species (the majority of them probably undescribed) occur in South Africa (D.H. Jacobs, personal communication). According to Carayon (1961), the most

common South African anthocorids belong to the Oriini tribe and the majority of them fall into the genus *Orius* . However, Hernández and Stonedahl (1999) suggested that only about two-thirds of the actual African *Orius* fauna is known.

Figure 3.1 Adult of F. serrata (left) and damaged young sugarcane plant by F. serrata (right) (Sallam 2009)

Most known anthocorid species are polyphagous predators (Péricart 1972) that feed on different life stages of a wide range of small arthropods, including springtails (Collembola), leaf hoppers (Hemiptera: Cicadellidae), psyllids (Hemiptera: Psyllidea), scales (Hemiptera: Coccidae), aphids, fly larvae (Diptera), grain beetles (Coleoptera), caterpillars (Lepidoptera), leaf-roller larvae (Lepidoptera: Tortricidae), psocids (Psocoptera), thrips (Carayon 1972; Kelton 1978; Hernández & Stonedahl 1999), and different mite (subclass: Acari) species, such as oribatids (Oribatida), phytoseiids (Mesostigmata: Phytoseiidae), and tetranychids (Trombidiformes: Tetranychidae) (Askari & Stern 1972a; Tawfik & Ata 1973; Lattin 1999). Anthocorids are also known to be omnivores, feeding on pollen and other plant materials besides arthropod prey (Coll 1998; Lattin 1999; Coll & Guershon 2002; Horton 2008).

Within the Anthocoridae family, species of the genus *Orius* Wolff are economically important predators of agricultural pests such as thrips, aphids, mites, whiteflies (Hemiptera: Aleyrodidae) and the eggs of Lepidoptera, both in greenhouses and field crops. However, they appear to show a preference for attacking larval and adult thrips over other available prey (**section 2.4.7.1)**.

Consequently, *Orius* species have been used successfully in biological control programs in greenhouse and open-field cropping systems against various thrips pests world-wide (**section 2.5.1**).

This paper presents the results from surveys conducted in 2008, 2009 and 2013 primarily in and around South African sugarcane fields, in an attempt to find anthocorid natural enemies of sugarcane thrips. Most observations were made in the provinces of Mpumalanga and KwaZulu-Natal, where sugarcane is the predominant crop. In 2013, surveys were also done in the Western Cape Province where other crops were monitored for the presence of anthocorids.

3.2 Materials and Methods

Specimens of *Orius* spp. and other anthocorids were collected from sugarcane flowers, spindles of young sugarcane and several pollen producing neighbouring (weedy) plants (Maes 2009; Cottenie 2010; Vangansbeke 2010). In 2008 and 2009, the focus was on the sugarcane fields in Mpumalanga and KwaZulu-Natal, representing the epicentre of the South African sugarcane production. In 2013, besides sugarcane, other crops including maize (*Zea mays* L.) were inspected for the presence of anthocorid thrips predators. In that year, the survey area was expanded to the non-sugarcane producing Western Cape Province, where was being monitored for the presence of anthocorids in a vineyard (*Vitis* sp.), an orange (*Citrus sinensis* (L.) Osbeck) plantation and in naturally occurring fynbos (the natural shrubland or heathland vegetation occurring in a small belt of the Western Cape of South Africa, mainly in winter rainfall coastal and mountainous areas with a Mediterranean climate). Sampling sites were chosen at random (see map; **Figure 3.2**) as the main objective of the surveys was to outline associations between anthocorids and plants rather than to assess their population densities or spatial distributions. A list of the sampling sites per province, including some information on their geography and climate, is given in **Table 3.1**. All sampling locations are situated in the warm temperate climate zone (Kottek et al. 2006).

Insects were collected by placing a large Ziplock plastic bag (24 x 35 cm) over the flower heads and shaking the bag plus flowering head vigourously. Dislodged anthocorids running inside the bag were then directly sucked up using an aspirator, placed in ventilated vials (10 cm high, 3 cm diameter), and taken back to the laboratory at the South African Sugarcane Research Institute (SASRI). There, they were placed in a 70% ethanol solution to be preserved for later identification. *Orius* specimens were

identified using the keys developed by Carayon (1961) and Hernández and Stonedahl (1999), based on an examination of male and female genitalia. Live adults of the two most abundant species were used from subsequent field collections to initiate laboratory colonies. Based on laboratory experiments on individuals of these colonies, their biology and biocontrol potential will be investigated (see all **following Chapters**).

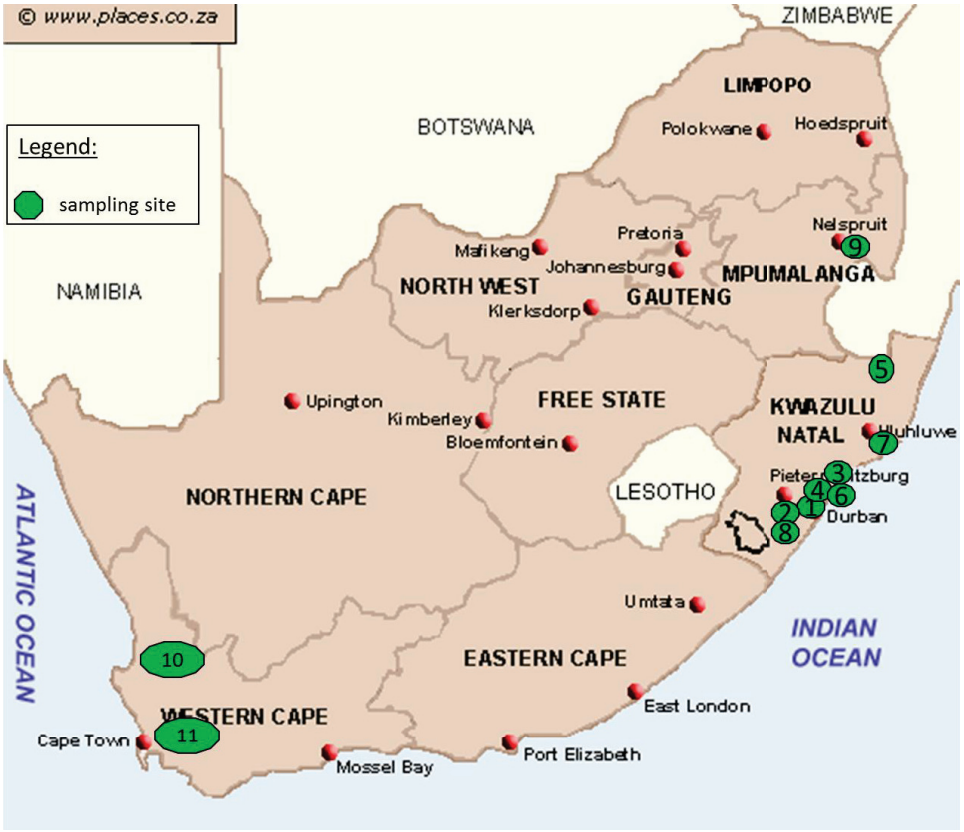

Figure 3.2. Map of South Africa with sampling sites shown (see also Table 3.1)

Table 3.1 List of the different sampling sites per province, including information on geography and climate

Province	Location[a]	Coordinates[b]	Altitude (m)[b]	Köppen-Geiger climate classification[d]	Annual mean min/max temperature (°C)[c]	Annual rainfall (mm/y)[c]
KwaZulu-Natal	Durban (1)	29°47'15.1"S 30°57'51.2"E	96	Cfa	16.6 / 26.4	1040
KwaZulu-Natal	Eston (2)	29°51'59.0"S 30°32'04.7"E	516	Cfa	13.2 / 27.9	863
KwaZulu-Natal	Ginginhlovu (3)	29°01'03.0"S 31°35'01.3"E	74	Cfa	17.8 / 24.7	1212
KwaZulu-Natal	Mt Edge-combe (4)	29°42'19.3"S 31°02'34.2"E	90	Cfa	17.6 / 25.0	925
KwaZulu-Natal	Pongola (5)	27°23'36.0"S 31°37'34.1"E	509	Cfa	15.4 / 28.1	678
KwaZulu-Natal	Stanger (6)	29°22'40.8"S 31°17'47.4"E	43	Cfa	16.6 / 26.4	1040
KwaZulu-Natal	Umfolozi (7)	28°19'26.3"S 32°14'17.0"E	74	Cfa	17.8 / 24.7	1212
KwaZulu-Natal	Umzimkulu (8)	30°15'37.1"S 29°55'27.1"E	763	Cfb	13.2 / 27.9	863
Mpumalanga	Malelane (9)	25°28'50.2"S 31°32'38.8"E	345	Cwa	19.0 / 28.5	644
Western Cape	Citrusdal (10)	32°35'34.9"S 19°01'49.0"E	170	Csb	10.8 / 25.2	120
Western Cape	Stellenbosch (11)	33°55'58.3"S 18°52'28.6"E	129	Csb	12.0 / 27.2	802

[a] the numbers between brackets are used to indicate the sampling locations on the map (**Figure 3.2**)

Sources: [b] Google Earth (2015); [c] World Weather Online (2015)

[d] C = warm temperate climate; s = dry summer; w = dry winter; f = fully humid; a = hot summer; b = warm summer (Kottek et al. 2006)

3.3 Results and Discussion

Our surveys extended the known distribution of *Orius naivashae* (Poppius) and *Orius tantillus* (Motchulsky) southwards into South Africa (**Tables 3.3 and 3.4**). *Orius naivashae* was previously only known from Kenya, preying on *Helicoverpa armigera* (Hübner) in cotton (Hernández & Stonedahl, 1999). *Orius tantillus* has been observed in several East African countries, more in particular in

association with *H. armigera* in sunflower and cotton in Kenya and on heads of *Pennisetum typhoides* (Burm.) in Tanzania (Hernández & Stonedahl 1999). *Orius thripoborus* (Hesse) (**Table 3.5**) had been observed before in South Africa, feeding on *H. armigera* eggs in cotton fields (Van Hamburg & Guest 1997), and as a natural enemy of the thrips species *S. aurantii*, *H. haemorrhoidalis* and *S. rubrocinctus* (Hesse 1940; Steyn et al. 1993). Dennil (1989) suggested that *O. thripoborus* might be a useful biological control agent against *H. haemorrhoidalis* and *S. rubrocinctus* in the old Eastern Transvaal Province in South Africa. In Kenya, *O. thripoborus* was found feeding on eggs of *H. armigera* in sunflower and cotton (van den Berg & Cock 1995; van den Berg et al. 1997).

The single record of *Orius brunnescens* (Poppius) confirms its presence in South Africa (**Table 3.2**). According to Carayon (1961), *O. brunnescens* is widely distributed in Africa, where it occurs from sea level to the alpine meadows at altitudes of 3000 m. Nevertheless, it appears to mainly occur in mountainous habitats rather than agricultural ecosystems (Carayon 1961). Likewise, during our survey, *O. brunnescens* was only found in Eston, which is regarded as the midlands of KwaZulu-Natal, and not at the coastal sugarcane sites sampled (**Tables 3.1 and 3.2**).

Table 3.2 Records of O. brunnescens from different locations and host plants in South Africa, from 2008 to 2013. Records are clustered by plant category

Host plant	(Neighbouring) crop system	Location	Date	Numbers[a]		
				Male	Female	Nymph
Grassland weedy forbs						
Senecio madagascariensis Poir.	sugarcane	Eston	July 2008	/	/	/

[a] Numbers of collected adults and nymphs were not registered when marked with '/'

Table 3.3 Records of O. naivashae from different locations and host plants in South Africa, from 2008 to 2013. Records are clustered by plant category

Host plant	(Neighbouring) crop system	Location	Date	Numbers[a]		
				Male	Female	Nymph
Grassland weedy forbs						
Amaranthus hybridus L.	sugarcane	Mt Edgecombe	Aug 2009	0	1	1
Ageratum conyzoides L.	sugarcane	Eston	July 2008	/	/	/
Ageratum conyzoides L.	sugarcane	Mt Edgecombe	Aug 2009	3	14	17

Ageratum conyzoides L.	sugarcane	Mt Edgecombe	Sept 2009	8	38	46
Ageratum conyzoides L.	sugarcane	Stanger	Sept 2009	1	0	1
Ageratum conyzoides L.	sugarcane	Mt Edgecombe	Oct 2013	2	9	4
Ageratum conyzoides L.	sugarcane	Pongola	Oct 2013	0	3	0
Ageratum conyzoides L.	sugarcane	Mt Edgecombe	Dec 2013	0	1	0
Athanasia trifurcata L.	citrus	Citrusdal	Oct 2013	0	1	0
Bidens pilosa L.	sugarcane	Mt Edgecombe	July 2009	1	8	9
Bidens pilosa L.	sugarcane	Umfolozi	July 2009	0	4	4
Bidens pilosa L.	sugarcane	Mt Edgecombe	Aug 2009	3	19	22
Bidens pilosa L.	sugarcane	Mt Edgecombe	Sept 2009	1	2	3
Bidens pilosa L.	sugarcane	Pongola	Oct 2013	0	4	0
Conyza bonariensis	sugarcane	Pongola	Oct 2013	0	1	0
Conyza sp.	sugarcane	Pongola	Oct 2013	0	3	0
Senecio madagascariensis Poir.	sugarcane	Eston	July 2008	/	/	/
Senecio madagascariensis Poir.	sugarcane	Umfolozi	July 2009	0	1	1
Senecio madagascariensis Poir.	sugarcane	Mt Edgecombe	Aug 2009	6	16	22
Senecio madagascariensis Poir.	sugarcane	Mt Edgecombe	Sept 2009	35	82	117
Senecio madagascariensis Poir.	sugarcane	Stanger	Sept 2009	0	1	1
Senecio madagascariensis Poir.	sugarcane	Pongola	Oct 2013	0	3	0
Senecio madagascariensis Poir.	sugarcane	Mt Edgecombe	Oct 2013	1	6	3
Senecio sp.	sugarcane	Pongola	Oct 2013	1	7	0
Tall grasses						
Pennisetum purpureum Schumach.	sugarcane	Stanger	Sept 2009	0	1	1
Saccharum officinarum L.	sugarcane	Ginginhlovu	July 2008	/	/	/
Saccharum officinarum L.	sugarcane	Mt Edgecombe	Aug 2009	0	1	1
Wetland tall grass reeds						
Phragmites australis (Cav.) Trin. Ex Steud.	sugarcane	Mt Edgecombe	Aug 2009	0	1	1
Indigenous trees						
Erythrina lyistemon Hutch.	sugarcane	Umfolozi	July 2009	0	2	2
	Totals collected[b]:			62	229	256

[a] Numbers of collected adults and nymphs were not registered when marked with '/'

[b] only for the years 2009 and 2013

Table 3.4 Records of O. tantillus from different locations and host plants in South Africa, from 2008 to 2013. Records are clustered by plant category

Host plant	(Neighbouring) crop system	Location	Date	Numbers[a]		
				Male	Female	Nymph
Grassland weedy forbs						
Amaranthus spinosa L.	sugarcane	Stanger	Sept 2009	0	1	1
Bidens pilosa L.	sugarcane	Malelane	Aug 2008	/	/	/
Bidens pilosa L.	sugarcane	Stanger	Sept 2009	0	1	1
Conyza sp.	sugarcane	Pongola	Oct 2013	0	1	0
Flaveria bidentis L.	sugarcane	Malelane	July 2009	0	1	1
Senecio madagascariensis Poir.	sugarcane	Eston	July 2008	/	/	/
Tall grasses						
Pennisetum purpureum Schumach.	sugarcane	Pongola	Aug 2008	/	/	/
Pennisetum purpureum Schumach.	sugarcane	Stanger	Aug 2009	13	44	57
Pennisetum purpureum Schumach.	sugarcane	Stanger	Sept 2009	32	24	56
Saccharum officinarum L.	sugarcane	Pongola	Aug 2008	/	/	/
Saccharum officinarum L.	sugarcane	Malelane	Aug 2008	/	/	/
Saccharum officinarum L. (flowering)	sugarcane	Pongola	Oct 2013	1	16	2
Sorghum sudanense Stapf.	sugarcane	Pongola	Sept 2008	/	/	/
Zea mays L.	sugarcane	Pongola	Aug 2008	/	/	/
Zea mays L.	sugarcane	Malelane	July 2009	4	21	25
Zea mays L.	sugarcane	Pongola	Oct 2013	2	13	0
Wetland tall grass reeds						
Cyperus fastigiatus Robbt.	sugarcane	Pongola	Aug 2008	/	/	/
Indigenous trees						
Acacia nigrescens Oliver	sugarcane	Malelane	Aug 2008	/	/	/
Bougainvillea sp.	sugarcane	Mt Edgecombe	Oct 2013	1	0	0
Erythrina lysistemon Hutch.	sugarcane	Malelane	Aug 2008	/	/	/
Melia azedarach L.	sugarcane	Pongola	Aug 2008	/	/	/
Indigenous shrubs						
Ochna atropurpurea (Hochst.) Walp.	sugarcane	Mt Edgecombe	Sept 2008	/	/	/
Totals collected[b]:				53	122	143

[a] Numbers of collected adults and nymphs were not registered when marked with '/'

[b] only for the years 2009 and 2013

Table 3.5 Records of O. thripoborus from different locations and host plants in South Africa, from 2008 to 2013. Records are clustered by plant category

Host plant	(Neighbouring) crop system	Location	Date	Numbers[a]		
				Male	Female	Nymph
Grassland weedy forbs						
Ageratum conyzoides L.	sugarcane	Eston	July 2008	/	/	/
Ageratum conyzoides L.	sugarcane	Mt Edgecombe	Sept 2009	2	1	3
Bidens pilosa L.	sugarcane	Umfolozi	July 2009	2	6	8
Bidens pilosa L.	sugarcane	Mt Edgecombe	Sept 2009	0	1	1
Flaveria bidentis L.	sugarcane	Malelane	July 2009	1	1	2
Indigofera sp.	vineyard	Stellenbosch	Nov 2013	1	0	2
Lantana camara L.	sugarcane	Pongola	Aug 2008	/	/	/
Parthenium hysterophorus L.	sugarcane	Mt Edgecombe	Oct 2013	0	1	0
Senecio madagascariensis Poir.	sugarcane	Eston	July 2008	/	/	/
Senecio madagascariensis Poir.	sugarcane	Mt Edgecombe	Aug 2009	0	1	1
Senecio madagascariensis Poir.	sugarcane	Mt Edgecombe	Sept 2009	0	2	2
Tagetes minuta L.	sugarcane	Eston	July 2008	/	/	/
Tall grasses						
Saccharum officinarum L.	sugarcane	Pongola	Aug 2008	/	/	/
Saccharum officinarum L.	sugarcane	Mt Edgecombe	July 2009	0	2	2
Saccharum officinarum L.	sugarcane	Malelane	July 2009	0	3	3
Saccharum officinarum L. (young spindle with *F.serrata*)	sugarcane	Mt Edgecombe	Nov 2013	1	2	0
Sorghum sudanense Stapf.	sugarcane	Pongola	Sept 2008	/	/	/
Zea mays L.	sugarcane	Malelane	Aug 2008	/	/	/
Zea mays L.	sugarcane	Pongola	Sept 2008	/	/	/
Zea mays L.	sugarcane	Malelane	July 2009	5	8	13
Indigenous trees						
Acacia robusta Burch.	sugarcane	Malelane	Aug 2008	/	/	/
Albizia adianthifolia (Schumach.) W.F. Wight	sugarcane	Umzimkulu	Sept 2008	/	/	/
Citrus limon L.	sugarcane	Pongola	Sept 2008	/	/	/
Erythrina lysistemon Hutch.	sugarcane	Umfolozi	July 2009	1	2	3
Erythrina lysistemon Hutch.	sugarcane	Durban	Sept 2009	1	2	3

Eucalyptus cladocalyx F. Muell.	sugarcane	Mt Edgecombe	Oct 2013	7	4	0
Jacaranda acutifolia auct. non-Humb. & Bonpl.	sugarcane	Mt Edgecombe	Oct 2013	0	4	0
Mangifera indica L.	sugarcane	Umzimkulu	Sept 2008	/	/	/
Maytenus oleoides Loes.	fynbos	Stellenbosch	Nov 2013	2	2	2
Melia azedarach L.	sugarcane	Pongola	Sept 2008	/	/	/
Persea americana Mill.	sugarcane	Umzimkulu	Sept 2008	/	/	/
Indigenous shrubs						
Boscia senegalensis (Pers.) Lam. *ex* Poir.	sugarcane	Malelane	Aug 2008	/	/	/
Brunia sp.	fynbos	Stellenbosch	Nov 2013	1	3	0
Erica glandulosa Thunb.	fynbos	Stellenbosch	Nov 2013	4	11	0
Ochna atropurpurea (Hochst.) Walp.	sugarcane	Mt Edgecombe	Sept 2008	/	/	/
Rubus cuneifolius Pursh	sugarcane	Malelane	Aug 2008	/	/	/
	Totals collected[b]:			28	56	45

[a] Numbers of collected adults and nymphs were not registered when marked with '/'

[b] only for the years 2009 and 2013

Both abiotic (e.g., climate) and biotic factors (e.g., presence and abundance of prey and flowering host plants) determine the seasonal occurrence of anthocorid predators in South Africa. As surveys in this study were mainly performed during winter and spring over three scattered years, caution is needed when trying to describe the habitat and climate preferences of the collected *Orius* species. Despite the fact that most collections were done in and around sugarcane fields, the surveys indicated that the recorded *Orius* species showed some degree of preference for certain plant categories (**Tables 3.2 to 3.5**). Nearly all of the predatory bugs were collected from the pollen producing parts of the plants. High numbers of *O. naivashae* were collected from pollen producing grassland weedy forbs, and occasionally from (wetland) tall grasses (Poaceae), including sugarcane (**Table 3.3**). *Orius thripoborus* was more prevalent in taller vegetation (indigenous shrubs and trees, and tall grasses), most of which were cash crops such as sugarcane, maize, lemon (*Citrus limon* (L.) Burm. f.), mango (*Mangifera indica* L.) and avocado (*Persea americana* Mill.). It was the only anthocorid observed in the pollen-free spindle of young sugarcane, with *F. serrata*. *Orius thripoborus* was more rarely observed on pollen producing

weeds (**Table 3.5**). *Orius tantillus* was prevalent on tall grasses, including maize and sugarcane. This species was only occasionally found on weeds, wetland tall grass reed, and indigenous shrubs and trees (**Table 3.4**).

The abundance of *O. naivashae* was highest in regions with hot summers and humid conditions throughout the year (e.g., Mount Edgecombe). A single record of *O. naivashae* was made in Citrusdal, a location characterised by warm and dry summers, though it was in an irrigated crop (citrus). *Orius thripoborus* was found at varying conditions within the widespread warm temperate climate zone. Latter species was located in both the Western Cape (e.g., Stellenbosch, with warm and dry summers), Mpumalanga (e.g., Malelane, with dry winters and hot summers) and KwaZulu-Natal (e.g., Mount Edgecombe, humid all year round, with hot summers; or Umzimkulu, humid all year round, with warm summers). Finally, *O. tantillus*, a species known from more tropical regions (Nakashima & Hirose 1997a, b), was mainly collected in regions where annual temperatures are overall higher, driven by hot summers, combined with high year-round humidity (e.g., Pongola), or with dry winters (e.g., Malelane). Given the single record of *O. brunnescens*, there is insufficient information to hypothesise about its host plant and climate preferences.

More female than male *Orius* individuals were observed in 2009 and 2013 (the numbers of collected males and females were not recorded in the survey year 2008). For *O. thripoborus*, *O. tantillus* and *O. naivashae*, 2, 2.3 and 3.7 times more females than males were recorded in the field, respectively (**Tables 3.3 to 3.5**). In most species, dispersal rates vary between sexes and male and female offspring may disperse from one habitat to another with different probabilities (Julliard 2000). Especially on sunny days, *Orius* females engage less in flight activity than males do, and invest more time in on-plant foraging and oviposition activities (Tuda & Shima 2002). As most of the field surveys took place when weather conditions were favourable, the probability to encounter female *Orius* bugs was therefore greater compared to males. The difference in observed sex ratio between *O. naivashae* on the one hand, and *O. thripoborus* and *O. tantillus* on the other, is likely due to a naturally occurring female bias in the sampled *O. naivashae* populations. Sex-determining mechanisms in invertebrates are of genetic and/or environmental origin, but also cytoplasmic factors, like the secondary endosymbionts *Wolbachia* and *Spiroplasma*, may be involved (Stouthamer et al. 1999; Cook 2002). Molecular studies

have shown that the bacterial endosymbiont *Wolbachia* was present in our *O. naivashae* population (J. Bonte, unpublished data), but the sex-determining mechanism of the endosymbiont in this anthocorid needs further investigation (**see Chapter 9**). The only other report of a skewed sex ratio in an *Orius* sp. is that by Shapiro et al. (2009), where a skewed field sex ratio was found for *O. insidiosus* in the favour of males; however, the authors stated that this was most likely the result of sampling error or differential hatch rate or survival of one sex.

Populations of *Orius* spp. may be supported by pollen-producing wild or cultivated plants in the vicinity of the crop. Habitats with different plant communities and phenologies attract alternative prey and can, whether or not in combination with pollen, support populations of omnivorous predators when target prey becomes scarce in a given crop system (Coll 1998; Lundgren 2009). This aspect can be used in the management of *Orius* spp. populations in an integrated pest management system against a pest such as *F. serrata*. The temporal resources provided by the pollen-producing plants can be appropriately synchronized with the predator and pest population buildup in nearby crops. Therefore, regular cutting of weeds for example, may force predators to move into crop fields (Coll 1998). Furthermore, the impact of *Orius* spp. may be broadened to other sugarcane pests, as many of the tall grasses (e.g., sugarcane, *Pennisetum purpureum* Schumach., *Z. mays* and *Sorghum* spp.) and wetland sedges (e.g., *Cyperus* spp.) on which *Orius* spp. were observed, are known hosts for *Eldana saccharina* Walker (Lepidoptera: Pyralidae). This stalk borer has been the most serious pest in sugarcane since 1970 (Conlong 2001; Keeping et al. 2007). It has a very cryptic life cycle, hiding its eggs behind dry leaf sheaths, which small predators such as *Orius* spp. can access. The neonate larvae move op the stalks of sugarcane once hatched, to 'parachute' off the green leaves to surrounding plants which they then infest (Conlong et al. 2007). During this dispersal phase they will be prone to predation by *Orius* spp. Augmentation of the relevant *Orius* spp. when eggs and neonate larvae are abundant in sugarcane may thus contribute to Integrated Pest Management of *E. saccharina.* Further, *Orius* spp. may also hold promise for the suppression of the yellow sugarcane aphid, *Sipha flava* (Forbes) (Homoptera: Aphididae), a recently discovered pest in sugarcane in South Africa (Conlong & Way 2014).

Chapter 4

Thermal biology of the predatory bugs *Orius thripoborus* and *O. naivashae*

Redrafter after:

Bonte, J., De Ro, M., Conlong, D. and De Clercq, P. 2012. Thermal biology of the predatory bugs *Orius thripoborus* and *O. naivashae* (Hemiptera: Anthocoridae). *Environmental Entomology* 41: 989-996.

4.1 Introduction

Insects are poikilothermic animals with limited ability to regulate their body temperature. For this reason, temperature can exert a major influence on such organisms: at a macrophysiological scale, determining distributions and abundance, and at a more localised level, affecting 'rate-based' processes such as development, reproduction, activity, predation and survival (Andrewartha & Birch 1954; Jervis & Copland 1996; Cocuzza et al. 1997a; Obrycki & Kring 1998; Schwartz 2003; Bale et al. 2008).

The efficiency of biocontrol systems is largely defined by the knowledge about temperature responses of an arthropod natural enemy. This knowledge is important for rearing the natural enemy, as well as for assessing its field performance. The present study was undertaken to determine the effects of temperature on the development and reproduction of *O. thripoborus* and *O. naivashae*. The relationship between temperature and development of both predators was expressed as developmental thresholds and degree-day accumulations. To achieve this, the widely applied and user-friendly linear model was used (Kontodimas et al. 2004).

4.2 Materials and Methods

4.2.1 Stock culture

Cultures of *O. thripoborus* and *O. naivashae* were started in 2008 and 2009, respectively, with nymphs and adults collected in and around sugarcane (*Saccharum officinarum* L.) fields in the South African provinces Mpumalanga and KwaZulu-Natal (**see Chapter 3**). Stock colonies of both anthocorids were established at Ghent University and maintained in climatic cabinets at 25 ± 1°C, 65 ± 5% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. The predators were cultured in cylindrical Plexiglas containers (9 cm diameter, 4 cm high) containing a sharp pepper plant (*Capsicum annuum* L. 'Cayenne Long Slim') as a water source and oviposition substrate (**Figure 4.1**). The food of nymphs and adults consisted of a mixture of frozen *Ephestia kuehniella* Zeller eggs (Koppert B.V., Berkel en Rodenrijs, The Netherlands); adults were also given dry honey bee pollen (N.V. Weyn's Honingbedrijf, Ghent, Belgium). Maintenance of the colony was being done every Monday, Wednesday and Friday. On these days, adults were transferred to a fresh container, supplied with *E. kuehniella* eggs and pollen, and allowed to oviposit for 2 or 3 days on the sharp pepper seedlings. On the next day of maintenance, the

surviving adults were moved to a new container and their number was complemented to 80 adults, using 4- to 5-day-old adults originating from the nymphal cultures. The remaining food in the old container was removed and thus it eventually only contained a sharp pepper plant with a high number of oviposited eggs which were allowed to develop and hatch during the following days. Upon egg hatch, *E. kuehniella* eggs, adhered to 1 cm² pieces of household paper, were added to the rearing unit as food for the predator nymphs; prey eggs were replenished on every maintenance day. The predators remained in the same container for their entire nymphal life, until they had reached the early adult stage. In this way the continuous culture of *O. thripoborus* and *O. naivashae* was assured. To reduce cannibalism, a wrinkled piece of wax paper was placed in each container (Bonte & De Clercq 2011) (**Figure 4.1**).

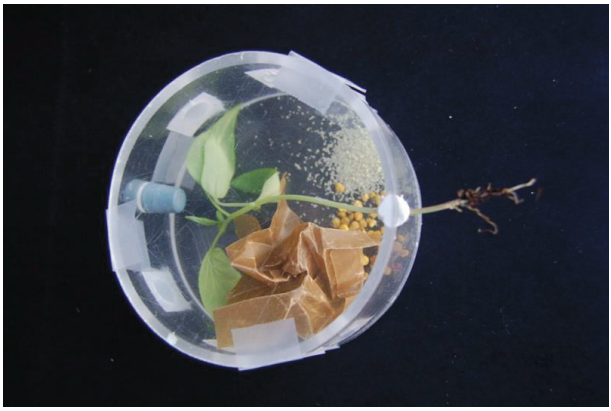

Figure 4.1 Orius sp. rearing unit, provided with a sharp pepper plant and a wrinkled piece of wax paper (photo: author)

4.2.2 Experiments

All experiments were done at Ghent University in climatic cabinets set at different constant temperatures, 65 ± 5% RH, and a 16 h photophase. In all treatments, both nymphs and adults were offered a diet of frozen eggs of *E. kuehniella* and a flat green bean pod (*Phaseolus vulgaris* L.) was provided as a source of water and extra nutrients, hiding place, and oviposition substrate. Depending on temperature, *E. kuehniella* eggs and bean pods were refreshed daily (29 to 35°C), or every other day (12 to 25°C).

4.2.2.1 Egg and nymphal development

Egg and nymphal development were determined at eight constant temperatures: 12, 15, 19, 23, 25, 29, 33 and 35°C. Eggs (< 24 h old) were collected from the stock colony (25°C) and transferred to an incubator set at the desired temperature. Incubation time of the eggs deposited in the pepper plants was monitored on a daily basis. For each treatment, 50 to 130 first instars (< 24 h old) were then caged in individual plastic containers (4.5 cm diameter, 3 cm high) sealed with a lid having a ventilation hole covered with a fine-mesh gauze. Development and survival of nymphs were monitored daily, and newly emerged adults were sexed and weighed using a Sartorius Genius ME 215P balance (Sartorius, Goettingen, Germany).

4.2.2.2 Reproduction

Adult reproduction was studied at 15, 19, 25 and 33°C. For this purpose, newly emerged adults (< 24 h old) were paired and transferred to similar plastic containers as those used for development experiments. Adults were exposed to the same temperature as during their nymphal life. Bean pods were checked daily for eggs to determine pre-oviposition period. After the first egg was laid, bean pods were replaced daily (25 and 33°C), or every other day (15 and 19°C). Lifetime oviposition and egg hatch were also monitored. As developmental experiments for *O. naivashae* did not yield sufficient male adults due to skewed sex ratios, females in different temperature treatments were paired with one to two day old males from the stock colony where needed, in order to obtain at least 10 replicates (couples) per treatment. Longevities of paired males and females were also examined.

4.2.3 Data analysis

4.2.3.1 Development and reproduction

For both species, means were compared by using pairwise comparison procedures (Kutner et al. 2005). In case the means were normally distributed (according to a Kolmogorov-Smirnov test), they were analysed using a one-way analysis of variance (ANOVA). When a Levene test indicated that their variances were homoscedastic, means were separated using a Tukey test; in case of heteroscedasticity, a Tamhane test was applied. A Kruskal-Wallis H test was applied to analyse means which were not normally distributed. In the latter case, means were pairwise separated with a Mann-Whitney U test. Parameters expressed as percentages, i.e. nymphal survival, egg hatch and proportion of ovipositing

females, were compared by means of a logistic regression. This regression is a generalised linear model using a probit (log odds) link and a binomial error function (McCullagh & Nelder 1989). Sex ratios were evaluated versus an equal male:female distribution (1:1 ratio) by means of a non-parametric Chi-Square test (IBM SPSS Statistics 19, IBM 2011).

4.2.3.2 Day degree model

The relationship between temperature and development rate (1/development time) of egg, total nymphal stage, and egg to adult stage of *O. thripoborus* and *O. naivashae* was described by a linear regression model (Arnold 1959). This model is expressed by the equation "$Y = a + bX$", where Y is the development rate, X is the rearing temperature, and a and b are parameters. The lower temperature thresholds of insect development were determined as the x-intercept ($t_0 = -a/b$) (Arnold 1959) and the thermal requirements (in degree-days or DD) were determined as the inverse of the slope ($K = 1/b$) of the regression lines (Campbell et al. 1974). Data points at extreme temperatures which deviated from the straight line through the other points were rejected for correct estimation of regression parameters (Campbell et al. 1974; De Clercq & Degheele 1992).

4.3 Results

4.3.1 Development

Developmental parameters of *O. thripoborus* and *O. naivashae* at seven constant temperatures are shown in **Tables 4.1 and 4.2**, respectively. As eggs of *O. thripoborus* and *O. naivashae* were unable to hatch at 12°C, the lowest temperature allowing full development of both species was 15°C.

For both anthocorids, nymphal survival was highest at 23°C, averaging 98.0% for *O. thripoborus* and 88.9% for *O. naivashae*, and lowest at the extreme temperatures tested (*O. thripoborus*: $\chi^2 = 60.96$; df = 5; $P < 0.001$; *O. naivashae*: $\chi^2 = 84.78$; df = 6; $P < 0.001$) (**Tables 4.1 and 4.2**). Survival of *O. thripoborus* nymphs at 19, 25 and 29°C was similar, whereas for *O. naivashae*, nymphal survival at 19°C did not differ significantly from that at temperatures between 25 and 35°C. Survival rates were below 50% for *O. thripoborus* nymphs reared at 33 and 35°C, and for nymphs of *O. naivashae* maintained at 15°C. At 35°C, no nymphs of *O. thripoborus* reached adulthood.

Table 4.1 Developmental parameters (mean ± SEM) of O. thripoborus at seven temperatures (65 ± 5% RH, 16:8 (L:D) h; E. kuehniella eggs + bean pod)

Temperature[a] (°C)	Nymphal survival[b] (%)	Developmental time (d)					Adult weight (mg)		Sex ratio[c] (male:female)
		Egg	Nymph		Total		Males	Females	
			Males	Females	Males	Females			
15	67.2 ± 6.1c (61)	12.05 ± 0.13e	33.8 ± 0.3f	32.6 ± 0.6f	45.9 ± 0.3f	44.7 ± 0.6f	0.352 ± 0.011a	0.430 ± 0.014a	1:1.28
19	84.1 ± 3.2b (132)	8.26 ± 0.08d	26.2 ± 0.2e	25.5 ± 0.2e	34.4 ± 0.2e	33.8 ± 0.2e	0.342 ± 0.005a	0.411 ± 0.006a	1:1.02
23	98.0 ± 2.0a (51)	5.94 ± 0.06c	15.2 ± 0.09d	14.8 ± 0.09d	21.1 ± 0.1d	20.7 ± 0.1d	0.287 ± 0.005b	0.375 ± 0.005b	1:0.92
25	84.5 ± 4.8b (58)	4.50 ± 0.08b	13.0 ± 0.4c	12.6 ± 0.3c	17.4 ± 0.5c	17.0 ± 0.4c	0.288 ± 0.011b	0.374 ± 0.010b	1:0.88
29	87.6 ± 3.7b (81)	3.46 ± 0.07a	10.2 ± 0.2a	9.9 ± 0.1a	13.6 ± 0.2a	13.3 ± 0.1a	0.272 ± 0.004b	0.333 ± 0.004c	1:0.97
33	44.7 ± 5.7d (76)	3.26 ± 0.06a	11.4 ± 0.6b	11.5 ± 0.4b	14.8 ± 0.2b	14.8 ± 0.4b	0.215 ± 0.009c	0.235 ± 0.012d	1:0.62
35	0.0 ± 0.0e (22)	4.32 ± 0.12b	/	/	/	/	/	/	/

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tamhane (adult weights), Mann-Whitney U (all developmental times) or probit test (nymphal survival))

[a] Eggs transferred from the stock colony to 12°C did not hatch; when placed at 35°C, eggs hatched, but nymphs were not able to develop and died during the first three instars

[b] The initial number of first instars tested is placed in parentheses

[c] Sex ratios did not differ significantly from a 1:1 ratio; with respective *P*-values of 0.435; 0.924; 0.777; 0.668; 0.906 and 0.170 (Chi-square test)

Table 4.2 Developmental parameters (mean ± SEM) of O. naivashae at seven constant temperatures (65 ± 5% RH, 16:8 (L:D) h; E. kuehniella eggs + bean pod)

Temperature[a] (°C)	Nymphal survival[b] (%)	Developmental time (d)					Adult weight (mg)		Sex ratio[c] (male:female)
		Egg	Nymph		Total		Males	Females	
			Males	Females	Males	Females			
15	26.8 ± 4.5c (97)	13.87 ± 0.11f	44.0 ± 1.9f	45.0 ± 0.7g	58.8 ± 1.7f	58.4 ± 0.8g	0.314 ± 0.025ab	0.380 ± 0.010ab	1:3.67*
19	70.6 ± 5.6b (68)	9.66 ± 0.15e	28.6 ± 0.3e	28.7 ± 0.4f	37.6 ± 0.4e	38.4 ± 0.4f	0.317 ± 0.013ac	0.379 ± 0.006b	1:2.62*
23	88.9 ± 4.0a (63)	6.06 ± 0.05d	15.9 ± 0.2d	14.9 ± 0.1e	22.0 ± 0.3d	20.9 ± 0.1e	0.296 ± 0.005bc	0.407 ± 0.005a	1:1.24
25	78.7 ± 4.8ab (75)	4.71 ± 0.06c	11.8 ± 0.5c	12.1 ± 0.1d	16.2 ± 0.7c	16.8 ± 0.1d	0.356 ± 0.024a	0.384 ± 0.008ab	1:10.8*
29	75.8 ± 5.5ab (62)	3.53 ± 0.08b	9.0 ± 0.0b	9.1 ± 0.07b	12.4 ± 0.4b	12.6 ± 0.1b	0.284 ± 0.018ab	0.380 ± 0.007b	1:8.4*
33	76.7 ± 5.0ab (73)	3.04 ± 0.06a	8.2 ± 0.2a	8.4 ± 0.1a	11.1 ± 0.2a	11.5 ± 0.2a	0.268 ± 0.013b	0.334 ± 0.006c	1:3.23*
35	66.4 ± 6.3b (59)	3.52 ± 0.07b	9.6 ± 0.3b	10.2 ± 0.2c	13.1 ± 0.3b	13.7 ± 0.2c	0.184 ± 0.011d	0.238 ± 0.010d	1:3.11*

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey (male adults weight), Tamhane (female adult weight), Mann-Whitney U (all developmental times) or probit test (nymphal survival))

[a] Eggs transferred from the stock colony to 12°C did not hatch

[b] The initial number of first instars tested is placed in parentheses

[c] Values with an asterisk differ significantly from a 1:1 ratio; *P*-values were 0.002; 0.002; 0.423; <0.001; <0.001; <0.001 and 0.002, respectively (Chi-square test)

Developmental times of males and females of both *Orius* species varied with temperature for eggs (*O. thripoborus*: χ^2 = 440.98; df = 6; P < 0.001; *O. naivashae*: χ^2 = 461.16; df = 6; P < 0.001), nymphs (*O. thripoborus* males: χ^2 = 167.78; df = 5; P < 0.001; *O. thripoborus* females: χ^2 = 161.11; df = 5; P < 0.001; *O. naivashae* males: χ^2 = 69.50; df = 6; P < 0.001; *O. naivashae* females: χ^2 = 233.88; df = 6; P < 0.001) and for total development (*O. thripoborus* males: χ^2 = 169.01; df = 5; P < 0.001; *O. thripoborus* females: χ^2 = 162.94; df = 5; P < 0.001; *O. naivashae* males: χ^2 = 70.02; df = 6; P < 0.001; *O. naivashae* females: χ^2 = 235.28; df = 6; P < 0.001) (**Tables 4.1 and 4.2**). Total developmental time of males and females decreased with increasing temperature from 45.9 and 44.7 days (15°C) to 13.6 and 13.3 days (29°C) for *O. thripoborus*, and from 58.8 and 58.4 days (15°C) to 11.1 and 11.5 days (33°C) for *O. naivashae*. As temperatures rose above latter maximum values, there was a significant decline of development rate for both *Orius* species. Only *O. thripoborus* eggs at 33°C developed as fast as those at 29°C (**Table 4.1**).

Linear regression analysis indicated a significant negative slope for the relationship between adult weight of *O. thripoborus* and temperature (**Figure 4.2**). Heaviest males and females emerged at 15 and 19°C, and lightest at 33°C (males: F = 50.49; df = 5, 168; P < 0.001; females: F = 43.66; df = 5, 176; P < 0.001) (**Table 4.1**). For *O. naivashae*, there was no linear relationship between adult weight and temperature (**Figure 4.2**), but at 35°C, both males and females had lower body weights than at the other temperatures (males: F = 13.93; df = 6, 67; P < 0.001; females: F = 42.08; df = 6, 241; P < 0.001) (**Table 4.2**). It deserves emphasis that in particular for *O. naivashae* adults, non-linear models would provide better fit to the data than the linear models used here.

Sex ratios of *O. naivashae* were all female biased, except at 23°C, at which the proportions of males and females were similar. Male:female ratios of *O. naivashae* stuck out at 25°C (1:10.8) and 29°C (1:8.4) (**Table 4.2**). For *O. thripoborus*, no significant deviations from a 1:1 sex ratio were observed at any temperature (**Table 4.1**).

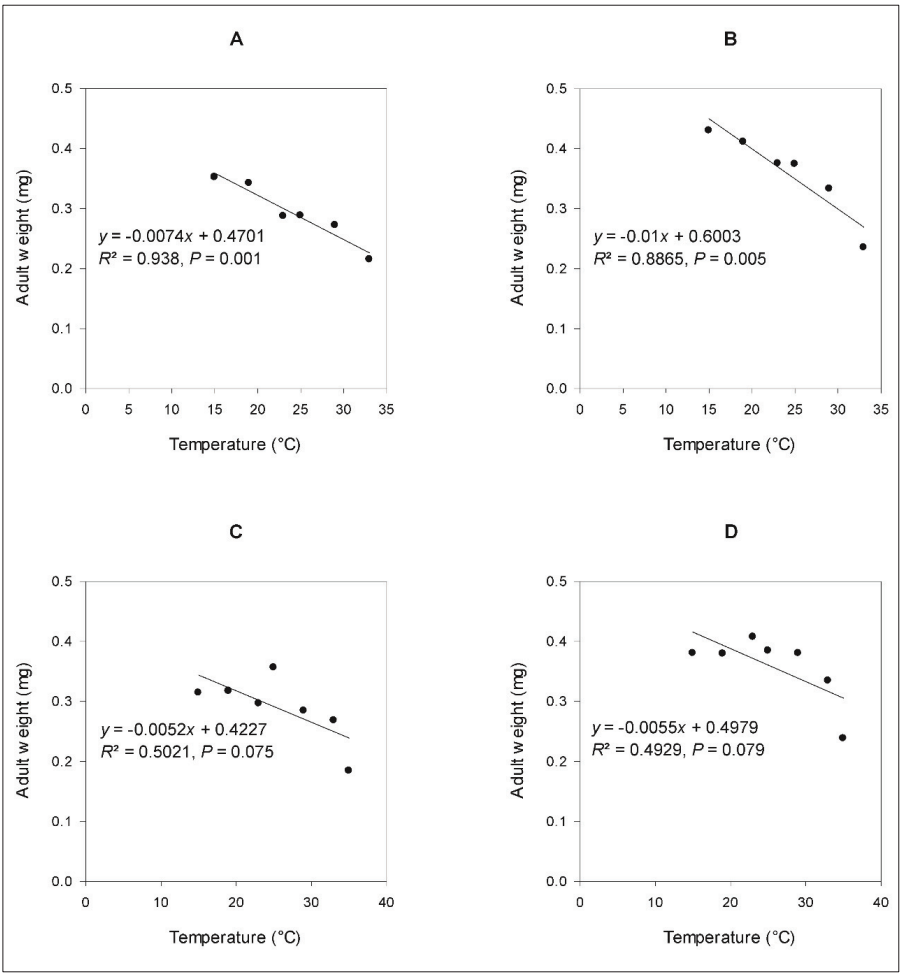

Figure 4.2 Adult weights of males and females of O. thripoborus and O. naivashae plotted against temperature. Curves were fitted to the data using linear regression analysis. (A) Orius thripoborus males. (B) Orius thripoborus females. (C) Orius naivashae males. (D) Orius naivashae females.

4.3.2 Day-degree model

Table 4.3 presents the lower development thresholds, degree-day requirements and linear regression equations for the immature stages of *O. thripoborus* and *O. naivashae*. The relationship between temperature and development rate for the total (egg to adult) development of *O. thripoborus* and *O. naivashae* is shown in **Figure 4.3**. Development rates at 33 and 35°C for *O. thripoborus* nymphs and at 35°C for eggs and nymphs of *O. naivashae* fell outside the linear part of the curve, and hence were not

included in the regression. Coefficients of determination (R^2) for each regression exceeded 97% (with all $P > 0.05$), indicating a good linear model fit in all cases. The total development of *O. thripoborus* required 258 DD with a lower threshold of 10.2°C, whereas for *O. naivashae*, the values of these parameters were 236 DD and 11.6°C, respectively. Mean DD estimates for egg, nymph and total development did not differ among species (Student's t-tests: egg: $t = 2.13$; df = 10; $P = 0.059$; nymph: $t = 1.16$; df = 9; $P = 0.135$; total: $t = 1.25$; df = 9; $P = 0.243$). All t_0-values of *O. naivashae* were consistently higher than those of *O. thripoborus*.

Table 4.3 Lower development thresholds (t_0), degree-day requirements (K, mean ± SEM) and linear regression equations with corresponding coefficients of determination (R^2) and P-values for development of the immature stages of O. thripoborus and O. naivashae at different constant temperatures

Species	Stage	t_0 (°C)	K (DD)	Regression equation	R^2	*P*
O. thripoborus	Egg	9.40	73.8 ± 2.4	$Y = 0.0136X - 0.1278$	0.970	<0.001
	Total nymphal	10.2	191.1 ± 10.8	$Y = 0.0052X - 0.0531$	0.975	0.002
	Egg to adult	10.2	258.4 ± 13.4	$Y = 0.0039X - 0.0398$	0.977	0.001
O. naivashae	Egg	11.3	65.2 ± 3.2	$Y = 0.0152X - 0.1711$	0.984	<0.001
	Total nymphal	11.8	168.2 ± 9.0	$Y = 0.0059X - 0.0699$	0.974	<0.001
	Egg to adult	11.6	236.3 ± 11.6	$Y = 0.0043X - 0.0497$	0.978	<0.001

4.3.3 Reproduction and longevity

Tables 4.4 and 4.5 show the reproductive parameters and longevities of *O. thripoborus* and *O. naivashae*, respectively, at four constant temperatures.

At 33°C, *O. thripoborus* females were not able to produce eggs (**Table 4.4**), while 68.8% of *O. naivashae* females oviposited at this temperature (**Table 4.5**). Between 15 and 25°C, proportions of ovipositing *O. thripoborus* females ranged between 50.0% (15°C) and 79.0% (25°C), though they did not differ among temperatures ($\chi^2 = 3.22$; df = 2; $P = 0.199$). At 25°C, a significantly higher proportion of *O. naivashae* females (92.9%) produced eggs as compared with 15 and 19°C (30.0 and 52.4%, respectively) ($\chi^2 = 10.26$; df = 3, 42; $P = 0.016$).

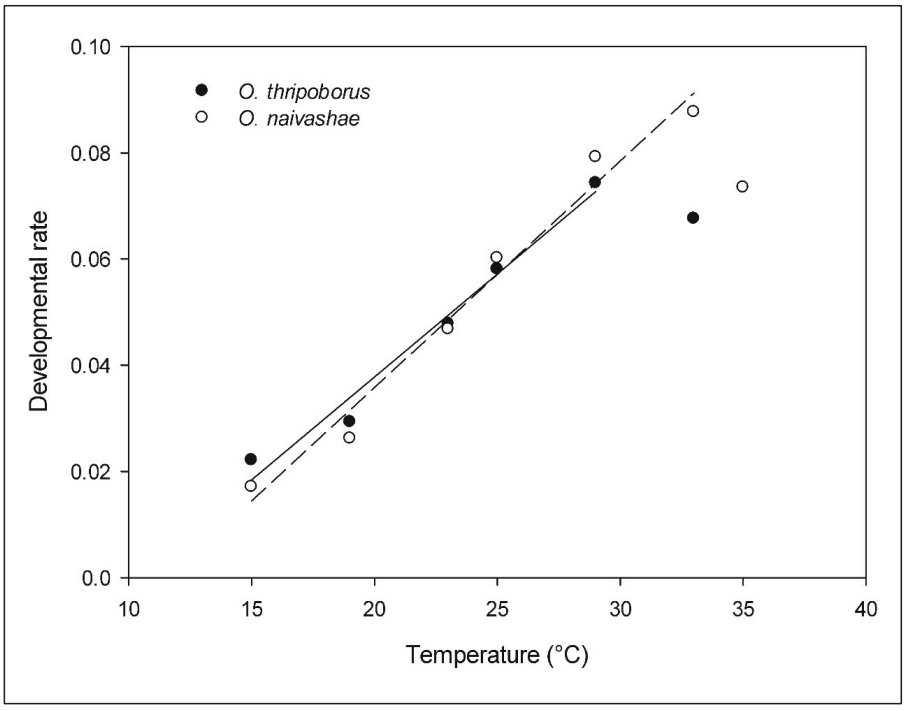

Figure 4.3 Relationship between temperature and developmental rate for total (egg to adult) development of O. thripoborus and O. naivashae at constant temperatures. Solid and broken lines represent linear regressions of all data from 15 to 29°C for O. thripoborus, and from 15 to 33°C for O. naivashae, respectively.

For *O. thripoborus*, the preoviposition period significantly decreased with increasing temperature (F = 9.76; df = 2, 42; P < 0.001), ranging from 22.8 days at 15°C to 9.3 days at 25°C (**Table 4.4**). Preoviposition periods of *O. naivashae* females varied between 5.5 days (33°C) and 32.3 days (15°C), but they only showed significant differences between 19°C and the highest two temperatures (F = 33.04 df = 3, 34; P < 0.001) (**Table 4.5**).

The total number of eggs produced by *O. thripoborus* females was higher at 25°C (100.7 eggs) than at 15°C (25.8 eggs), but was similar to their fecundity at 19°C (46.8 eggs) (F = 5.95; df = 2, 67; P = 0.004) (**Table 4.4**). Fecundities of *O. naivashae* at 19 and 33°C (15.9 and 13.5 eggs, respectively) were similar and both significantly lower than that at 25°C (68.1 eggs) (F = 12.47; df = 3, 57; P < 0.001). At 15°C the fecundity of the latter species was substantially lower (0.9 eggs) than that at all higher temperatures (**Table 4.5**).

Table 4.4 Reproductive parameters and longevities (mean ± SEM) of O. thripoborus at four constant temperatures (65 ± 5% RH, 16:8 (L:D) h; E. kuehniella eggs + bean pod)

Temperature[a] (°C)	Proportion of ovipositing females[b] (%)	Preoviposition period (d)	Oviposition period (d)	Total fecundity	Egg hatch (%)	Longevity (d)	
						Males	Females
15	50.0 ± 12.9a (16)	22.8 ± 2.7c	41.2 ± 11.1a	25.8 ± 10.1b	40.0 ± 2.4c	68.5 ± 9.1a	91.1 ± 7.5a
19	62.9 ± 8.3a (35)	15.6 ± 1.7b	33.0 ± 4.0a	46.8 ± 10.4ab	60.9 ± 1.2b	68.9 ± 3.8a	61.5 ± 3.4b
25	79.0 ± 9.6a (19)	9.3 ± 1.4a	25.0 ± 3.4a	100.7 ± 21.6a	65.8 ± 1.1a	24.3 ± 2.6b	42.8 ± 3.9c
33	/	/	/	/	/	8.2 ± 1.6c	14.0 ± 2.1d

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey (preoviposition period), Tamhane (oviposition period, total fecundity and longevity) or probit test (ovipositing females and egg hatch))

[a] At 33°C, no females produced eggs

[b] The number of adult pairs tested at each temperature is placed in parentheses

Table 4.5 Reproductive parameters and longevities (mean ± SEM) of O. naivashae at four constant temperatures (65 ± 5% RH, 16:8 (L:D) h; E. kuehniella eggs + bean pod)

Temperature (°C)	Proportion of ovipositing Females[a] (%)	Preoviposition period (d)	Oviposition period (d)	Total fecundity	Egg hatch (%)	Longevity (d)	
						Males	Females
15	30.0 ± 15.3b (10)	32.3 ± 6.2ab	6.7 ± 2.8b	0.9 ± 0.6c	0.0 ± 0.0c	41.2 ± 12.1ab	70.3 ± 9.0a
19	52.4 ± 11.2b (21)	16.2 ± 1.6b	38.1 ± 4.1a	15.9 ± 4.9b	41.4 ± 2.7b	51.1 ± 6.9a	71.6 ± 5.8a
25	92.9 ± 7.1a (14)	7.8 ± 1.0a	23.7 ± 3.4a	68.1 ± 15.2a	70.4 ± 1.5a	33.6 ± 4.9a	37.2 ± 4.9b
33	68.8 ± 12.0ab (16)	5.5 ± 0.7a	6.0 ± 0.9b	13.5 ± 3.0b	39.8 ± 3.3b	11.5 ± 2.0b	12.5 ± 1.3c

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Tamhane (preoviposition and oviposition period, total fecundity and longevity) or probit test (ovipositing females and egg hatch))

[a] The number of adult pairs tested at each temperature is placed in parentheses

Egg hatch was highest at 25°C for both *O. thripoborus* and *O. naivashae* (65.8 and 70.4%, respectively), and decreased with temperature (*O. thripoborus*: χ^2 = 124.27; df = 3; P < 0.001; *O. naivashae*: χ^2 = 90.96; df = 2; P < 0.001) (**Tables 4.4 and 4.5**). At 15°C, *O. naivashae* eggs did not hatch, while at 33°C, their hatchability was similar to that at 19°C.

Longevities of paired females ranged from 91.1 (15°C) to 14.0 days (33°C) for *O. thripoborus* (F = 31.94; df = 3, 76; P < 0.001) and from 70.3 (15°C) to 12.5 days (33°C) for *O. naivashae* (F = 28.38; df = 3, 55; P < 0.001). For paired males, life-spans were between 68.9 (19°C) and 8.2 (33°C) days for *O. thripoborus* (F = 30.07; df = 3, 70; P < 0.001) and between 71.6 (19°C) and 12.5 days (33°C) for *O. naivashae* (F = 6.85; df = 3, 57; P = 0.001) (**Tables 4.4 and 4.5**). Longevities decreased with increasing temperature, except for *O. naivashae* males. At 15 and 19°C, life-spans were similar, except for *O. thripoborus* males, which showed a very high longevity at 15°C.

Oviposition periods of *O. thripoborus* ranged from 25.0 days at 25°C to 41.2 days at 15°C, though differences were not significant (F = 1.83; df = 2, 42; P = 0.173). For *O. naivashae*, longest oviposition periods were found at both 19 and 25°C (38.1 and 23.7 days, respectively), and shortest periods were observed at the extreme temperatures tested (F = 19.33; df = 3, 34; P < 0.001).

4.4 Discussion

No previous studies have addressed the thermal biology of *O. thripoborus* and *O. naivashae*. Developmental data of the present study suggest that *O. thripoborus* is adapted to a cooler temperature range as compared with *O. naivashae*. The duration of egg and nymphal development of *O. thripoborus* and *O. naivashae* decreased significantly with an increase in temperature between 15 and 29°C. At 15°C, *O. naivashae* showed a poor nymphal survival and a pronounced prolongation of nymphal development as compared to that at medium temperatures. On the other hand, *O. thripoborus* developed well at 15°C, but suffered greater nymphal mortality at the high end of the tested temperature range (≥ 33°C). Furthermore, *O. thripoborus* and *O. naivashae* developed fastest at 29 and 33°C, respectively, with a deceleration of egg to adult development as temperature rose further. Our findings suggest that the upper threshold temperature for development (i.e. the temperature above which the rate of development starts decreasing (De Clercq & Degheele 1992)) of the egg and nymphal stages of *O. naivashae* was between 33 and 35°C, whereas for *O. thripoborus*,

this was between 33 and 35°C for eggs and between 29 and 33°C for nymphal and total development. At these higher temperatures, adult body weights of both *Orius* species were substantially lower than at the medium temperatures. For practical reasons, eggs were used which had been deposited by females kept at 25°C for the development tests. It can therefore not be excluded that survival and development rate of resulting nymphs were influenced by maternal effects (Gilchrist & Huey 2001).

Whereas the respective degree-day requirements were similar among the studied species, estimated lower thresholds for immature development of *O. naivashae* were consistently higher than those of *O. thripoborus*. Linear models have been documented to be suitable for calculation of lower development thresholds and thermal constants of arthropods within a limited temperature range (usually 15 to 30°C) (e.g. Campbell et al. 1974; De Clercq & Degheele 1992; Jarošik et al. 2002; Kontodimas et al. 2004; Jalali et al. 2010). However, the estimated threshold is an extrapolation of the linear portion of the relationship into a region where the relationship is unlikely to be linear (Jervis & Copland 1996), which may yield ecologically inaccurate estimates (Kontodimas et al. 2004). In our study, the linear model estimated the lower thermal threshold for egg development of *O. thripoborus* and *O. naivashae* to be 9.4 and 11.3°C, respectively, whereas our observations showed that eggs of both species did not develop successfully at a constant temperature of 12°C. Non-linear models (e.g., Brière and Lactin) may enable a more accurate description of the relationship between arthropod development and temperature (Kontodimas et al. 2004; Jalali et al. 2010); on the negative side, they do not permit the calculation of thermal constants (Kontodimas et al. 2004).

Whereas for *O. thripoborus* sex ratios at all temperatures were essentially 1:1, skewed sex ratios in *O. naivashae* were observed. In the latter species, sex ratios were female biased, except at 23°C. Strikingly, 11 and 8 times more females than males emerged at 25 and 29°C, respectively. Molecular studies showed that the endosymbiont *Wolbachia* is involved in this sex-ratio distortion mechanism (J. Bonte, unpublished data; see also **Chapter 9**).

In the present study, fecundities and egg hatch rates were superior at 25°C for both species, and *O. thripoborus* produced more eggs than *O. naivashae*. At 15°C, the fecundity of *O. naivashae* females was greatly reduced as compared with higher temperatures (> 19°C), and the eggs deposited at this temperature did not hatch. Further, only about half of the females of both species produced eggs at

15°C. The higher proportion of non-ovipositing females at this low temperature may be due to the induction of reproductive diapause, or to a decrease of mating activity as reported for other *Orius* spp. (Alauzet et al. 1994; van den Meiracker 1994; Nagai & Yano 1999). However, there is no information on the diapause sensivity of *O. thripoborus* and *O. naivashae*. At the high end of the temperature range, not a single *O. thripoborus* female was able to produce eggs at 33°C. This could be due to unsuccessful mating under high temperatures, as was observed in *Orius strigicollis* (Poppius), *Orius sauteri* (Poppius) and *Orius minutus* (Linnaeus) (Kakimoto et al. 2005).

The differing thermal adaptation of *O. thripoborus* and *O. naivashae* is partly reflected by the recorded distribution of both *Orius* species in South Africa (**see Chapter 3**). With winter temperatures averaging 11°C at night and 23°C at day in KwaZulu-Natal, South Africa (South African Weather Service 2011), t_0-values of both studied predators do not seem to limit their winter development. This is corroborated by observations of nymphs of both *O. thripoborus* and *O. naivashae* in sugarcane fields in July and August 2008 and 2009 in the South African provinces Mpumalanga and KwaZulu-Natal (**Chapter 3**). Moreover, t_0-values of local overwintering populations may even be lower than those estimated in this laboratory study under long day conditions and with insects directly taken from rearing stocks. Musolin and Ito (2008) found that in *O. sauteri*, temperature dependence of pre-adult development was smaller and a lower development threshold was estimated under a 12:12h (L:D) photoperiod than under long-day conditions.

The present study may provide useful information to understand the potential role of these predators in augmentation and conservation biological control programs in southern Africa. First, 25°C appears to be the optimal rearing temperature for both studied species. This temperature, in combination with a diet of *E. kuehniella* eggs and green bean pods, resulted in rapid development, good survival, and high reproduction. Second, as both species are complementary in terms of temperature adaptation, there may be opportunities for their use at different times of the season. Whereas *O. thripoborus* may have potential when cool temperatures prevail, *O. naivashae* may perform better in hot summer situations. In **Chapter 3**, the population dynamics of both predators and their focal prey in sugarcane and other crops in southern Africa were studied. In sugarcane, thrips survey data collected over the years 2005 to 2009 indicated that infestations in the South African Umfolozi region followed an annual

pattern. Numbers of *F. serrata* were relatively low during autumn and winter (from March to August) and peaked in the middle of summer (December) (van den Berg et al. 2009). As there is no information on the predation capacities of both *Orius* species on *F. serrata* and other key thrips pests during different parts of the season, more research on their predation ecology is warranted (**see Chapter 6**).

Chapter 5

Diapause and winter survival of two *Orius* species from southern Africa

Redrafter after:

Bonte, J., Musolin, D.L., Conlong, D. and De Clercq, P. 2015. Diapause and winter survival of two *Orius* species from southern Africa. *BioControl* (submitted).

5.1 Introduction

Because of their cryptic lifestyle and fast development of resistance to pesticides, thrips are difficult to control. Moreover, many invasive thrips species may remain active outdoors during mild winters as they often lack obligate diapause (Morse & Hoddle 2006). For instance, *F. occidentalis* has been observed to survive the mild to cold winters in the southern regions of USA and Europe, and in Australia (Kirk & Terry 2003). Therefore, information on the winter ecology of their natural enemies is indispensable in order to achieve an effective biological control. In **Chapter 4**, we showed that *O. thripoborus* is adapted to a slightly cooler temperature range as compared with *O. naivashae*. Based on a linear degree-day model, lower threshold temperatures for total development were estimated to be 10.2°C for *O. thripoborus* and 11.6°C for *O. naivashae*, with thermal requirements of 258.4 and 236.3 DD, respectively (**Chapter 4**). Further, very little is known concerning the behavior of *O. thripoborus* and *O. naivashae* during southern African winters. Southern Africa has a wide variety of climatic conditions ranging from Mediterranean in the south-western corner, to temperate on the interior plateau (Highveld), subtropical in the northeast, and desert in the northwest. At higher elevation in the interior part of South Africa, average winter temperatures can be low (e.g., 7°C in Lesotho Highlands) and occasionally drop to below freezing during winter nights (Brand South Africa 2015; South African Weather Service 2015).

Diapause is an essential life-cycle element underlying the overwintering success of many temperate and colder climate arthropod species. In most *Orius* species studied so far, only adult females overwinter in a state of reproductive diapause and day length appears to play a key role in diapause induction in these insects. However, for (sub)tropical anthocorids, diapause responses are poorly studied. In general, insects show a weakened diapause response towards the tropics and subtropics (**section 2.4.4**).

In the present study, the cold hardiness and diapause responses of *O. thripoborus* and *O. naivashae* were studied in order to assess their overwintering strategies in southern Africa. Cold hardiness was evaluated by determining the supercooling point (SCP) and lower lethal times (LTs) in the laboratory (e.g., Hart et al. 2002a, b; Hatherly et al. 2005, 2008; Berkvens et al. 2010; Maes et al. 2014; Van Damme et al. 2014). SCP measurements evaluate an insect's resistance to a brief cold exposure,

whereas LT measurements assess its cold hardiness when faced with a long-term cold exposure (Chown & Terblanche 2006). Further, our study assessed the effect of photoperiod and temperature on the development and reproduction of *O. thripoborus* and *O. naivashae* in order to improve insights in the diapause potential of these predators.

5.2 Materials and methods

5.2.1 Stock culture

Stock colonies of *O. thripoborus* and *O. naivashae* were reared as described in **section 4.2.1**.

5.2.2 Diapause induction

To study the diapause responses of *O. thripoborus* and *O. naivashae*, the anthocorids were exposed to three photoperiods at 18°C during their entire nymphal and adult life. The tested photoperiods are close to the average day lengths that occur during the four seasons in South Africa, i.e. 14:10 (L:D) h in summer, 12:12 (L:D) h in spring and autumn, and 10:14 (L:D) h in winter. The selected temperature (18°C) represents the mean autumn/spring temperature in the southwestern part of South Africa. Relative humidity in the incubators was maintained at 65%.

In all treatments, both nymphs and adults were offered frozen eggs of the flour moth *E. kuehniella* and a flat green bean pod (*Phaseolus vulgaris* L.) was provided as a water source and oviposition substrate. Flour moth eggs and bean pods were refreshed every three days. Eggs (< 24 h old) were collected from the stock colony and transferred to an incubator set at 18°C and one of the three photoperiods. For each treatment, 70 to 120 nymphs (< 24 h old) were then caged in individual plastic containers (4.5 cm diameter, 3 cm high) sealed with a lid having a ventilation hole covered with a fine mesh-gauze. Development and survival of nymphs were monitored daily and newly emerged adults were sexed and weighed using a Sartorius Genius ME 215P balance (Sartorius, Goettingen, Germany). Adults (< 24 h old) were paired and transferred to similar plastic containers and placed in the same incubator as during their nymphal life. Bean pods were checked daily for eggs in order to determine the preoviposition period. When the first egg was laid, bean pods were replaced every three days and eggs were counted. On day 20 after adult emergence, supercooling points of females and males were assessed (see further). Afterwards, females were dissected to quantify oocyte development (Callebaut

et al. 2004; **Chapter 7**). Ovipositing females and females carrying mature eggs in the ovaries upon dissection were treated as non-diapausing females (Ruberson et al. 1991; Kohno 1997).

Additionally, to better understand the process of diapause induction, the experiment was repeated with both species being exposed to a 12:12 (L:D) h photoperiod at 23°C during their entire nymphal and adult life.

5.2.3 Cold hardiness

The SCP is reached when body fluids of freeze-intolerant individuals freeze in response to exposure to below zero temperatures. However, chill injury caused by temperatures above the insect's SCP can lead to death as well (Bennett & Lee 1989; Denlinger 1991; Bale 1993). Therefore, LTs have been used as an additional index of cold hardiness (Bale et al. 1988; Watanabe 2002) and were quantified at different temperatures, i.e., 0 and 5°C.

5.2.3.1 Acclimation

In part of the experiments, individuals were allowed to acclimate to lower temperatures. For this purpose, newly moulted adults (< 24 h) from the stock colony (25°C) were transferred to an incubator set at 10°C and a photoperiod of 16:8 (L:D) h for seven days. During this period, adults were kept in an insect breeding dish and provided with *E. kuehniella* eggs and a piece of bean pod. Free water was also provided by way of a moist cotton plug fitted into a 1.5 cm (diameter) plastic dish.

5.2.3.2 Supercooling point

The supercooling capacity was determined separately for males and females of *O. thripoborus* and *O. naivashae*, subjected to six different experimental conditions. Three treatment groups consisted of 20-day-old adults obtained from the diapause induction experiments at 18°C and the three different photoperiods. Their responses were compared with those of 20-day-old adults collected from the stock colony (16:8 (L:D) h and 25°C). Two further groups were set up to test the influence of acclimation; these consisted of 7-day-old acclimated insects (exposed to 10°C before testing, **see 5.2.3.1**) and 7-day-old adults directly taken from the stock colony.

The SCP was measured using a Picotech TC-08 thermocouple datalogger (Pico Technology, UK) and a low temperature programmable Haake Phoenix II CP30 alcohol bath (Thermo Electron Corporation, USA). Insects were placed individually in a 1.0 ml pipette tip with a thermocouple attached to the

dorsal side of the body using Vaseline (Unilever, UK). After the pipette tip was sealed with Parafilm M, it was placed in an individual glass test tube and subsequently immersed in the alcohol bath (Berkvens et al. 2010). For each treatment 18 to 61 adults of each sex were tested. The starting temperature was set at 18°C (as in the diapause induction experiment), 25°C (the rearing temperature) or 10°C (the acclimation temperature) and then lowered to -25°C at a rate of 0.5°C min^{-1}. The SCP of each individual was detected by the release of exothermal heat when the insect's body fluids froze.

5.2.3.3 Lower lethal time

Lower lethal time estimates how many days are needed to kill 10 (LT_{10}), 50 (LT_{50}) or 90% (LT_{90}) of the tested adults at a certain temperature. Lethal times were determined at 0 and 5°C for acclimated male and female adults of *O. thripoborus* and *O. naivashae* (see below). Adults were placed in closed polystyrene insect breeding dishes (10 cm diameter, 4 cm high) (SPL Life Sciences, Republic of Korea), with a mesh hole (4 cm diameter) in the lid. Throughout the predators' exposure to 0 or 5°C, eggs of *E. kuehniella* were provided as food, but none of the predators were observed to feed on them. Water was supplied by means of a moist piece of cotton wadding. For exposure to 0 or 5°C, 20 (*O. naivashae*) or 30 (*O. thripoborus*) breeding dishes each containing eight *Orius* adults (four males and four females) were transferred to climatic cabinets (Type ET 2028, Weiss Technik, Belgium) set at the respective temperatures. No light was provided and relative humidity was not controlled during the cold exposure, although humidity was likely in a range of 55 to 75%. After 12 h for *O. naivashae* at 0°C and after 48 h for all other treatments, two containers of either species were removed from the cabinets every 24 h. To avoid temperature shock, the containers were first held for 1 h at 10°C in complete darkness. The insects were finally transferred to an incubator set at 25°C and 16:8 (L:D) h and maintained for 24 h with water but without food, after which survival was recorded. The anthocorids were deemed to have died if they were incapable of moving upon prodding with a fine brush. All adults were allowed to acclimate before being subjected to 0 or 5°C (**see 5.2.3.1**). As for both species no differences in LTs between male and female adults were detected at either temperature, data of males and females were pooled resulting in four remaining data sets.

5.2.4 Statistical analysis

Data analysis was carried out using IBM SPSS Statistics 21 (IBM Corp. 2012).

If the data were continuous and a Kolmogorov-Smirnov test indicated that the data were normally distributed, the parameter was analysed using analysis of variance (ANOVA). When continuous data were not normally distributed, a non-parametric Kruskal-Wallis H test was used. In case of non-continuous data, a generalised linear model was used with a link function and error distribution depending on the nature of the data. Each analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. Countable data were analysed using a generalised linear model, with a Poisson distribution if applicable or a negative binomial distribution in case of overdispersion, as determined by the deviance and Pearson goodness-of-fit statistics (Hilbe 2011). If none of the generalised linear models were applicable, a non-parametric model was applied. Parameters expressed as percentages (binary) were compared by means of a logistic regression. This regression is a generalised linear model using a probit (log odds) link and a binomial error function (McCullagh & Nelder 1989). For all studied parameters, a two-factor analysis was applied using the appropriate model (2-way ANOVA or generalised linear model). In case a factor with two degrees of freedom (df) was found to be significant, a post-hoc analysis was performed to separate means. When a significant interaction between the factors was found, means were compared pairwise. Sex ratios were tested versus an equal female:male distribution (1:1 ratio) by means of Chi-square tests. Lethal times were analysed using Probit analysis in order to estimate the time required to kill 10, 50 and 90% of the population at a temperature of 0 and 5°C. Significant differences were identified by non-overlapping fiducial limits (Hart et al. 2002b).

5.3 Results

5.3.1 Diapause induction

A two-factor analysis at 18°C with *Orius* species and photoperiod as factors indicated no interaction between these factors for the parameters male and female adult weight, preoviposition period and proportion of ovipositing females (**Table 5.1**). For the remaining parameters at 18°C, and for those observed at a 12:12 (L:D) h photoperiod, means were compared pairwise given significant interactions (**Tables 5.2 and 5.3**).

At 18°C, survival rates of nymphs ranged from 80.8 to 92.2% for *O. thripoborus* and from 44.1 to 74.6% for *O. naivashae* (**Table 5.2**; $\chi^2 = 67.118$; df = 5; $P < 0.001$). Hence, at this temperature, nymphal survival

of *O. thripoborus* was higher than that of *O. naivashae*, except at a 14 h photoperiod ($P = 0.207$). For *O. thripoborus* nymphs reared at 18°C, the highest survival occurred under short day conditions (10 h) and the lowest under a 14-h photoperiod. For *O. naivashae* at 18°C, the lowest nymphal survival (44.1%) was observed at a 12:12 (L:D) h photoperiod; the highest mortality was recorded during the final (fifth) instar. However, when at the same photoperiod temperature was set to 23°C, nymphal survival was more than doubled for *O. naivashae* and increased by 10% as well for *O. thripoborus*, resulting in a similar survival rate of both anthocorids under the latter conditions ($P = 0.434$).

At 18°C, male and female nymphal developmental times were shorter for *O. thripoborus* than for *O. naivashae* (all $P < 0.001$), and fluctuated between 27.1 and 30.6 days for *O. thripoborus* and between 31.3 and 40.6 days for *O. naivashae*. For *O. thripoborus* reared at 18°C, the fastest development was observed at a 10-h light regime, whereas development was slowest at 12 h light. For *O. naivashae*, no differences between developmental times were observed when reared at 10 or 14 h light and 18°C (males: $P = 0.135$; females: $P = 0.890$), but primarily due to a prolongation of the fifth nymphal stadium, nymphal development of *O. naivashae* was extended by 25% when the predator was maintained at a 12 h day length. At the latter photoperiod and 23°C, nymphal developmental times of both anthocorids were much shorter than at 18°C (all $P < 0.001$), and development of *O. naivashae* was as long as (males; $P = 0.216$) or shorter than (females: $P = 0.001$) that of *O. thripoborus* (**Table 5.2**).

No effect of photoperiod on adult weight was observed at 18°C (**Table 5.2**). For all tested photoperiods at the latter temperature, *O. naivashae* males and females were heavier than those of *O. thripoborus*. However, when reared at a 12 h photoperiod, *O. naivashae* females were heavier at 23 than at 18°C ($P < 0.001$), whereas for *O. thripoborus* males the opposite was observed ($P < 0.001$) (**Table 5.2**).

Sex ratios of *O. naivashae* were female biased at a 14 h day length at 18°C and at 12 h light and 23°C. For all other treatments, no significant deviations from a 1:1 sex ratio were observed (**Table 5.2**).

Preoviposition period at 18°C was influenced by species and photoperiod (**Table 5.1**). At this temperature, first eggs were always laid earlier by *O. thripoborus* than by *O. naivashae* females. *Orius naivashae* females did not oviposit during the 20-day-observation period at a 12 h day length and 18 °C, although 16 % of them showed a preoviposition period exceeding 20 days. This regime led to the longest preoviposition period for O. thripoborus and O. naivashae, although only for the latter species

Table 5.1 Results of a logistic regression or a two-way ANOVA indicating the effect of species (O. thripoborus and O. naivashae) and photoperiod (14:10, 12:12 and 10:14 (L:D) h) on developmental and reproductive parameters of Orius species reared at 18°C

Parameter	Species			Photoperiod			Species × photoperiod			Error term
	F/χ^2	df	*P*	F/χ^2	df	*P*	F/χ^2	df	*P*	df
Nymphal survival[a]	31.131	1	< 0.001	12.229	2	0.002	9.164	2	0.010	-
Female developmental time[b]	356.545	1	< 0.001	103.209	2	< 0.001	28.210	2	< 0.001	180
Male developmental time[b]	313.682	1	< 0.001	118.503	2	< 0.001	52.239	2	< 0.001	168
Female adult weight[b]	26.090	1	< 0.001	0.029	2	0.972	0.722	2	0.487	178
Male adult weight[b]	16.782	1	< 0.001	1.724	2	0.181	1.809	2	0.167	168
Preoviposition period[c]	25.156	1	< 0.001	6.345	2	0.042[e]	0.021	1	0.884	-
Number of oviposited eggs during the first 20 days[d]	16.21	1	< 0.001	68.228	2	< 0.001	15.775	2	< 0.001	-
Number of oocytes in females dissected on day 20[b]	0.377	1	0.007	4.510	2	0.014	5.347	2	0.007	83
Proportion of non-diapausing females[a]	12.986	1	< 0.001	12.685	2	0.002[f]	1.580	2	0.454	-

[a] Probit (Wald Chi-square); [b] Two-way ANOVA; [c] Poisson (Wald Chi-square); [d] Negative binomial (Wald Chi-square).

[e] Poisson post-hoc test: 14:10 vs. 12:12 h: $\chi^2 = 6.344$, df = 1, $P = 0.012$; 14:10 vs. 10:14 h: $\chi^2 = 1.584$, df = 1, $P = 0.208$; 12:12 vs. 10:14 h: $\chi^2 = 2.722$, df = 1, $P = 0.099$.

[f] Probit post-hoc test: 14:10 vs. 12:12 h: $\chi^2 = 7.689$, df = 1, $P = 0.006$; 14:10 vs. 10:14 h: $\chi^2 = 0.452$, df = 1, $P = 0.501$; 12:12 vs. 10:14 h: $\chi^2 = 11.313$, df = 1, $P = 0.001$.

a significant difference between its preoviposition periods at 12 and 14 h light was found. Whereas *O. naivashae* females did not oviposit during the 20-day-observation period at a 12 h day length and 18°C, this regime led to the longest preoviposition period for *O. thripoborus*, although only for the latter species a significant difference between its preoviposition periods at 12 and 14 h light was found. At 23°C and 12 h light, preoviposition periods of both *Orius* species were similar ($P = 0.065$) and about twice shorter than those observed for *O. thripoborus* at 18°C and 12 h light (both $P < 0.001$) (**Table 5.3**).

In *O. thripoborus* females at 18°C, the number of eggs produced within the first 20 days of adult life did not differ between the three photoperiods. This number was also higher than egg numbers produced by *O. naivashae* females at both temperatures (18°C: $P < 0.001$; 23°C: $P = 0.006$) (**Table 5.3**). Likewise, the number of oocytes in females dissected on day 20 at 18°C was higher for *O. thripoborus* than for *O. naivashae*, except at a 12 h light period. At the latter regime, oocyte counts in dissected *O. naivashae* females were as high as those in *O. thripoborus* females ($P = 0.302$), despite the fact that the former species did not oviposit during the first 20 days after adult emergence. For *O. naivashae*, differences in the number of oviposited eggs occurred between photoperiods, but the total potential egg production by day 20 (i.e., numbers of oviposited eggs plus oocyte counts) was not affected ($F = 0.062$; $df = 2$; $P = 0.939$. At 23°C, a 12 h photoperiod led to similar oocyte numbers as those counted at 18°C for both species ($\chi^2 = 2.128$; $df = 3$; $P = 0.546$), but more eggs were oviposited by day 20 at the higher temperature (both $P < 0.001$) (**Table 5.3**).

Species and photoperiod influenced the proportion of non-diapausing females at 18°C (**Table 5.1**). At this temperature, the relative number of egg producing couples was higher in *O. thripoborus* than in *O. naivashae,* ranging from 57.7 to 83.9% in *O. thripoborus* and from 15.8 to 57.1% in *O. naivashae*. Whereas for both anthocorids no difference in the proportion of non-diapausing females between the photoperiods 10 and 14 h was recorded, a day length of 12 h at 18°C was associated with the lowest proportion of ovipositing females. However, at 12 h light and 23°C, the proportion of egg producing females was equally high in both *Orius* species ($P = 0.959$).

Table 5.2 Developmental parameters (mean ± SE) of O. thripoborus and O. naivashae at two temperatures and three photoperiods (65 ± 5% RH; E. kuehniella eggs + bean pod)

Temperature (°C)	Photoperiod (L:D) (h)	Species	n[a]	Nymphal survival (%)	Female		Male		Sex ratio (m:f)[c]
					Developmental time (d)	Adult weight (mg)[b]	Developmental time (d)	Adult weight (mg)[b]	
18	10:14	*O. thripoborus*	77	92.2 ± 3.1a	27.1 ± 0.19a	41.1 ± 0.77	27.3 ± 0.23a	34.5 ± 0.73	1:1.03
		O. naivashae	71	74.6 ± 5.2bc	32.8 ± 0.51d	37.2 ± 0.88	32.7 ± 0.67d	30.7 ± 0.64	1:1.41
	14:10	*O. thripoborus*	73	80.8 ± 4.6bc	29.0 ± 0.29b	40.5 ± 0.61	29.5 ± 0.32b	32.3 ± 0.61	1:1.18
		O. naivashae	75	72.0 ± 5.2c	32.7 ± 0.53d	38.3 ± 0.62	31.3 ± 0.32d	31.0 ± 0.65	1:2.12*
	12:12	*O. thripoborus*	84	85.7 ± 3.8abB	30.6 ± 0.35cC	40.9 ± 0.65A	30.5 ± 0.25cB	32.8 ± 0.53A	1:0.64
		O. naivashae	118	44.1 ± 4.6dC	40.6 ± 0.57eD	37.7 ± 0.84B	40.3 ± 0.66eC	30.8 ± 0.92AB	1:1.55
23	12:12	*O. thripoborus*	80	96.8 ± 2.2A	15.6 ± 0.19B	40.4 ± 0.60A	15.5 ± 0.12A	29.7 ± 0.54B	1:0.79
		O. naivashae	63	98.8 ± 1.2A	14.9 ± 0.13A	41.7 ± 0.57A	15.3 ± 0.21A	31.1 ± 0.50AB	1:2.04*

Means within a column at 18°C followed by the same lowercase letter are not significantly different ($P > 0.05$): Mann-Whitney U test (developmental times); or binary probit test (nymphal survival).

Means within a column at 12:12 (L:D) h followed by the same uppercase letter are not significantly different ($P > 0.05$): Tukey test (adult weights); Mann-Whitney U test (developmental times); or binary probit test (nymphal survival).

[a] Initial number of first instars tested.

[b] Statistical differences (at 18°C), as indicated by a two-factor analysis and post-hoc analysis, see Table 1.

[c] Values with an asterisk differ significantly from a 1:1 ratio; χ^2 and P values were 0.906, 0.014; 0.216, 1.528; 0.515, 0.424; 0.001, 10.383; 0.059, 3.556; 0.123, 2.373; 0.370, 0.803; and 0.002, 9.228, respectively (Chi-square test, df = 1).

Table 5.3 Reproductive parameters (mean ± SE) of O. thripoborus and O. naivashae at two temperatures and three photoperiods (65 ± 5% RH; E. kuehniella eggs + bean pod)

Temperature (°C)	Photoperiod (L:D) (h)	Species	n[a]	Preoviposition period (d)[b]	No. of oviposited eggs during the first 20 days[c]	No. of oocytes in females dissected on day 20[c]	Proportion of non-diapausing females (%)[b]
18	10:14	*O. thripoborus*	31	10.00 ± 0.50	32.6 ± 4.3a	6.53 ± 0.53a	83.9 ± 6.7
		O. naivashae	21	15.50 ± 1.23	2.1 ± 0.9c	4.23 ± 0.82bc	57.1 ± 11.1
	14:10	*O. thripoborus*	27	9.11 ± 0.63	32.2 ± 5.7a	6.18 ± 0.73ab	74.1 ± 8.6
		O. naivashae	25	13.78 ± 1.00	7.0 ± 2.0b	3.43 ± 0.54c	56.0 ± 10.1
	12:12	*O. thripoborus*	26	11.73 ± 0.86B	30.7 ± 4.4aC	6.00 ± 0.57aA	57.7 ± 9.9B
		O. naivashae	19	-[d]	0.0 ± 0.0dD	9.67 ± 3.38acA	15.8 ± 8.6C
23	12:12	*O. thripoborus*	26	5.32 ± 0.60A	89.1 ± 9.6A	6.7 ± 0.79A	88.5 ± 6.4A
		O. naivashae	25	6.68 ± 0.32A	54.9 ± 5.4B	5.7 ± 0.66A	88.0 ± 6.6A

Means within a column and at 18°C followed by the same lowercase letter are not significantly different ($P > 0.05$): generalized linear model with negative binomial distribution (oviposited eggs); or Mann-Whitney U test (oocytes in dissected females).

Means within a column and at 12:12 (L:D) h followed by the same uppercase letter are not significantly different ($P > 0.05$): generalized linear model with Poisson distribution (preoviposition period); Mann-Whitney U test (oviposited eggs; oocytes in dissected females); or binary probit test (non-diapausing females).

[a] Initial number of first instars tested.

[b] Statistical differences (at 18°C), as indicated by a two-factor analysis and post-hoc analysis, see Table 1.

[c] Only females were included which produced at least a single egg and/or had oocytes on day 20 after emergence.

[d] No eggs were deposited in this treatment.

Table 5.4 Supercooling points (SCP) (mean ± SE) of 20-day-old females and males of O. thripoborus and O. naivashae reared at 18°C and three photoperiods

Photoperiod (L:D) (h)	Species	Female		Male	
		n[a]	SCP (°C)	n[a]	SCP (°C)
10:14	*O. thripoborus*	32	-18.79 ± 0.29a	30	-19.80 ± 0.30a
	O. naivashae	27	-18.03 ± 0.24ab	21	-19.07 ± 0.36a
12:12	*O. thripoborus*	26	-19.21 ± 0.57aab	27	-19.14 ± 0.28a
	O. naivashae	18	-17.09 ± 0.33b	18	-19.53 ± 0.48a
14:10	*O. thripoborus*	29	-18.91 ± 0.33a	24	-19.76 ± 0.47a
	O. naivashae	21	-18.32 ± 0.42ab	23	-18.41 ± 0.34a

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tamhane test)

[a] number of adults tested

Table 5.5 Supercooling points (SCP) (mean ± SE) of 7- and 20-day-old females and males of O. thripoborus and O. naivashae reared at 25°C and a 16:8 (L:D) h photoperiod

Age (d)	Species	Female		Male	
		n[a]	SCP (°C)	n[a]	SCP (°C)
7	*O. thripoborus*	61	-18.32 ± 0.18a	30	-19.07 ± 0.37b
	O. naivashae	39	-18.70 ± 0.27a	27	-20.51 ± 0.24ab
20	*O. thripoborus*	28	-17.16 ± 0.34a	30	-18.71 ± 0.24ab
	O. naivashae	26	-17.85 ± 0.33a	25	-18.28 ± 0.61a

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tamhane test)

[a] number of adults tested

Table 5.6 Supercooling points (SCP) (mean ± SE) of 7-day-old acclimated and non-acclimated females and males of O. thripoborus and O. naivashae reared at a 16:8 (L:D) h photoperiod

Temperature (°C)	Species	Female		Male	
		n[a]	SCP (°C)	n[a]	SCP (°C)
25	*O. thripoborus*	61	-18.32 ± 0.18c	30	-19.07 ± 0.37b
	O. naivashae	39	-18.70 ± 0.27bc	27	-20.51 ± 0.24ab
25/10[b]	*O. thripoborus*	30	-20.16 ± 0.33ab	33	-21.01 ± 0.24a
	O. naivashae	34	-20.47 ± 0.31a	34	-20.03 ± 0.37ab

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tamhane test)

[a] number of adults tested

[b] acclimated treatment group, consisting of newly moulted adults (< 24 h) from the stock colony (25°C and 16:8 (L:D) h), transferred to an incubator set at 10°C and a photoperiod of 16:8 (L:D) h for 7 days before determination of SCP

5.3.2 Cold hardiness

5.3.2.1 Supercooling point

Supercooling points of *Orius* males and females were affected by experimental conditions (F = 8.017, df = 5, 310, $P < 0.001$ and F = 16.717, df = 5, 359, $P < 0.001$ for males and females, respectively), but not by species (F = 1.709, df = 1, 310, $P = 0.192$ and F = 3.295, df = 1, 359, $P = 0.070$, respectively). The interaction between both factors was significant (F = 4.086, df = 5, 310, $P = 0.001$ and F = 4.346, df = 5,359, $P = 0.001$ for males and females, respectively). At all tested conditions, no differences were found between average SCP values of *O. thripoborus* and *O. naivashae*. When reared at 25°C and a 16 h light regime, SCP values of (non-acclimated) 7- and 20-day-old adults did not differ for both species and sexes (**Table 5.5**). At 18°C, no differences in SCP values were detected between the three tested regimes, for both species and sexes (**Table 5.4**). Lowest SCP values were found in acclimated adults of both species, but test significances among SCPs at non-acclimating conditions were inconsistent. Whereas SCP values of acclimated 7-day-old females of *O. naivashae* were significantly lower compared to those of non-acclimated 7-day-old females, no differences were found between acclimated and non-acclimated *O. naivashae* males (**Table 5.6**). Acclimated 7-day-old *O. thripoborus* adults had lower SCP values than those of non-acclimated 7-day-old males and females and of 20-day-old adults of both sexes reared at 25°C and a 16 h photoperiod (**Table 5.6**). Also, the SCP of 7-day-old *O. thripoborus* males was higher when they were transferred from 18°C and 12 h light without acclimation as compared with those which were allowed to acclimate at 10°C ($P < 0.001$).

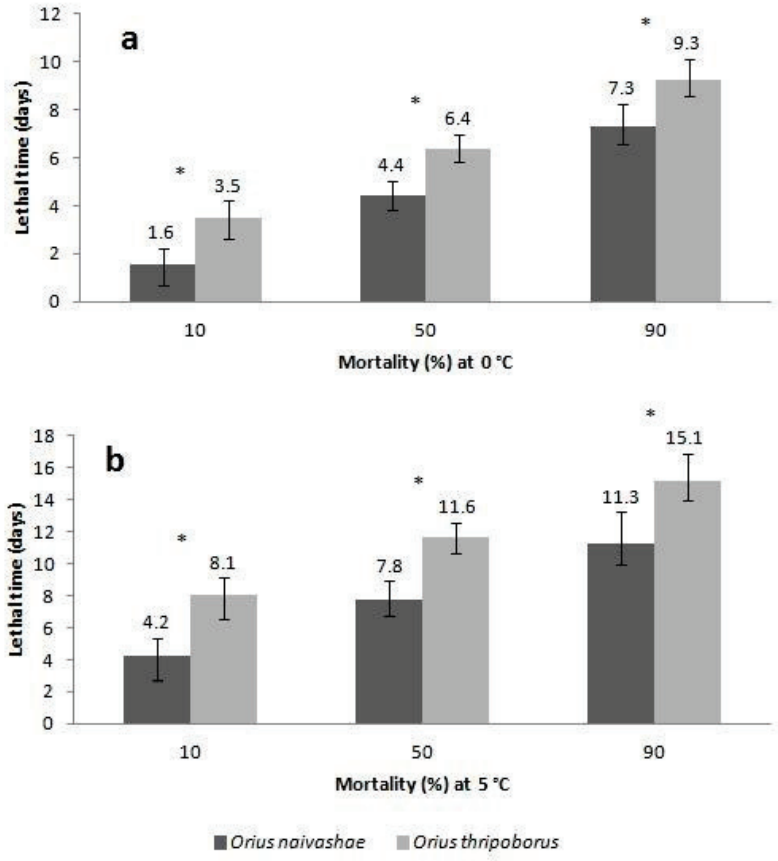

Figure 5.1 Lethal times (LT_{10}, LT_{50} and LT_{90}) (means ± 95% fiducial limits) at 0°C (a) and 5°C (b) for acclimated adults of O. thripoborus and O. naivashae. Asterisks indicate significant differences in lethal time values among species

5.3.2.2 Lower lethal time

Adults of both *Orius* species survived longer at 5°C (**Figure 1b**) than at 0°C (**Figure 1a**), but LTs were overall lower in *O. naivashae* than in *O. thripoborus*. At 5°C, the time required to kill 50% of the population of *O. naivashae* and *O. thripoborus* was 7.8 and 11.6 days, respectively, whereas at 0°C it took 4.4 and 6.4 days, respectively.

5.4 Discussion

In the subtropics, where the mean winter temperature falls close to the developmental threshold of a particular species very occasionally, the benefit of diapause varies from year to year, and therefore the species may not achieve and maintain accurate seasonal synchronisation and adaptation to local

seasonal conditions (Masaki 1990). In many *Orius* species the photoperiodic response becomes weaker towards the (sub)tropics and some populations do not enter diapause at all (Tommasini & Nicoli 1995, 1996; Ito & Nakata 2000; Musolin & Ito 2008; Saulich & Musolin 2009). In the present study, however, the low percentage of egg producing females of *O. naivashae* reared at 18°C and a 12 h day length indicated a relatively high incidence of reproductive diapause in this species, at least under some environmental conditions. The latter conditions, representing the southern African autumn, induced diapause in twice as much females of *O. naivashae* as in those of *O. thripoborus* (84% as compared with 42% of females, respectively). At 18°C, shorter and longer days induced reproductive diapause in 43 and 44% of the *O. naivashae* population, respectively, whereas for *O. thripoborus* diapause incidence remained below 26%. Most *Orius* species enter diapause under short-day conditions, but critical day length varies from species to species as well as within the same species for populations occurring at different latitudes (Tauber et al. 1986; Danks 1987; Leather et al. 1995; Shimizu & Fujisaki 2006; Musolin and Ito 2008). Based on our results, critical day length for diapause induction in the studied populations of *O. naivashae* and *O. thripoborus* appears to be around 12 h. As shorter days did not increase the proportion of diapausing females, we assume that, when the autumn photoperiod prevails in South Africa, temperature further enhances diapause induction and then prevents premature diapause termination in these species. In temperate zones, both thermal and photoperiodic conditions are well known to influence the incidence of reproductive diapause in *Orius* species (e.g., Kingsley & Harrington 1982; van den Meiracker 1994; Kohno 1998; Musolin et al. 2004; Cho et al. 2005). Yet, in subtropical climates, diapause incidence was low (< 20%) or nonexistent in the southern Japanese species *Orius tantillus* (Motschulsky) (Nakashima & Hirose 1997b; Shimizu & Kawasaki 2001) and *Orius strigicollis* (Poppius) (Shimizu & Kawasaki 2001), and in *Orius albidipennis* (Reuter) from the Canary Islands (van den Meiracker 1994) and Israel (Chyzik et al. 1995).

Photoperiodic control of developmental time is a well-known phenomenon in many insects (Saunders 2002; Beck 1980). Short day lengths have been observed to cause decelerating or accelerating effects to some extent in a number of heteropteran species (e.g., Ruberson et al. 1991; Musolin & Saulich 1997; Lopatina et al. 2007; Saulich & Musolin 2009), but can also lead to a pronounced prolongation of development in other true bugs (Kiritani 1985; Musolin & Saulich 1997; Tanaka & Zhu 2003). In this study, a 12 h light regime at 18°C prolonged development of *O. naivashae*, accompanied by a low

nymphal survival (44%), mainly in the last instar. However, at 23°C development of *O. naivashae* at a 12 h photoperiod was more successful and data were comparable with those reported for this species in previous experiments performed at long day conditions (16:8 (L:D) h) and 23°C (**Chapter 4**). For *O. thripoborus* nymphs reared at 18°C, a photoperiod of 12 h also resulted in the slowest development, but differences in developmental times among the three photoperiods were less pronounced than those observed for *O. naivashae*. Moreover, at 18°C short day conditions accelerated growth of *O. thripoborus* nymphs compared to long days, whereas for *O. naivashae* no differences in developmental times were observed. Short-day acceleration of nymphal growth at low temperatures (18 to 20°C) has been reported earlier for *Orius insidiosus* (Say) (Ruberson et al. 1991), *Orius majusculus* (Reuter) (van den Meiracker 1994), *O. strigicollis* (Musolin et al. 2004), *Orius sauteri* (Poppius) and *Orius minutus* (L.) (Musolin & Ito, 2008), but overall trends were inconsistent and geographically driven (Parker 1975; Tauber et al. 1986; Tommasini & Nicoli 1995, 1996). These seasonal adaptations are always local and differ among species and populations (Saulich & Musolin 2009).

After 20 days at 18°C and 12 h light, not a single *O. naivashae* female produced eggs, although upon dissection 16% of females had vitellogenic oocytes in their ovarioles. These females were not considered to be in reproductive diapause (Musolin & Ito 2008), but demonstrated a delayed oviposition. At long and short day conditions (both at 18°C), half of the non-diapausing *O. naivashae* females also showed delayed oviposition. For *O. thripoborus*, however, delayed oviposition was rarely observed.

In **Chapter 4**, it was shown that the overall performance of *O. thripoborus,* in terms of its developmental rates and reproduction, is generally superior to that of *O. naivashae*. In this study, developmental and reproductive parameters of *O. thripoborus* were better than those of *O. naivashae* at all tested photoperiods at 18°C. At 23°C (12:12 (L:D) h), both predators showed similar developmental success, but *O. thripoborus* had a better reproductive output. Studying the thermal biology of *O. thripoborus* and *O. naivashae*, we indicated that within a moderate temperature range (19 to 25°C) the overall performance of both anthocorids improved with increasing temperature (**Chapter 4**). Likewise, developmental and reproductive parameters obtained in the present study were better at 23°C than at 18°C in both anthocorids.

Lethal times (50% mortality) were 6.4 and 4.4 days at 0°C and 11.6 and 7.8 days at 5°C, for acclimated adults (males and females combined) of *O. thripoborus* and *O. naivashae*, respectively. To our knowledge, lower LTs have never been assessed for any anthocorid species even though responses to cold storage have been studied in a few *Orius* species (Bueno et al. 2014; Zhang et al. 2008). Half of the (non-acclimated) *O. insidiosus* adults of a population originating from Brazil survived a 14-day-storage period at 5°C (Bueno et al. 2014), suggesting a higher cold tolerance than that observed for the *Orius* species in the present study. When transferred from 22°C and short days (11:13 (L:D) h) to 0°C, Ito and Nakata (1998a) recorded longevities of ca. 90 and 140 days for 50% of the diapausing females of *O. minutus* and *O. sauteri*, respectively. Based on recorded LT values, *O. thripoborus* has a better cold tolerance than *O. naivashae*. However, this is not reflected in the SCP values of the species, which were similar for all treatments and ranged between -21 and -17°C.

Several factors related to the experienced rearing conditions or origin of the population under study may influence an insect's cold tolerance. Maes et al. (2012) noted that acclimation period, infection status with endosymbionts and diet may influence the supercooling ability of the predator *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Also physiological changes associated with diapause, reproductive maturation or ageing can affect an insect's cold tolerance (Bowler & Terblanche 2008; Saulich & Musolin 2009). For example, a decline in cold tolerance over adult life has been observed in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and *Dacus tryoni* (Frogatt) (Diptera: Tephritidae) (Meats 1973; David et al. 1998; Jensen et al. 2007). In the present study, no influence of adult age on SCP was found, and values recorded for males and females were similar. Females in treatment groups showing higher diapause incidence did not have lower supercooling points. The only factor influencing the SCP was the 7-day-acclimation of adults at 10°C. Acclimated adults tended to have lower SCPs, but this was statistically significant only for *O. naivashae* females. A similar trend towards lower SCP values for acclimated individuals was found in *M. pygmaeus* (Maes et al. 2012). However, for another predatory mirid bug, *Nesidiocoris tenuis* Reuter, no significant decrease in SCP was detected after acclimation for seven days at 10°C (Hughes et al. 2009). Also bacteria can affect an insect's freezing tolerance as they may act as heterogeneous ice nucleators inside the body of their host (Lee et al. 1991; Worland & Block 1999). As a result, intracellular freezing may occur at higher temperatures when hosts are infected with bacterial endosymbionts. Maes et al. (2012)

reported that *M. pygmaeus* infected with *Wolbachia pipientis* and *Rickettsia* spp. had higher SCP values than uninfected conspecifics. Given that these bacterial endosymbionts were also detected in the studied *O. naivashae* population (J. Bonte, unpublished data), it remains to be investigated whether SCPs of uninfected adults are lower than those measured in the present study. It again deserves emphasis that SCP data alone are not sufficiently reliable and comprehensive indicators of cold tolerance since the vast majority of species are freeze avoiding and SCP temperatures are rarely experienced by individuals in their natural habitats (Bale 1996). Lethal time data are therefore believed to best indicate naturally occurring cold stress as they do not only test temperature but also exposure time (Allen 2010).

In many *Orius* species studied so far, only females (and usually fertilised ones) can properly accumulate fat body and enter diapause to ensure successful overwintering, whereas males usually mate before winter and do not survive until spring (Ito & Nakata 1998a, b; Kobayashi & Osakabe 2009; Saulich & Musolin 2009; Shimizu & Kawasaki 2001). However, in this study, LTs did not differ between *Orius* males and females and it is thus expected that both sexes survive southern African mild winters with reasonably similar success. During field observations at the end of winter (July) in the provinces of Mpumalanga and KwaZulu-Natal male and female adults as well as nymphs of both *Orius* species were collected (J. Bonte, unpublished data). This implies that, in these areas, at least some nymphs and adults of both sexes remain active in winter and do not enter reproductive diapause, or that diapause in these individuals is very weak. In other words, winter diapause is not likely to be a critical trait in populations of *O. thripoborus* and *O. naivashae* occurring in regions with less pronounced temperature extremes. Our experiments showed that only a fraction of the studied *Orius* populations are able to enter diapause: at 18°C and 12 h day length, 86 and 44% of the nymphs developed successfully without entering diapause, and additionally, 58 and 16% of the adults were able to reproduce, for *O. thripoborus* and *O. naivashae*, respectively. However, diapause may be induced in both anthocorids during autumn in cooler regions. Further, it is worth noting that research on overwintering strategies based solely on laboratory populations may not be representative of field situations. Prolonged laboratory rearing of *O. thripoborus* and *O. naivashae* at a 16 h photoperiod and 25°C could have reduced their diapause response and tolerance to cold conditions. Field observations can elucidate

whether the studied predators remain active and are able to control crop pests in different parts of southern Africa during different parts of the season.

In the previous **Chapter** (**4**), we showed that *O. thripoborus* is adapted to a slightly cooler temperature range as compared with *O. naivashae*. Our present findings indicate that *O. naivashae* is less cold tolerant and has a stronger tendency to enter reproductive diapause as compared with *O. thripoborus*. In regions of southern Africa where average autumn/winter temperatures are around or below 18°C, *O. thripoborus* thus appears to have stronger potential for use in biological control than *O. naivashae*. However, for successful biocontrol programs using these anthocorids not only species-specific (e.g., type of diapause), but also population-specific diapause-related traits (e.g., critical day length) should be taken into consideration (Musolin et al. 2004). Our laboratory study indicates that a combination of a 12 h day length and low temperatures (e.g., 18 °C) induces (weak) diapause in *O. thripoborus* and *O. naivashae*, but year-round field observations are warranted to elucidate whether the studied predators remain active and are able to contribute to the suppression of crop pests in different parts of southern Africa during different parts of the season.

Chapter 6

Predation capacity, development and reproduction of *Orius thripoborus* and *Orius naivashae* on various prey

Based on:

Bonte, J., De Hauwere, L., Conlong, D. and De Clercq, P. 2015. Predation capacity, development and reproduction of the southern African flower bugs *Orius thripoborus* and *Orius naivashae* (Hemiptera: Anthocoridae) on various prey. *Biological Control* 86: 52-59

6.1 Introduction

Predatory bugs of the genus *Orius* (Hemiptera: Anthocoridae) are omnivores, feeding on a wide array of arthropod prey as well as on plant materials such as pollen and plant juices. They are used worldwide for the control of different thrips (Thysanoptera: Thripidae) pests, but are also known to attack a variety of soft-bodied arthropods such as aphids (Hemiptera: Aphididae), whiteflies (Hemiptera: Aleyrodidae), mites (Arachnida: Acari), young lepidopterous larvae and small arthropod eggs (**section 2.4.7.1**).

The little-studied southern African species *Orius thripoborus* (Hesse) and *Orius naivashae* (Poppius) have been suggested as potential biological control agents of various thrips pests, which include the sugarcane thrips *Fulmekiola serrata* Kobus, the citrus thrips *Scirtothrips aurantii* Faure, the two avocado thrips pests *Heliothrips haemorrhoidalis* (Bouché) and *Selenothrips rubrocinctus* (Giard), and the western flower thrips *Frankliniella occidentalis* (Pergande) (Hesse 1940; Dennil 1992; Way et al. 2006a, b; EPPO 2014). However, little is known on the prey range of *O. thripoborus* and *O. naivashae*. Given their good performance when reared on factitious or even artificial diets (**see Chapters 7 and 8**), it is likely that their natural prey range reaches beyond the Thysanoptera. Therefore, it is warranted to investigate whether these *Orius* species may also contribute to the suppression of non-thrips arthropod pests in southern Africa.

The western flower thrips, *F. occidentalis*, the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) and the green peach aphid *Myzus persicae* Sulzer, including its subspecies *M. persicae nicotianae* Blackman, are economically important pests on a wide range of agricultural and ornamental plants worldwide. In South Africa, *F. occidentalis* and *T. urticae* are key pests in vineyards (Schwartz 1990; De Villiers & Pringle 2007, 2011; Allsopp 2010), tomatoes and other fruit and vegetable crops, while *M. persicae* is a known vector of two potato viruses in the area (van der Waals et al. 2013). Their propensity to develop resistance to chemical pesticides greatly complicates the control of the above pests, necessitating the development of alternative control strategies.

Laboratory experiments were conducted to assess the development, reproduction, intrinsic growth rates and predation capacities of *O. thripoborus* and *O. naivashae* on different life-stages of *F. occidentalis*, *T. urticae* and *M. persicae nicotianae*. Predation capacities of female adults of *O. thripoborus* and *O. naivashae* on *F. serrata* adults were also quantified. In **Chapter 4**, it was suggested

that, based on their developmental and reproductive performance at different constant temperatures, *O. thripoborus* is adapted to a slightly cooler temperature range as compared with *O. naivashae*. The second objective of the present chapter was to elucidate whether a similar temperature effect on the predation capacity of these predators can be observed. Therefore, predation by female adults of *O. thripoborus* and *O. naivashae* was examined at 19, 25 and 29°C, using larvae of *F. occidentalis* as prey. These experiments are aimed to allow a better insight into the potential of the anthocorids as biological control agents of a range of agricultural pests in southern Africa.

6.2 Materials and Methods

6.2.1 Stock culture

6.2.1.1 Orius thripoborus and O. naivashae

Stock colonies of *O. thripoborus* and *O. naivashae* were reared as described in **section 4.2.1**.

6.2.1.2 Frankliniella occidentalis

A laboratory population of *F. occidentalis* was established in 2011 using insects collected on rose plants (*Rosa* spp.) in Belgian greenhouses. The thrips were reared on green bean pods (*Phaseolus vulgaris* L.), serving as an oviposition substrate and food source, placed on a layer of vermiculite in vented plastic boxes. The diet of adult *F. occidentalis* was supplemented with dry honeybee pollen to enhance reproduction. Rearing containers were kept in an incubator set at 23 ± 1°C, 65 ± 5% RH and a 16:8 (L:D) h photoperiod.

6.2.1.3 Myzus persicae subsp. nicotianae

A colony of *M. persicae nicotianae* was started in 2012 with individuals provided by Koppert B.V. and maintained at ambient laboratory conditions on sharp pepper plants.

6.2.1.4 Tetranychus urticae

Two-spotted spider mites were collected from castor bean (*Ricinus communis* L.) at the Faculty of Bioscience Engineering of Ghent University and a laboratory colony was set up using broad bean (*Vicia faba* L.) plants. The infested plants were kept in ventilated Plexiglas containers (60 x 60 x 60 cm) at ambient laboratory conditions.

6.2.2 Experiments

Except for the assessment of the predation capacity on *F. serrata*, all experiments were performed at Ghent University in climatic cabinets set at 25 ± 1°C, 65 ± 5% RH and a 16:8 (L:D) h photoperiod. Only for the experiments described in **section 6.2.2.2**, two additional temperatures, i.e., 19 and 29°C, were tested. Predation on *F. serrata* was assessed in the laboratories of the SASRI, Mount Edgecombe, South Africa, in a climatised room set at 25 ± 1°C, 75 ± 5% RH and a 14:10 (L:D) h photoperiod.

For each experiment, similar plastic containers were used (4.5 cm diameter, 2 cm high), the lids of which had a ventilation hole covered with fine-mesh gauze.

6.2.2.1 Predation capacity on different prey

Predation capacities of the 2nd and 4th instars, and female adults of *O. thripoborus* and *O. naivashae* were assessed on different stages and species of prey: 2nd instars and adults of *F. occidentalis*, 2nd to 3rd instars of *M. persicae nicotiana*e, and eggs and deutonymphs of *T. urticae*. The predator/prey combination between 2nd instar *Orius* nymphs and *F. occidentalis* adults was not examined, as preliminary experiments indicated that the thrips adults were too agile to serve as prey for the small predator nymphs.

Newly moulted (< 24 h) second and fourth instars, and 3- to 5-day-old female adults (i.e., reproductively active) of *O. thripoborus* and *O. naivashae* were collected randomly from stock cultures. Fourth instars and adults were individually starved for 24 h, during which time water was provided by way of a moist piece of cotton wadding fitted into a 1.5 cm plastic dish. Second instars of the predators were only starved for 16 h. After starvation, each predator was transferred to an individual plastic cup containing an excess of prey and a plant substrate serving as food for the prey and a moisture source for the predator. The number of prey presented in each predator/prey combination was determined based on preliminary experiments and is given in **Table 6.1**. In these preliminary experiments, individual predators confined in 1.5 cm dishes (5-10 replicates per treatment) were offered ad libitum prey during 24 h, after which the number of dead prey was counted. In all treatments, more prey was provided than could be consumed.

When *F. occidentalis* were used as prey, a piece of bean pod was added to the container. The pod was cut between two seeds and fixed on a small pile of Pritt Buddies (N.V. Henkel, Brussel, Belgium), in order to limit hiding places for the prey. For *M. persicae nicotianae*, a reversed *C. annuum* leaf was

placed on water-soaked cotton. *T. urticae* eggs and nymphs were offered on a circular bean leaf disc (4 cm diameter) placed upside down on a 7 mm layer of agar (1% w/w). *T. urticae* eggs were obtained by placing 8 to 10 females for 24 h on the leaf discs (**Figure 6.1**). Females were removed and the surplus eggs were pierced with a fine needle. All other prey stages were directly transferred from the stock colonies using a fine brush.

Table 6.1 Species and numbers of prey offered for the different predator/prey combinations

Predator stage	Prey species and stage				
	F. occidentalis		*M. persicae nicotianae*	*T. urticae*	
	Adults	Larvae	Nymphs	Eggs	Nymphs
2nd Instar	-	20	10	20	20
4th Instar	15	30	15	30	20
Adult female	20	40	20	40	20

Figure 6.1 Arenas used for assessing the predation capacity of Orius sp. on F. occidentalis (left) , M. persicae nicotianae (middle) and T. urticae (right) (photos: L. De Hauwere)

After 24 h the number of dead and live prey were counted. Data from predators that died during the 24 h test period were omitted from analysis. The number of replicates per treatment varied from 15 to 25. To check natural mortality of the prey in the absence of the predator, 10 to 15 containers were set up as a control treatment using the prey densities given in **Table 6.1**. If control mortality exceeded 5%, the number of prey consumed by the predator in 24 h was corrected using Abbott's formula (Abbott 1925).

For assessing the predation capacity of 1- to 2-day old females of *O. thripoborus* and *O. naivashae* on adults of *F. serrata*, 20 field-collected adult thrips (Mount Edgecombe, South Africa; **see Chapter 3**) and a piece of sugarcane stalk were added to each container. The predators used in these experiments

originated from the stock colonies established at Ghent University and were reared as described in **section 4.2.1**, in the facilities of the SASRI. Predation capacities were quantified as explained above for *Orius* adults on *F. occidentalis* adults.

6.2.2.2 Predation capacity at different temperatures

Predation capacities of 4th instars and female adults of *O. thripoborus* and *O. naivashae* were assessed on 2nd instars of *F. occidentalis* at 19, 25 and 29°C. The experimental setup was similar as described in **section 6.2.2.1**. The number of prey presented for each temperature/predator stage combination was determined based on preliminary experiments and is given in **Table 6.3**.

6.2.2.3 Effect of prey on development and reproduction

The developmental and reproductive performance of *O. thripoborus* and *O. naivashae* fed on the same prey species as in **section 6.2.2.1** was studied here. Unlike in the first experiment, mixed stages of each prey species were offered as food, i.e., 1st and 2nd instars of *F. occidentalis*, nymphs and adults of *M. persicae nicotianae*, and eggs, nymphs and adults of *T. urticae*. In each treatment, a fresh, flat green bean pod was provided as a water source and substrate for predators and prey. All prey were supplied ad libitum and replenished every other day.

6.2.2.3.1 Development

In all treatments, 50 to 100 first instars (<24 h old) of the two *Orius* species were caged in individual plastic containers. Development and survival of nymphs were monitored and recorded daily, and newly emerged adults were sexed and weighed using a Sartorius Genius ME215P balance (Sartorius, Goettingen, Germany).

6.2.2.3.2 Reproduction

On each tested prey, newly emerged adults (<24 h old) were paired and transferred to individual plastic containers. The adults were offered the same prey as during their nymphal life. Bean pods were checked daily for eggs to determine the pre-oviposition period. When the first egg was laid, bean pods were replaced every other day with fresh ones, until the female died. Lifetime oviposition and egg hatch were also monitored. As developmental experiments for *O. naivashae* did not yield sufficient males because of strongly skewed sex ratios (**see other Chapters**), 20 to 45% of the females over the different treatments were paired with 1- to 2-day-old males from the stock colony in order to have at

least 20 replicates (= couples) per treatment. Longevities of paired males and females were also examined.

6.2.2.3.3 Intrinsic rate of increase

Daily age-specific survival and age-specific fecundity were used to calculate the intrinsic rate of increase (r_m) of the two predatory species, expressed as the number of females per female per day, using the formula of Birch (1948): $\sum l_x m_x e^{-r_m x} = 1$, where x equals the female age (days), l_x is the age specific survival of the females at age x and m_x is the number of daughters produced per female in the age interval x. The latter parameter is obtained by multiplying the mean number of eggs laid per female by the proportion of female progeny produced at age x. The Jackknife procedure was used according to Meyer et al. (1986) and Hulting et al. (1990) to calculate the standard error of r_m.

6.2.3 Data analysis

If the data were continuous and a Kolmogorov-Smirnov test indicated that the data were normally distributed, the parameter was analysed using analysis of variance (ANOVA). When continuous data were not normally distributed, a non-parametric Kruskal-Wallis H test was used. In the case of non-continuous data, a generalised linear model was used with the link function and error distribution depending on the nature of the data. Each analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. Countable data were analysed using a generalised linear model, with a Poisson distribution if applicable or a negative binomial distribution in case of overdispersion, as determined by the deviance and Pearson goodness-of-fit statistics (Hilbe 2011). If none of the generalised linear models were applicable, a non-parametric model was applied. Parameters expressed as percentages (binary) were compared by means of a logistic regression. This regression is a generalised linear model using a probit (log odds) link and a binomial error function (McCullagh and Nelder 1989). For all studied parameters, a two-factor analysis was applied using the appropriate model (2-way ANOVA or generalised linear model). In case a factor with two degrees of freedom (df) was found to be significant, a post-hoc analysis was performed to separate means. When interaction between the factors was found, means were compared pairwise. Predation capacities on *F. serrata* were compared using a t-test for equality of means. Sex ratios were tested versus an equal male:female distribution (1:1 ratio) by means of Chi-square tests. All of the

above statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp 2012). In case predation capacities had to be corrected with Abbott's formula (Abbott 1925), the statistical program R 3.0.1 (R Core Team 2014) was used to perform a generalised linear model (Poisson or negative binomial) with non-integer values.

6.3 Results

6.3.1 Predation capacity on different prey

Numbers of prey killed per day by nymphs and adults of *O. thripoborus* and *O. naivashae* are presented in **Table 6.2**. A two-factor analysis with predator species and life-stage as factors indicated no interaction between these when *T. urticae* deutonymphs were offered as prey ($F = 0.533$, $df = 2, 97$, $P = 0.588$). Predation of *T. urticae* deutonymphs was affected by predator species ($F = 9.348$, $df = 1, 97$, $P = 0.003$), with all tested stages of *O. thripoborus* killing more *T. urticae* deutonymphs than the corresponding stages of *O. naivashae*. Predator life-stage also influenced the predation capacity of *O. thripoborus* and *O. naivashae* on *T. urticae* deutonymphs ($F = 21.434$, $df = 2, 97$, $P < 0.001$). Adults killed more *T. urticae* deutonymphs than did the predator nymphs, but no significant difference in predation was observed between second and fourth nymphal instars of the predators. Predation on *T. urticae* eggs was affected by predator life-stage ($\chi^2 = 74.570$, $df = 2$, $P < 0.001$), but not by predator species ($\chi^2 = 0.422$, $df = 1$, $P = 0.516$). There was also a significant combined effect of predator life-stage and species on the predation of *T. urticae* eggs ($\chi^2 = 6.733$, $df = 2$, $P = 0.035$). Fourth instar nymphs and females of *O. thripoborus* killed similar numbers of *T. urticae* eggs, but the predation capacity of its second instar nymphs was significantly lower ($\chi^2 = 80.667$, $df = 5$, $P < 0.001$). For *O. naivashae,* on the other hand, the predation of *T. urticae* eggs increased with age of the predator. No differences in predation capacity on *T. urticae* eggs between corresponding life-stages of *O. thripoborus* and *O. naivashae* were found.

Predation on *F. occidentalis* adults was affected by predator species, predator life-stage and their interaction ($F = 27.343$, $df = 1, 71$, $P < 0.001$; $F = 17.341$, $df = 1, 71$, $P < 0.001$ and $F = 18.671$, $df = 1, 71$, $P < 0.001$, respectively). Predation capacities on *F. occidentalis* larvae were affected by predator life-stage ($\chi^2 = 416.642$, $df = 2$, $P < 0.001$), but not by predator species ($\chi^2 = 0.107$, $df = 1$, $P = 0.744$). Also, the interaction effect of predator life-stage and species on the predation on *F. occidentalis* larvae was

Table 6.2 Predation capacities (means ± SE), expressed as the number of prey killed in 24 hours, by different life-stages of O. thripoborus and O. naivashae on different prey species and stages (25°C, 16:8 (L:D) h and 65 ± 5% RH)

Prey		*O. thripoborus*			*O. naivashae*		
Species	**Life-stage**	**2nd instar**	**4th instar**	**Adult female**	**2nd instar**	**4th instar**	**Adult female**
F. occidentalis	Larvae (2nd instar)	2.85 ± 0.33e (19)[a]	11.17 ± 1.02cA (18)	23.94 ± 1.07aA (17)	4.10 ± 0.43d (19)[a]	9.20 ± 0.81cA (15)	17.64 ± 1.18bA (25)
	Adults	-[b]	4.73 ± 0.26bB (22)	7.87 ± 0.41aB (15)	-[b]	4.39 ± 0.28bB (23)	4.33 ± 0.57bB (15)
M. persicae nicotianae	Nymphs (2nd-3rd instar)	1.62 ± 0.15c (16)	5.38 ± 0.56a (16)	5.06 ± 0.76a (18)	2.61 ± 0.22bc (18)	5.71 ± 0.37a (17)	3.60 ± 0.67b (15)
T. urticae	Eggs	6.29 ± 1.09cA (17)	15.39 ± 1.37abA (18)	15.32 ± 1.41abA (25)	4.70 ± 0.86cA (20)	12.00 ± 1.67bA (17)	21.05 ± 2.44aA (20)
	Deutonymphs	5.94 ± 0.51bA (18)	6.82 ± 0.70bB (17)	9.71 ± 0.48aB (17)	4.81 ± 0.45dA (16)	5.94 ± 0.43dB (17)	7.78 ± 0.56cB (18)

Means within a row followed by the same lowercase letter are not significantly different ($P > 0.05$): generalized linear model with Poisson distribution (*F. occidentalis* larvae; *M. persicae nicotianae* nymphs); generalized linear model with negative binomial distribution (*T. urticae* eggs); Mann-Whitney U test (*F. occidentalis* adults); or Tukey's HSD test (*T. urticae* deutonymphs)

Means within a column and a prey species followed by the same uppercase letter are not significantly different ($P > 0.05$): Mann-Whitney U test (*O. thripoborus* adults on *F. occidentalis*; *O. naivashae* 4th instars on *F. occidentalis*); generalized linear model with Poisson distribution (*O. thripoborus* 4th instars on *F. occidentalis*); or generalized linear model with negative binomial distribution (*O. naivashae* adults on *F. occidentalis*; all predators on *T. urticae*)

The number of individuals tested is placed in parentheses

[a] Corrected with Abbott's formula (Abbott, 1925)

[b] Not done

significant (χ^2 = 13.925, df = 2, *P* = 0.001). The younger (and smaller) the life-stage of *F. occidentalis* and *T. urticae*, the more prey individuals were killed by fourth instar nymphs and female adults of both *Orius* species. This is in contrast to when *T. urticae* were used as prey, as then second instar nymphs of *O. thripoborus* and *O. naivashae* had similar predation capacities on *T. urticae* eggs and deutonymphs. Overall, for both anthocorids, the predation capacity on larvae (χ^2 = 514.352, df = 5, *P* < 0.001) and adults (χ^2 = 27.253, df = 3, *P* < 0.001) of *F. occidentalis* increased with developmental life-stage of the predator. As the only exception, female adult *O. naivashae* did not kill more *F. occidentalis* adults, compared to their fourth instar nymphs.

Predation capacities on nymphs of *M. persicae nicotianae* were affected by predator life-stage (χ^2 = 47.904, df = 2, *P* < 0.001), but not by predator species (χ^2 = 0.340, df = 1, *P* = 0.560). The interaction between both factors was significant (χ^2 = 7.828, df = 2, *P* = 0.020). Fourth instar nymphs of both *Orius* species killed significantly more nymphs of *M. persicae nicotianae* than did their second instar nymphs, and performed as well as, if not better on *M. persicae nicotianae* than did the predatory adults (χ^2 = 52.427, df = 5, *P* < 0.001). Predation capacities on *M. persicae nicotianae* nymphs only differed between the anthocorid species in the adult life-stage, with *O. thripoborus* females killing more *M. persicae nicotianae* than those of *O. naivashae* (5.06 and 3.60 nymphs per day, respectively).

Predation capacities on *F. serrata* adults by 1- to 2-day old females of *O. thripoborus* (10.53 ± 0.64 adults/24 h; 21 replications) and *O. naivashae* (9.06 ± 0.75 adults/24 h; 20 replications) were similar (*P* = 0.137).

6.3.2 Predation capacity at different temperatures

Numbers of *F. occidentalis* larvae killed per day by 4th instars and female adults of *O. thripoborus* and *O. naivashae* at 19, 25 and 29°C are shown in **Figure 6.2**.

A multi-factor analysis with temperature, predator species and predator stage as factors indicated that predation on *F. occidentalis* larvae was affected by temperature, predator species, predator stage, the temperature x predator species interaction and the temperature x predator stage interaction (χ^2 = 385.127, df = 2, *P* < 0.001; χ^2 = 38.909, df = 1, *P* < 0.001; χ^2 = 320.510, df = 1, *P* < 0.001; χ^2 = 22.053, df = 2, *P* < 0.001 and χ^2 = 22.478, df = 2, *P* < 0.001, respectively), but not by the species x predator stage interaction and the threefold interaction between all factors (χ^2 = 3.050, df = 1, *P* = 0.081 and χ^2 =

4.790, df = 2, *P* = 0.091, respectively). Predation increased with increasing temperature, except for *O. naivashae*, which killed no more thrips larvae at 29°C than at 25°C. Irrespective of temperature and predator species, female adults of the *Orius* spp. killed twice as many *F. occidentalis* larvae per day as did their 4th instars. However, when comparing the two predator species, some marked predator stage-dependent differences in predation capacity occurred. Predation by 4th instars of both *Orius* spp. was similar at 19 and 25°C, but at 29°C, 4th instars of *O. thripoborus* killed substantially more thrips larvae than those of *O. naivashae*. For female adults, however, predation capacity of *O. thripoborus* was higher than that of *O. naivashae*, except at 25°C (**Figure 6.2**).

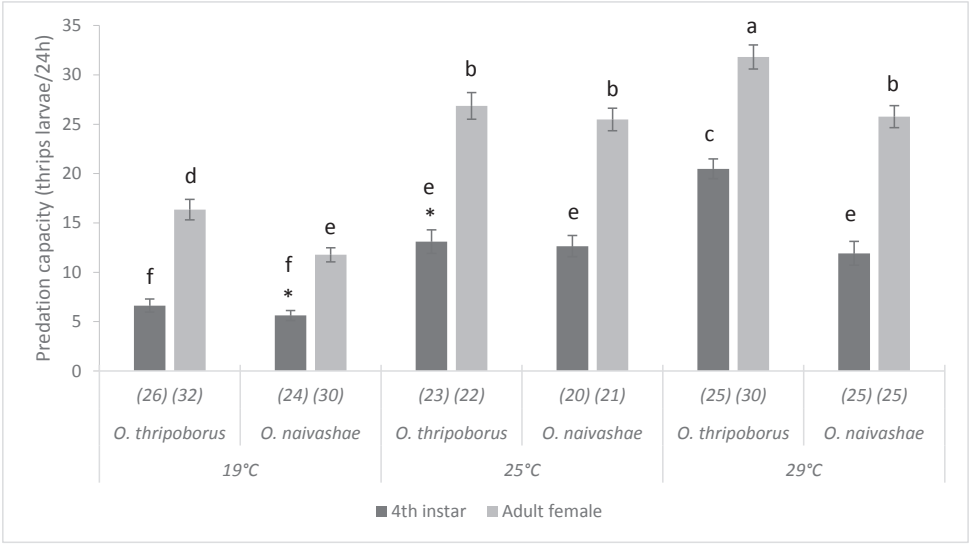

Figure 6.2 Predation capacities (means ± SE), expressed as the number of thrips larvae killed in 24 hours, by fourth instars and adult females of O. thripoborus and O. naivashae on 2nd instars of F. occidentalis at three temperatures, 16:8 (L:D) h photoperiod and 65 ± 5% RH.

Bars with the same letter are not significantly different (P > 0.05): generalized linear model with negative binomial distribution. For bars with an asterisk, the predation capacity was corrected with Abbott's formula (Abbott 1925).

The number of individuals tested is placed in parentheses below each bar

6.3.3 Development

Developmental parameters of *O. thripoborus* and *O. naivashae* as a function of prey species are shown in **Table 6.3**. The species of prey and species of predator affected nymphal survival and male adult weight (nymphal survival: χ^2 = 8.885; df = 2, *P* = 0.012 and χ^2 = 5.489, df = 1, *P* = 0.019; male adult

weight: F = 14.727, df = 2, 126, *P* < 0.001 and F = 20.646, df = 1, 126 and *P* < 0.001 for prey and predator species, respectively). The interaction between the factors, however, was not significant (χ^2 = 3.678, df = 2, *P* = 0.159 and F = 2.238, df = 2, 126, *P* = 0.111 for nymphal survival and male adult weight, respectively). Nymphal survival until adulthood ranged from 73.8 to 80.8% for *O. thripoborus,* and from 61.4 to 83.1% for *O. naivashae*. Regardless of prey species, survival of *O. thripoborus* nymphs to adulthood was higher than that of *O. naivashae*. For both anthocorids, feeding on *F. occidentalis* resulted in the highest nymphal survival to adulthood, whilst *M. persicae nicotianae* and *T. urticae* as prey resulted in lower survival rates.

Orius naivashae males were always heavier than those of *O. thripoborus*. Further, body weights of male *Orius* of both species were highest when fed on *F. occidentalis* and lowest on *M. persicae nicotianae*. In both anthocorids, males presented with *T. urticae* had intermediate weights between those of males fed on the other two prey species (**Table 6.3**).

Body weights of female adults were affected by predator species, predator stage and their interaction (F = 22.211; df = 2, 184, *P* < 0.001; F = 23.088; df = 1, 184, *P* < 0.001 and F = 11.702, df = 1, 184, *P* < 0.001, respectively). *O. naivashae* females were heavier than those of *O. thripoborus* fed on a diet of *F. occidentalis* or *T. urticae*, but female weights of *O. thripoborus* (0.319 mg) and *O. naivashae* (0.310 mg) on *M. persicae nicotianae* did not differ (F = 21.867; df = 5, 184; *P* < 0.001) (**Table 6.3**). For *O. naivashae,* similar effects of prey type on adult body weights as observed for males, were reflected in female body weight. Adult weights of *O. thripoborus* females, on the other hand, were highest when fed on *F. occidentalis* (0.342 mg), and lowest when fed on *T. urticae* (0.298 mg), but when fed on *M. persicae nicotianae* their weights did not differ from those when fed on the other two prey species.

A two-way ANOVA indicated a significant effect of predator species and its interaction with prey species on male (F = 5.955, df = 1, 126, *P* = 0.016 and F= 11.702, df = 2, 126, *P* < 0.001, respectively) and female developmental times (F = 1.412, df = 1, 184, *P* < 0.001 and F = 68.442, df = 2, 184, *P* < 0.001, respectively). The influence of prey species on predator developmental time was significant for males (F = 50.503, df = 2, 126, *P* < 0.001), but not for females (F = 65.090, df = 2, 184, *P* = 0.236). Developmental times of *O. thripoborus* at 25°C fluctuated between 13.4 and 14.4 days for females and between 13.3 and 14.6 days for males; for *O. naivashae*, they ranged from 11.6 to 16.3 days for females

Table 6.3 Developmental parameters (means ± SE) of O. thripoborus and O. naivashae on different prey species (25°C, 16:8 (L:D) h and 65 ± 5% RH)

Predator species	Prey species	n[a]	Nymphal survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio (male:female)[b]
				Females	Males	Females	Males	
O. thripoborus	*F. occidentalis*	52	80.8 ± 5.5a	13.4 ± 0.3bc	13.3 ± 0.1b	0.342 ± 0.009bc	0.280 ± 0.006c	1:0.75
	M. persicae nicotianae	75	80.0 ± 4.6c	13.7 ± 0.2c	14.2 ± 0.2c	0.319 ± 0.006cd	0.240 ± 0.007f	1:1.31
	T. urticae	65	73.8 ± 5.5c	14.4 ± 0.2d	14.6 ± 0.2c	0.298 ± 0.005d	0.251 ± 0.005e	1:1
O. naivashae	*F. occidentalis*	77	83.1 ± 4.3b	11.6 ± 0.09a	12.1 ± 0.2a	0.387 ± 0.005a	0.290 ± 0.004a	1:1.78*
	M. persicae nicotianae	101	61.4 ± 4.9d	16.3 ± 0.3e	15.7 ± 0.4d	0.310 ± 0.01cd	0.265 ± 0.007d	1:2.65*
	T. urticae	73	63.0 ± 5.7d	13.0 ± 0.2b	12.9 ± 0.1b	0.355 ± 0.006b	0.286 ± 0.006b	1:1.55

Means within a column followed by the same letter are not significantly different ($P > 0.05$): Tamhane's test (female adult weights); Mann-Whitney U test (developmental times); binary probit test (nymphal survival); or Tukey's HSD test (male adult weights)

[a] Initial number of first instars tested

[b] Values with an asterisk differ significantly from a 1:1 ratio; χ^2 and P values were 0.857, 0.355; 1.067, 0.302; < 0.001, 1.000; 5.063, 0.024; 12.645, < 0.001; and 2.174, 0.140, respectively (Chi-square test, df = 1)

and from 12.1 to 15.7 days for males (**Table 6.3**). Males (χ^2 = 76.793; df = 5; P < 0.001) and females (χ^2 = 133.431; df = 5; P < 0.001) of *O. naivashae* developed faster than those of *O. thripoborus* when fed on *F. occidentalis* or *T. urticae*, but on *M. persicae nicotianae* the opposite was observed. In both anthocorids, developmental times were shortest when *F. occidentalis* were offered. There was no significant difference in developmental time of *O. thripoborus* females fed on *F. occidentalis* or *M. persicae nicotianae*. Longest development times of *O. thripoborus* were recorded when it was fed on *T. urticae*, although nymphal development of males fed on *M. persicae nicotianae* was similar to that with *T. urticae* as prey. Developmental times of *O. naivashae* were longest when fed on *M. persicae nicotianae* (**Table 6.3**).

Sex ratios of *O. naivashae* were female biased when presented with *F. occidentalis* or *M. persicae nicotianae*. For *O. thripoborus*, no significant deviation from a 1:1 sex ratio was observed (**Table 6.3**).

6.3.4 Reproduction

Reproductive traits and longevities of *O. thripoborus* and *O. naivashae* reared on the three different prey types are presented in **Table 6.4**. The proportion of ovipositing females ranged between 84.0 and 96.2% for *O. thripoborus* and between 58.6 and 85.2% for *O. naivashae* (**Table 6.4**). Prey and predator species influenced the percentage of ovipositing females (χ^2 = 7.656, df = 2, P = 0.022 and χ^2 = 5.394, df = 1, P = 0.020 for prey and predator species, respectively), whereas their combined effect was not significant (χ^2 = 1.064, df = 2, P = 0.587). In general, proportionally more *O. thripoborus* than *O. naivashae* females produced eggs. The best prey in terms of this parameter were *T. urticae* and *F. occidentalis*. *Myzus persicae nicotianae* as prey resulted in the lowest proportion of egg producing females, especially for *O. naivashae* (58.6%).

Pre-oviposition period was only affected by prey species (χ^2 = 7.958, df = 2, P = 0.019; χ^2 = 3.572, df = 1, P = 0.059 and χ^2 = 3.698, df = 2, P = 0.157 for prey species, predator species, and their interaction, respectively). Pre-oviposition periods for females of both *Orius* species were similar on *F. occidentalis* and *T. urticae*, whereas *M. persicae nicotianae* as prey resulted in the longest pre-oviposition period.

The oviposition period was influenced by prey and predator species (χ^2 = 29.063, df = 2, P < 0.001 and χ^2 = 53.621, df = 1, P < 0.001, respectively), but not by their interaction (χ^2 = 5.150, df = 2, P = 0.076) ranging from 15.3 to 18.9 days for *O. thripoborus* and from 5.3 to 15.3 days for *O. naivashae* (**Table 6.4**). Regardless of prey, oviposition periods of *O. thripoborus* females were longer than those of *O.*

naivashae. *Frankliniella occidentalis* always resulted in longer oviposition periods than did *M. persicae nicotianae* or *T. urticae*.

Lifetime oviposition was affected by prey and predator species (χ^2 = 50.174, df = 2, P < 0.001 and χ^2 = 58.390, df = 1, P < 0.001, respectively), but not by their interaction (χ^2 = 3.416, df = 2, P = 0.181). Higher fecundities were observed for *O. thripoborus* (40.2 to 122.6 eggs) than for *O. naivashae* (5.3 to 67.5 eggs) (**Table 6.4**). Female *O. thripoborus* and *O. naivashae* reared on *F. occidentalis* produced 2 and 6 times more eggs than when reared on *M. persicae nicotianae*, and 3 and 8 times more than when reared on *T. urticae*, respectively.

Egg hatch of the studied *Orius* species never exceeded 70% and was affected by predator species, predator life-stage and their interaction (χ^2 = 21.005, df = 2, P < 0.001; χ^2 = 11.529, df = 1, P < 0.001 and χ^2 = 7.822, df = 2, P = 0.020, respectively). *Orius thripoborus* egg hatch percentages were higher when fed on *F. occidentalis* and *M. persicae nicotianae*, than when fed on *T. urticae*. *Orius naivashae* egg hatchability when fed on *F. occidentalis* or *T. urticae* was higher than that when fed on *M. persicae nicotianae* (χ^2 = 28.037, df = 5, P < 0.001) (**Table 6.4**).

Male and female longevities were affected by predator species, predator life-stage and their interaction (males: χ^2 = 47.001, df = 2, P < 0.001; χ^2 = 14.697, df = 1, P < 0.001 and χ^2 = 17.202, df = 2, P < 0.001, respectively; females: χ^2 = 27.499, df = 2, P < 0.001; χ^2 = 40.654, df = 1, P < 0.001 and χ^2 = 29.202, df = 2, P < 0.001, respectively). Male and female longevities were longer for *O. thripoborus* than for *O. naivashae*, except on a diet of *F. occidentalis* which yielded similar longevities (males: χ^2 = 66.202, df = 5, P < 0.001; females: χ^2 = 94.170, df = 2, P < 0.001). Adult *O. naivashae* lived longest when fed on a diet of *F. occidentalis*, half as long when fed *M. persicae nicotianae* and shortest when fed *T. urticae* as prey. Female *O. thripoborus* lived significantly longer when fed on *M. persicae nicotianae* compared to the other two prey species. Male *O. thripoborus* had similar longevities when fed on *F. occidentalis* or *M. persicae nicotianae* as prey; longevity of males of this predatory species fed on *T. urticae* was similar to that on *M. persicae nicotianae*, but shorter than that on *F. occidentalis* (**Table 6.4**).

There was a significant effect of the factors prey species and predator species on the intrinsic rate of increase (r_m) (F = 76.755, df = 2, 145, P < 0.001 and F = 82.543, df = 1, 145, P < 0.001, respectively), and the interaction between the factors was also significant (F = 24.469, df = 2, 145, P < 0.001). The intrinsic

Table 6.4 Reproductive parameters, longevity and intrinsic rate of increase (r_m) (means ± SE) of O. thripoborus and O. naivashae on different prey species (25°C, 16:8 (L:D) h and 65 ± 5% RH)

Predator species	Prey species	Proportion of ovipositing females (%)[a]	Pre-oviposition period (days)	Oviposition period (days)	Lifetime oviposition	Egg hatch (%)	Longevity (days)		r_m
							Males	Females	
O. thripoborus	*F. occidentalis*	88.9 ± 7.6a (18)	4.06 ± 0.42a	18.9 ± 1.4a	122.6 ± 11.5a	70.0 ± 1.0a	25.6 ± 2.2a	21.2 ± 2.3b	0,1232 ± 0,0050a
	M. persicae nicotianae	84.0 ± 7.5c (25)	5.86 ± 0.37c	18.7 ± 2.4b	67.7 ± 10.0b	67.2 ± 1.3ab	22.8 ± 2.2ab	29.6 ± 2.3a	0,0966 ± 0,0061b
	T. urticae	96.2 ± 3.8a (26)	5.08 ± 0.58a	15.3 ± 1.7b	40.2 ± 6.2d	65.2 ± 1.5b	18.9 ± 1.7bc	22.0 ± 2.4b	0,0725 ± 0,0056c
O. naivashae	*F. occidentalis*	85.2 ± 7.0b (27)	5.83 ± 0.67b	15.3 ± 1.4c	67.5 ± 8.7c	67.2 ± 1.2ab	29.5 ± 2.1a	23.5 ± 1.7ab	0,1311 ± 0,0076a
	M. persicae nicotianae	58.6 ± 9.3d (29)	6.82 ± 0.70d	6.2 ± 1.3d	11.1 ± 2.7e	52.5 ± 3.7c	14.2 ± 2.5c	14.9 ± 1.7c	-0,0017 ± 0,0120d
	T. urticae	84.0 ± 7.5b (25)	4.95 ± 0.20b	5.3 ± 0.7d	5.3 ± 0.7f	58.7 ± 5.7bc	9.0 ± 2.0d	8.8 ± 0.4d	-0,0254 ± 0,0087d

Means within a column followed by the same letter are not significantly different ($P > 0.05$): binary probit test (proportion of ovipositing females; egg hatch); generalized linear model with Poisson distribution (pre-oviposition period); generalized linear model with negative binomial distribution (oviposition period; lifetime oviposition; longevity); or Mann-Whitney U test (r_m)

[a] The number of adult pairs tested is placed in parentheses.

rates of increase of *O. thripoborus* and *O. naivashae* were highest when reared on *F. occidentalis,* with similar values of 0.123 and 0.131 females/female/day, respectively (χ^2 = 96.568; df = 5; *P* < 0.001). On the other prey, *O. thripoborus* showed significantly higher r_m-values than *O. naivashae*. Overall, growth rates of both *Orius* species when fed on *M. persicae nicotianae* were higher than when fed on *T. urticae*, although differences were only significant for *O. thripoborus* (**Table 6.4**).

6.4 Discussion

Although *Orius* species are considered generalist predators attacking a wide array of arthropod prey, the results of our study on *O. thripoborus* and *O. naivashae* show that the type of prey can have considerable impact on the performance of the predators. Predation capacities and intrinsic rates of increase of the studied anthocorids showed large variations when they were offered different prey species and life-stages. Our results indicate that *F. occidentalis* larvae are the most suitable as food, amongst the arthropod prey tested, for both *O. thripoborus* and *O. naivashae*. This is demonstrated by high survival rates, short developmental times, and favourable reproductive parameters of the two predatory species when fed *F. occidentalis*. The estimated r_m-values of *O. thripoborus* and *O. naivashae* in the present study (0.123 and 0.131, respectively) approach those obtained for other thermophilic *Orius* species reared on various thrips species under similar climatic conditions (i.e., 25°C and a 16 h photoperiod). These include *Orius sauteri* (Poppius) on *Thrips palmi* Karny (Thysanoptera: Thripidae) larvae (r_m = 0.128) (Nagai & Yano 1999) and *Orius albidipennis* (Reuter) on *F. occidentalis* adults (r_m = 0.121) (Cocuzza et al. 1997a).

Several studies have shown that *E. kuehniella* eggs constitute a nutritionally superior food for *Orius* species (e.g., Cocuzza et al. 1997b; Ferkovich & Shapiro 2004; Bonte & De Clercq 2008; De Clercq et al. 2013; **and other Chapters**). The population growth rates of *O. thripoborus* and *O. naivashae* reared on *E. kuehniella* eggs (Van de Walle 2014; J. Bonte, unpublished data) compared well with those reared on *F. occidentalis* (this study). *Orius naivashae* (r_m = 0.138 on *E. kuehniella* eggs) performed similarly on both hosts, but *O. thripoborus* had a much higher intrinsic growth rate when fed on *E. kuehniella* (r_m = 0.159). Even though the r_m-values of *O. thripoborus* and *O. naivashae* fed on *F. occidentalis* were similar, *O. thripoborus* produced almost twice as many eggs as did *O. naivashae*. This is related to the faster development rate of the latter species and its female biased sex ratio (**see other Chapters**).

In addition to favourable intrinsic rates of increase on *F. occidentalis* larvae, *O. thripoborus* and *O. naivashae* killed higher numbers of *F. occidentalis* larvae than any of the other prey types tested. This is reflected in the findings for other *Orius* species, such as *O. sauteri* (Kohno & Kashio 1998), *Orius insidiosus* (Say) (Tommasini & Nicoli 1994) and *Orius laevigatus* (Fieber) (Bonte & De Clercq 2010b). High predation capacities are, however, not always an indication of prey suitability. Mendes et al. (2002) found that high prey consumption by a predatory anthocorid may occur to fill a nutritional gap caused by low quality prey. In contrast, a relatively small amount of *E. kuehniella* eggs, a nutritionally high quality prey type, is sufficient to successfully rear *Orius* species (Yano et al. 2002; Mendes et al. 2002). However, other factors such as prey mobility, or prey defense tactics are important factors to consider in prey selection and attack by a predator (De Clercq & Degheele 1994; Eubanks & Denno 2000). Butler and O'Neil (2006) and Desneux and O'Neil (2008) recorded defensive mechanisms of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), against *O. insidiosus*, which were more obvious and effective than those observed for *F. occidentalis*.

Within a limited temperature range, the activity of an insect increases with increasing temperature, leading to higher levels of predation in insect predators (Rabbinge 1976; McCaffrey & Horsburgh 1986; Cocuzza et al. 1997a; Nagai & Yano 1999). In this study, the number of *F. occidentalis* larvae consumed by fourth instars and female adults of *O. thripoborus* increased with increasing temperature between 19 and 29°C. However, predation of *O. naivashae* at 29°C did not increase compared to that at 25°C. Based on this study, it seems that the optimal temperature for predation fell between 25 and 29°C for *O. naivashae*, whereas this was higher for *O. thripoborus*. This is not in line with the lower temperature preference of *O. thripoborus* compared to *O. naivashae* in terms of development and reproduction (**Chapter 4**).

In the present study, nymphs and adults of both *Orius* species only killed low numbers of *F. occidentalis* adults. Tommasini et al. (2004) found similar predation patterns, ranging from 3.0 to 4.6 *F. occidentalis* adults per day killed by fourth instars of different *Orius* species at 26°C. However, the same authors recorded predation rates for 8-day-old *Orius* adults of between 10.2 and 14.9 *F. occidentalis* adults per day, which are higher than those observed in our study. Three- to five-day-old female adults of *O. thripoborus* in our study killed more *F. occidentalis* in 24 hours than those of *O. naivashae*. However, when offered adults of the sugarcane thrips, *F. serrata,* no marked differences in predation capacity

between the two anthocorids was found, averaging 10.5 and 9.1 adult *F. serrata* per day for *O. thripoborus* and *O. naivashae* respectively. The higher predation on adults of *F. serrata* compared to that on *F. occidentalis* may be due to the smaller size of *F. serrata* (Dixon & Russel 1972; Reitz et al. 2006). Nutritional value and behavior of the prey though, may also influence predator response (Evans 1976; Isenhour & Yeargan 1981b, c; Eubanks & Denno 2000; Mendes et al., 2002; Reitz et al. 2006), as indicated above. Adult thrips are winged, move fast and thus have better ability to escape from predators, such as *Orius* adults. As attacking this very agile type of prey uses more energy than can be gained from feeding on it, the predator may cease hunting it before being satiated (van den Meiracker & Sabelis 1999).

In our study, nymphs of *O. thripoborus* and *O. naivashae* killed only half as many *M. persicae nicotianae* nymphs than they did *F. occidentalis* larvae. Further, adults of both *Orius* species killed similar or even lower numbers of *M. persicae nicotianae* nymphs, compared to those killed by their fourth instars. Nymphal survival and developmental time of *O. thripoborus* on *M. persicae nicotianae* did not differ from that on *F. occidentalis*, but its reproductive performance was lower on *M. persicae nicotianae* than on the *F. occidentalis*. As a result, the intrinsic rate of increase of *O. thripoborus* on *M. persicae nicotianae* (r_m = 0.0966) was significantly lower than on *F. occidentalis*. *Orius naivashae*, on a diet of *M. persicae nicotianae,* yielded a slightly negative growth rate (r_m = -0.0017). This population decrease was due to poor nymphal survival, slow development, low number of ovipositing females, an extended pre-oviposition period, low fecundity and egg hatchability, and shortened longevity on the *M. persicae nicotianae*. The quality of aphids in general as prey for generalist predators has been shown to be lower relative to other prey types (Toft 2005). Mendes et al. (2002) reared *O. insidiosus* on nymphs of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), and on adults of *Caliothrips phaseoli* (Hood) (Thysanoptera: Thripidae) at 25°C and a 12 h photoperiod, and found a faster nymphal development on the latter prey. Yet, in the same study on *O. insidiosus*, prey consumption by nymphs (all stages) and total female fecundity of the predator were two and three times higher on *A. gossypii* than on *C. phaseoli*, respectively. At 25°C and a 15 h photoperiod, Bush et al. (1993) recorded developmental times for *O. insidiosus* on *A. gossypii* and on the greenbug aphid, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), similar to those obtained by Mendes et al. (2002) on *A. gossypii*. However, total egg production for *O. insidiosus* on *A. gossypii* observed by Mendes et al. (2002) was

higher than that reported by Bush et al. (1993) (ca. 70 vs. 19.4 eggs). Besides aphid species (Sengonca et al. 2008), also plant-aphid interactions (Goggin 2007), omnivore-plant relations (Coll & Ridgeway 1995; Coll 1998; Coll & Guershon 2002; Rutledge & O'Neil 2005), (in)direct interactions between aphids and other prey (Desneux a& O'Neil 2008; Messelinck et al. 2013), and intraguild predation (Rosenheim et al. 1995) may influence the behavior of an arthropod predator towards its aphid prey.

Tetranychus urticae also caused negative growth of *O. naivashae* in the present study, as was observed on a diet of *M. persicae nicotianae*. Although most developmental and reproductive parameters of *O. naivashae* were better on *T. urticae* than on *M. persicae nicotianae*, the poor fecundity and longevity on the former prey substantially lowered the predator's intrinsic growth rate. Reduced adult longevities, as noted for *O. naivashae* fed on *M. persicae nicotianae* or *T. urticae*, may be caused by allocation of energy to reproduction in the predator rather than to survival (Nakashima & Hirose 1999). However, *O. thripoborus* showed no such tradeoff between longevity and reproduction.

Orius thripoborus killed more *T. urticae* deutonymphs than *O. naivashae*, but no differences in predation on *T. urticae* eggs were found. Even though *T. urticae* were the worst prey for *O. thripoborus* in terms of development and reproduction, their consumption by the predator still resulted in a positive growth rate (r_m = 0.0725). Intrinsic growth rates reported for other *Orius* species fed on *T. urticae* strongly diverge from that found in the present study but have never been reported to be negative (e.g., 0.0126 females/female/day for *O. laevigatus* at 25°C (Venzon et al. 2002), 0.1279 for *O. albidipennis* at 26°C (Sobhy et al. 2010), 0.097 for *O. minutus* (L.) at 24°C, and 0.039 for *O. niger* Wolff at 24°C (Fathi 2009)). Similar to *O. laevigatus* (Venzon et al. 2002), our study showed that overall fitness of *O. thripoborus* and *O. naivashae* was better when fed on *F. occidentalis* than when fed on *T. urticae*. Under natural conditions, however, prey species may co-occur and influence a predator's responses. Venzon et al. (2000), for example, found that thrips larvae reduced the risk of being attacked by *O. laevigatus* by residing inside the webbing produced by spider mites. In future studies, prey preferences of *O. thripoborus* and *O. naivashae* could be tested by offering these predators assemblages of several prey species both in small scale arenas and on plants .

The anthocorids in the present study killed prey without fully consuming it. This behavior has been observed for other predatory anthocorids, and has been suggested to increase their effectiveness as biological control agents (Isenhour & Yeargan 1981b; De Clercq & Degheele 1994; Kohno & Kashio

1998; Meyling et al. 2003; Yano et al. 2005; Fantinou et al. 2008). However, predator attack rates measured in small arenas with high prey densities may not be realistic. Under such laboratory conditions, prey handling time is the most limiting factor, whereas in a field setting, attack rates will be limited more by searching behavior of the predators (Isenhour & Yeargan 1981b; De Clercq & Degheele 1994; van den Meiracker & Sabelis 1999).

A predator's predation capacity, as well as its developmental and reproductive performance on a given prey, are indicative of its potential value as a biological control agent (van Lenteren & Manzaroli 1999; Grenier & De Clercq 2003). In general, predation capacities of *O. thripoborus* measured in our laboratory experiments were slightly better than those of *O. naivashae*, which may be related to the higher mobility of the former species, based on laboratory observations. High predation and favourable life history characteristics are supportive for considering *O. thripoborus* and *O. naivashae* as biocontrol agents of *F. occidentalis* and likely also other thrips species in southern Africa. For the suppression of *M. persicae nicotianae* and *T. urticae*, however, our findings suggest that only *O. thripoborus* would have potential, as only this species was able to achieve a positive population growth on these prey types. Again, findings from laboratory experiments using small arenas and high densities of prey may not reflect what happens in the field (Isenhour & Yeargan 1981b, c; De Clercq & Degheele 1994). Therefore, semi-field and field trials are needed to fully understand the ecology and ecosystem service potential of the studied anthocorids in different cropping systems in southern Africa.

Chapter 7

Moisture source and diet affect development and reproduction of *Orius thripoborus* and *Orius naivashae*

Redrafter after:

Bonte, J., Vangansbeke, D., Maes, S., Bonte, M., Conlong, D. and De Clercq, P. 2012. Moisture source and diet affect development and reproduction of *Orius thripoborus* and *Orius naivashae*, two predatory anthocorids from southern Africa. *Journal of Insect Science* 12: 1-16.

7.1 Introduction

In order to optimise and rationalise the mass production and release of *O. naivashae* and *O. thripoborus*, it is crucial to understand their nutritional ecology. Important aspects of a successful mass rearing system for these *Orius* spp. include the availability of alternative food sources, adequate water sources and suitable oviposition substrates (**section 2.5.2**).

Plant feeding has been shown to provide moisture and nutrients to numerous predatory heteropterans. Additionally, *Orius* spp. are zoophytophagous, allowing them to benefit from plant materials and animal prey. Several *Orius* spp. are able to develop on certain pollens as a sole food source (**section 2.4.7.2**).

In commercial insectaries, *Orius* bugs are mainly reared on eggs of the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), which constitutes an effective but expensive factitious (i.e., unnatural) food. This has resulted in a search for cheaper alternative foods, such as brine shrimp (*Artemia* sp.) cysts (**section 2.5.2.1**) and various artificial diets (**section 2.5.2.2**).

To evaluate the quality of insects, several biological parameters such as immature developmental time and survival, body weight, fecundity and longevity are routinely used (**section 2.5.4**). In many synovigenic insects, determining lifetime fecundity is a tedious and time-consuming activity. Bonte and De Clercq (2008) proposed a method to assess fecundity of *Orius laevigatus* Fieber based on oocyte counts in dissected female adults. In the latter study, oocyte counts at day eight were strongly correlated with lifetime oviposition of the predator reared on different diets. Similar methods have been developed for *Macrolophus* spp. by Callebaut et al. (2004) and Vandekerkhove et al. (2006).

In the present study, we hypothesized that both *O. thripoborus* and *O. naivashae* are amenable to mass production and may have potential as biological control agents in southern Africa. First, the effect of moisture source on the development of *O. thripoborus* and *O. naivashae* was determined. Second, the impact of several factitious foods and bee pollen on developmental and reproductive parameters of both *Orius* species was studied. Finally, the reliability of the dissection test designed by Bonte and De Clercq (2008) to predict the influence of diet on the reproductive potential of *O. laevigatus*, was investigated for these two little studied *Orius* species from southern Africa.

7.2 Materials and Methods

7.2.1 Stock culture

Stock colonies of *O. naivashae* and *O. thripoborus* were reared as described in **section 4.2.1**.

7.2.2 Influence of water source on development

All experiments were conducted in an incubator set at 23 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 (L:D) h. In a first experiment, the effect of two water sources on the developmental performance of *O. thripoborus* and *O. naivashae* was assessed. In the first treatment, water was provided in hemispherical domes (70 µl) made of Parafilm M using an encapsulation device (ARS, Gainesville, FL, USA). Stretching the Parafilm M before encapsulation facilitated stylet penetration by early instars of the insect. The domes were sealed using transparent tape (Scotch 3M Packaging Super Tape, St. Paul, MN, USA). In the second treatment, a flat green bean pod (*Phaseolus vulgaris* L.) was used as a source of water. Green bean pods were thoroughly washed before being used in the experiment to avoid contamination with pesticide residues. The bean pod was cut between two seeds into 2 to 3 cm pieces to prevent nymphs from hiding inside the bean. As this plant substrate also provides nutrients besides being a source of moisture, 'water source' is an operational term and is not meant to be physiologically defining. Frozen eggs of *E. kuehniella* were supplied as food in both treatments. *Ephestia kuehniella* eggs and water sources were refreshed every other day.

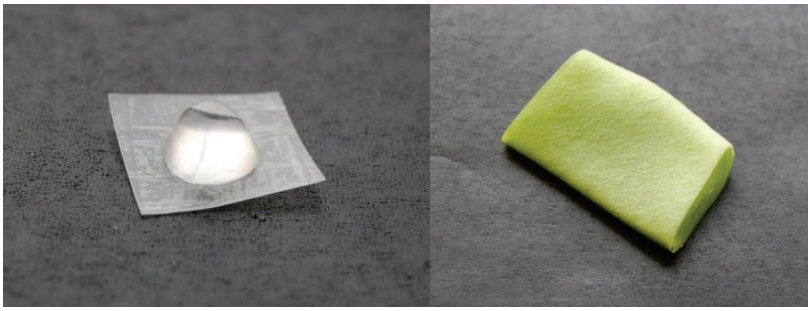

Figure 7.1 Water sources tested on Orius sp.: water-filled Parafilm dome (left) and green bean pod (right) (photos: author)

For each treatment, 40 first instars (<24 h old) were caged in individual plastic containers (4.5 cm diameter, 3 cm high) sealed with a lid having a ventilation hole covered with a fine-mesh gauze.

Development and survival of nymphs were monitored daily, and newly emerged adults were sexed and weighed using a Sartorius Genius ME215P balance (Sartorius, Goettingen, Germany).

A two-way ANOVA was conducted to evaluate whether water source had a different effect on developmental time and body weights of adult males and females of *O. thripoborus* and *O. naivashae*. As no interaction occurred between the main factors water source and species for all tested parameters, means within each factor were separated using the Tukey pairwise comparison procedure (Kutner et al. 2005). Survival rates were compared by means of a logistic regression. This regression is a generalised linear model using a probit (log odds) link and a binomial error function. Each test consists of a regression coefficient that is calculated and tested for being significantly different from zero, for which *P*-values are presented (McCullagh & Nelder 1989). *P*-values smaller than or equal to 0.05 are considered significant. Sex ratios were evaluated versus an equal male:female distribution (1:1 ratio) by means of a nonparametric Chi-Square test (SPSS-Inc. 2006).

7.2.3 Effect of diet on development and reproduction

7.2.3.1 Diets

In a second experiment, three foods were tested on both *Orius* species: two factitious prey types and one plant diet (**Figure 7.1**). The first factitious food consisted of frozen eggs of *E. kuehniella*, which were supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands). A second factitious food consisted of hydrated decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg (Crustacea: Artemiidae), originating from Great Salt Lake (Utah, USA) and supplied by the *Artemia* Reference Center at Ghent University in Ghent, Belgium. The cysts were hydrated by placing them in tap water for 2 h, after which excess water was removed. The plant diet was composed of moist frozen honey bee pollen, also supplied by Koppert B.V. The pollen pellets were finely crushed before being offered to the predator.

In each treatment, a flat green bean pod was provided as a water source, substrate (hiding place) and extra nutrient source. All foods were supplied ad libitum and replenished every other day, except for *A. franciscana* cysts, which were refreshed on a daily basis.

Figure 7.2 Diets tested on Orius species: E. kuehniella eggs (left), bee pollen (middle), A. franciscana cysts (right) (photos: author)

7.2.3.2 Nymphal development

For each diet, 120 first instars (<24 h old) of both *Orius* species were individually caged in 5 cm diameter containers. Developmental performance of the predators was assessed as described above.

A two-way ANOVA was conducted to evaluate effects of diet on developmental time and body weight of adult males and females of both predators. Where no interaction was found means within each factor were separated using a Tukey test. When interactions were significant pairwise multiple comparison procedures were used (Kutner et al. 2005). In case a Kolmogorov–Smirnov test indicated that these means were normally distributed, the parameter was analysed using a one-way analysis of variance (ANOVA). When means were not normally distributed, a non-parametric Kruskal-Wallis H test was used. Survival rates were compared by means of a logistic regression (SPSS-Inc. 2006).

7.2.3.3 Reproduction

For each diet, newly emerged adults (<24 h old) were paired and transferred into 5 cm diameter containers. The adults were offered the same diet as in their nymphal life. Half of the females were dissected, whereas the other half was held to determine lifetime oviposition. The latter group of females were offered a piece of green bean pod as an oviposition substrate. The bean pods were checked daily for eggs to determine the preoviposition period. When the first egg was laid, bean pods were replaced every other day until the female died. Lifetime oviposition and egg hatch were monitored. Eight days after adult emergence, the second half of the females was dissected to quantify

oocyte development. For dissection, the females were pinned down on their dorsal side. The ovipositor together with the last two abdominal segments was carefully separated from the abdomen, exposing the ovaries. The number of oocytes (follicles) in the ovaries and oviducts was counted and scored according to the method described by Callebaut et al. (2004): late vitellogenic to mature oocyte, 1; early to mid vitellogenic oocyte, 0.5; previtellogenic oocyte, 0.25; no observable oocyte, 0; early previtellogenic oocytes that were not clearly discernible under the dissection microscope (magnification 25x) were not scored. During the 8-d period before dissection, oviposition of this cohort was monitored. As the presence of an oviposition substrate could affect oocyte counts for this cohort, the bean pod was replaced by a water dome to provide moisture. Whereas Shapiro and Ferkovich (2006) used water-filled Parafilm domes to collect *Orius insidiosus* (Say) eggs, females of *O. laevigatus* (Bonte & De Clercq 2010a), *O. thripoborus* and *O. naivashae* were rarely observed to deposit eggs into water domes.

To evaluate whether diet had a different effect on reproduction of *O. thripoborus* and *O. naivashae*, measures of reproduction were subjected to a two-way ANOVA. As no interaction between diet and species was found for the parameters preoviposition period, lifetime oviposition, weighted sum of oocytes and longevity, means within each factor were separated using the Tukey pairwise comparison procedure (Kutner et al. 2005). Means for egg hatch were compared by way of a logistic regression. To evaluate the relationship between lifetime fecundity and oocyte counts, a Pearson's correlation test was performed (SPSS-Inc. 2006).

7.3 Results

7.3.1 Influence of water source on development

Nymphal survival was significantly affected by water source but not by species (**Table 7.1**). Survival rate of nymphs of both *O. thripoborus* and *O. naivashae* fed *E. kuehniella* eggs was about 30% higher when a bean pod was offered as a water source than when a water dome was offered (**Table 7.2**).

Both male and female developmental time was influenced by species. Regardless of water source, *O. thripoborus* developed faster than *O. naivashae* (**Tables 7.1 and 7.2**). Male developmental time was also influenced by water source. When a bean pod was offered, males of both *O. thripoborus* and *O. naivashae* developed faster than when a water dome was offered (**Table 7.1**).

Table 7.1 Results of a logistic regression and a two-way ANOVA indicating the effect of water source (water dome or bean pod) and species (O. thripoborus and O. naivashae) on developmental parameters

Source		Nymphal survival (%)[a]	Developmental time (days)[b]		Adult weight (mg)[b]	
			Males	Females	Males	Females
Water source	F	-	10.988	1.182	0.036	3.802
	df	-	1	1	1	1
	P	<0.001	0.002	0.281	0.851	0.056
Species		-	36.884	6.665	0.279	2.027
	df	-	1	1	1	1
	P	0.073	<0.001	0.012	0.600	0.160
Water source x Species	F	-	0.382	1.079	0.083	0.304
	df	-	1	1	1	1
	P	0.767	0.540	0.303	0.774	0.583
Error term	df	-	44	59	44	59

[a] Probit (Wald Chi-square); [b] Two-way ANOVA

Neither of the tested factors influenced adult weight of both males and females (**Table 7.1**).

Sex ratios of both species within all treatments did not deviate essentially from a 1:1 ratio (**Table 7.2**), although *O. naivashae* produced more females than males in both treatments.

7.3.2 Effect of diet on development and reproduction

7.3.2.1 Nymphal development

Table 7.3 presents the results of a two-way ANOVA assessing the effect of diet and species on developmental parameters.

Regardless of the species, nymphal survival of predators fed *E. kuehniella* eggs was not significantly different from that of predators reared on *A. franciscana* cysts (logistic regression; $P = 0.377$). On the other hand, nymphal survival of the anthocorids fed either factitious food (*E. kuehniella* eggs or *A. franciscana* cysts) was significantly better than that of those fed pollen ($P < 0.001$ and $P = 0.008$, respectively). For both anthocorids, nymphal survival ranged from 66.3 to 86.6% on the tested diets (**Table 7.4**).

Table 7.2 Developmental parameters of O. thripoborus and O. naivashae on two water sources (23℃, 16:8 (L:D) h and 65 ± 5% RH; E. kuehniella eggs)

Species	Water source	n[a]	Nymphal survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio[b] (male:female)
				Males	Females	Males	Females	
O. thripoborus	Water dome	39	64.1 ± 7.8b	16.3 ± 0.3b	15.6 ± 0.2ab	0.31 ± 0.02a	0.41 ± 0.02a	1:1.08
	Bean pod	38	94.7 ± 3.7a	15.7 ± 0.1a	15.3 ± 0.1a	0.31 ± 0.01a	0.36 ± 0.01a	1.25:1
O. naivashae	Water dome	34	50.0 ± 8.7b	18.0 ± 0.4c	15.8 ± 0.2b	0.33 ± 0.03a	0.43 ± 0.03a	1:2.4
	Bean pod	39	84.6 ± 5.9a	17.0 ± 0.3bc	15.8 ± 0.2b	0.31 ± 0.03a	0.40 ± 0.02a	1:2

Means ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey (male adult weight), Tamhane (female adult weight), Mann-Whitney U (developmental time) or probit test (survival))

[a] n = initial number of first instars tested

[b] Sex ratios did not differ significantly from a 1:1 ratio, with respective *P*-values of 0.841; 0.505; 0.090 and 0.056 (Chi-square test)

Table 7.3 Results of a logistic regression and a two-way ANOVA indicating the effect of diet (E. kuehniella eggs, A. franciscana cysts or bee pollen) and species (O. thripoborus and O. naivashae) on developmental parameters

Source		Nymphal survival (%)[a]	Developmental time (days)[b]		Adult weight (mg)[b]	
			Males	Females	Males	Females
Diet	F	-	125.606	252.810	20.140	34.451
	df	-	2	2	2	2
	P	0.001	<0.001	<0.001	<0.001	<0.001
Species	F	-	47.715	59.543	10.493	30.216
	df	-	1	1	1	1
	P	0.900	<0.001	<0.001	0.001	<0.001
Diet x Species	F	-	12.715	16.762	2.158	18.027
	df	-	2	2	2	2
	P	0.053	<0.001	<0.001	0.118	<0.001
Error term	df	-	190	336	190	336

[a] Probit (Wald Chi-square); [b] Two-way ANOVA

For developmental time, interactions were found to be significant (two-way ANOVA); here, the treatment means were compared pairwise using multiple comparison procedures. As developmental times were not normally distributed they were analysed using a non-parametric Kruskal-Wallis H test. Development of males ($\chi^2 = 144.843$; df = 5; $P < 0.001$) and females ($\chi^2 = 244.275$; df = 5; $P < 0.001$) of either species was faster on the factitious foods than on pollen. Predators fed flour moth eggs had shorter developmental times than those fed brine shrimp cysts (**Table 7.4**). When pollen was offered as food, development took longer than when cysts were offered, except for *O. naivashae* males. In the latter case, no significant differences in developmental time were observed between pollen and *A. franciscana* cysts.

As there was no interaction between diet and species for weights of male adults (two-way ANOVA), means within each factor were separated using a Tukey test. Both diet and species influenced male adult weight. In general, *O. naivashae* males were heavier than *O. thripoborus* males (**Table 7.3**). *Artemia* cysts and pollen yielded males with similar adult weights ($P = 0.295$) which were in turn lighter than those reared on *E. kuehniella* eggs (both $P < 0.001$).

Table 7.4 Developmental parameters of O. thripoborus and O. naivashae on three diets (23°C, 16:8 (L:D) h and 65 ± 5% RH; E. kuehniella eggs + bean pod)

Species	Diet	n[a]	Nymphal survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio[b] (male: female)
				Males	Females	Males	Females	
O. thripoborus	*E. kuehniella*	119	86.6 ± 3.1a	15.8 ± 0.09a	15.5 ± 0.1a	0.31 ± 0.007b	0.36 ± 0.007ab	1:1.15
	A. franciscana	108	87.0 ± 3.2a	17.2 ± 0.2b	17.1 ± 0.2b	0.28 ± 0.006a	0.37 ± 0.007ab	1:0.81
	Pollen	101	66.3 ± 4.7b	19.2 ± 0.2c	19.1 ± 0.3c	0.27 ± 0.009a	0.33 ± 0.01a	1:1.09
O. naivashae	*E. kuehniella*	117	85.5 ± 3.3a	15.9 ± 0.2a	15.5 ± 0.1a	0.35 ± 0.01c	0.45 ± 0.007c	1:4.56*
	A. franciscana	110	79.1 ± 3.9a	20.0 ± 0.4cd	19.2 ± 0.2c	0.30 ± 0.01ab	0.38 ± 0.007b	1:3.83*
	Pollen	112	77.9 ± 4.0ab	20.8 ± 0.4d	20.6 ± 0.2d	0.27 ± 0.007a	0.34 ± 0.007a	1:2.11*

Means ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey (male adult weights), Tamhane (female adult weights), Mann-Whitney U (developmental time) or probit test (survival))

[a] n = initial number of first instars tested

[b] Values with an asterisk differ significantly from a 1:1 ratio; *P*-values were 0.490; 0.302; 0.714; <0.001; <0.001 and 0.001, respectively (Chi-square test)

For female adult weight, interactions were found to be significant (two-way ANOVA). Consequently, the treatment means were compared pairwise using multiple comparison procedures; here, body weights of female adults were normally distributed and therefore analysed using a one-way analysis of variance (ANOVA). Their variances of means were heteroscedastic and hence separated using a Tamhane test ($P = 0.05$). Female adult weights of *O. thripoborus* were similar to those of *O. naivashae* on cysts and pollen, but adults of the latter species produced on *E. kuehniella* eggs were heavier than those of the former (one-way ANOVA; $F = 33.881$; $df = 5, 336$; $P < 0.001$). Within *O. naivashae*, flour moth eggs yielded heavier females than brine shrimp cysts, which in turn yielded heavier females than did pollen (**Table 7.4**).

Sex ratios of *O. naivashae* were female biased on all diets ($P \leq 0.001$). For *O. thripoborus*, no significant deviations from a 1:1 sex ratio were observed (**Table 7.4**).

7.3.2.2 Reproduction

Reproduction characteristics of both *Orius* species reared on different diets are given in **Table 7.6**. Females of both species were able to produce viable eggs on all diets.

The diet x species interaction was only significant for the parameter egg hatch (**Table 7.5**). Egg hatch exceeded 83% in all treatments. Hatching rate of *O. thripoborus* eggs was superior to that of *O. naivashae* eggs except on *A. franciscana* cysts. For *O. thripoborus*, *E. kuehniella* eggs resulted in the highest egg hatch, whereas *A. franciscana* cysts yielded the lowest hatching rate. In *O. naivashae*, egg hatch did not differ among diets (**Table 7.6**).

Preoviposition period was affected by species but not by diet. Females of *O. thripoborus* had shorter preoviposition periods than those of *O. naivashae* (**Tables 7.5 and 7.6**).

Both diet and species influenced lifetime oviposition. Regardless of diet, *O. thripoborus* produced more eggs than *O. naivashae* (**Table 7.5**). Variability of lifetime oviposition was high, with coefficients of variation ranging from 70.7 to 89.3%. Overall, females of both species fed pollen laid 26 to 51% of the number of eggs deposited by those fed *E. kuehniella* eggs or *A. franciscana* cysts (**Table 7.6**).

Oocyte counts were similar in all treatments (**Table 7.5**) and varied between 7.6 and 11.2 (**Table 7.6**).

A strong significant correlation was found between lifetime oviposition and the weighted sum of oocytes at dissection for *O. thripoborus* (r = 0.999; *P* = 0.022; n = 3), but for *O. naivashae* the correlation was not significant at the 0.05 level, despite a high magnitude of the correlation coefficient (r = 0.992; *P* = 0.082; n = 3).

Longevity of females varied between 50.5 and 61.1 days and did not differ among treatments (**Tables 7.5 and 7.6**). Male longevity depended on both diet and species. In general, *O. naivashae* males lived longer than those of *O. thripoborus* (**Table 7.5**). Regardless of the species, males lived longer on *E. kuehniella* eggs than on *A. franciscana* cysts (*P* = 0.011).

Table 7.5 Results of a logistic regression and a two-way ANOVA indicating the effect of diet and species (O. thripoborus and O. naivashae) on reproductive parameters

Source		Preoviposition period (days)[a]	Lifetime oviposition[a]	Weighted sum of oocytes[a]	Egg hatch (%)[b]	Longevity (days)[a]	
						Males	Females
Diet	F	2.004	7.736	2.345	-	3.862	0.980
	df	2	2	2	-	2	2
	P	0.141	0.001	0.101	<0.001	0.024	0.379
Species	F	4.133	21.004	0.002	-	6.734	2.607
	df	1	1	1	-	1	1
	P	0.045	<0.001	0.966	<0.001	0.011	0.110
Diet x	F	2.129	0.581	0.267	-	0.664	0.261
Species	df	2	2	2	-	2	2
	P	0.125	0.561	0.766	0.001	0.517	0.771
Error term	df	84	105	107	-	92	96

[a] Two-way ANOVA; [b] Probit (Wald Chi-square)

Table 7.6 Reproductive parameters of O. thripoborus and O. naivashae on three diets (23°C, 16:8 (L:D) h and 65 ± 5% RH; E. kuehniella eggs + bean pod)

Species	Diet	n[a]	Preoviposition period (days)	Lifetime oviposition	Weighted sum of oocytes[b]	Egg hatch (%)	Longevity (days)	
							Males	Females
O. thripoborus	*E. kuehniella*	22	8.0 ± 1.4ab	122.5 ± 21.7a	10.9 ± 1.8a (22)	93.2 ± 0.5a	59.4 ± 3.8ab	61.1 ± 5.6a
	A. franciscana	19	5.9 ± 0.5a	110.3 ± 17.9a	10.4 ± 1.3a (20)	85.2 ± 0.8c	46.0 ± 5.2ab	52.6 ± 3.8a
	Pollen	15	6.8 ± 0.5ab	56.7 ± 11.6ac	7.6 ± 1.2 a (14)	90.0 ± 1.1b	45.0 ± 3.4b	58.5 ± 6.5a
O. naivashae	*E. kuehniella*	25	8.3 ± 1.1ab	68.2 ± 9.9ab	11.2 ± 1.2a (24)	86.8 ± 0.9c	64.5 ± 4.5a	53.9 ± 2.7a
	A. franciscana	14	7.9 ± 1.1ab	36.6 ± 8.3bc	9.2 ± 1.4a (17)	84.6 ± 1.6c	55.5 ± 2.3ab	50.7 ± 2.6a
	Pollen	16	12.0 ± 1.4b	17.6 ± 3.9c	8.5 ± 0.7a (16)	83.3 ± 2.4c	60.2 ± 3.8ab	50.5 ± 4.4a

Means ± SE within a column followed by the same letter are not significantly different (P > 0.05; Tukey (preoviposition period and female longevity), Tamhane (deposited eggs, oocyte counts and male longevity) or probit test (egg hatch))

[a] n = number of adult pairs tested to determine preoviposition period, lifetime oviposition and longevity

[b] Number of oocytes (follicles) in ovaries and oviducts; the number of dissected females is placed in parenthes

7.4 Discussion

In a preliminary experiment, only a single *O. thripoborus* nymph (out of 20) reached the adult stage when *E. kuehniella* eggs were offered without a supplementary water source. Despite the ca. 68% water content of *E. kuehniella* eggs (De Clercq et al. 2005), a supplementary source of water was needed to sustain the development of the predator when presented with this food. In most rearing systems for heteropteran predators, water is supplied via plant materials. Besides being a source of moisture (Grenier et al. 1989; Richards & Schmidt 1996a), plants also serve as an oviposition substrate (Castañé & Zalom 1994; Coll 1996; Richards & Schmidt 1996b; Lundgren & Fergen 2006) and provide hiding places, thus reducing cannibalism (van de Veire 1995; Cocuzza et al. 1997b). In addition, it has been shown that several heteropteran predators, including *Orius* spp., may also derive supplemental nutrients from plant materials (Lundgren 2009). *Orius insidiosus* gains water from the plant xylem, and may ingest small amounts of starches, sugars, and amino acids from the mesophyll (Armer et al. 1998). The influence of plant materials on the performance of predatory bugs varies greatly (Naranjo & Gibson 1996; Lundgren 2009). Overall, supplementing prey diet with plant material has been reported to accelerate nymphal development, increase nymphal survival and adult longevity, and enhance fecundity (Coll 1998). In our experiments, nymphal survival was higher and development of males faster when a piece of bean pod was added as water source to *E. kuehniella* eggs, for both *O. thripoborus* and *O. naivashae*. Richards and Schmidt (1996a) stated that bean pods were an important source of moisture, greatly affecting the proportion of nymphs of *O. insidiosus* reaching the adult stage. Kiman and Yeargan (1985), on the other hand, observed no differences in nymphal survival or developmental time of this species when a bean pod was supplemented to *Heliothis virescens* (Fabricius) eggs. Bush et al. (1993) noted a faster development and better fecundity but similar survival when *O. insidiosus* were offered a bean pod in addition to *H. virescens* eggs. In contrast, nymphs of *O. laevigatus* developed slower when a bean pod was added to a diet of *E. kuehniella* eggs as compared with free water as a moisture source (Bonte & De Clercq 2010a).

Our findings suggest that the presence of the bean pod had a positive influence on some of the developmental parameters of the tested *Orius* species. Bonte & De Clercq (2010a) pointed out that the use of plant materials to provide moisture in rearing systems for predatory heteropterans has

several drawbacks. Artificial sources of water, like the Parafilm domes used in our study, may contribute to rationalising the rearing process. However, for some species it may be advisable to compensate for the extra nutrients which are normally gained from plant materials, when only free water is provided.

Several studies have shown that eggs of the Mediterranean flour moth *E. kuehniella* constitute a nutritionally superior food for *Orius* bugs (e.g., Cocuzza et al. 1997b; Ferkovich & Shapiro 2004; Bonte & De Clercq 2008). Arijs & De Clercq (2001b) and Bonte and De Clercq (2008) demonstrated that hydrated decapsulated cysts of *A. franciscana* also sustained development and reproduction of *O. laevigatus*, with similar or slightly inferior results as compared with *E. kuehniella* eggs. The current study indicates that *Artemia* cysts were also an acceptable food for *O. thripoborus* and *O. naivashae* and may thus have value for use in the mass production of these species as well. However, *Artemia* cysts may not be a suitable food to solely support long term cultures of *Orius* spp. (De Clercq et al. 2005) and may thus have more potential to replace the more expensive *E. kuehniella* eggs in part of the rearing process.

Orius thripoborus and *O. naivashae* were able to complete their development on moist honey bee pollen. With nymphal survival percentages of 66% for *O. thripoborus* and 78% for *O. naivashae*, mortality on honey bee pollen was higher than on the tested factitious foods. However, these survival rates are similar or even better than those reported for other *Orius* spp. reared on pollen from various sources in combination with plant tissue (Kiman & Yeargan 1985; Richards & Schmidt 1996a; Vacante et al. 1997; Venkatesan et al. 2008; Lundgren 2009; Bonte & De Clercq 2010a).

Overall, developmental fitness of both tested *Orius* species on factitious foods was better than on pollen. There is considerable variation in the performance of *Orius* nymphs on pollen among studies. Reported developmental times of different *Orius* spp. are generally longer on vegetal diets (e.g., pollen) than on insect prey (e.g., Kiman & Yeargan 1985; Funao & Yoshiyasu 1995; Richards & Schmidt 1996a). Lundgren (2009) showed that pollen from certain hybrids of *Zea mays* L. did not support development in *O. insidiosus* at all, whereas that from others only allowed a small number of individuals to complete development. In contrast, Duan et al. (2007) reported good survival and rapid development of *O. insidiosus* on a bee pollen diet consisting of 40% water and 60% pollen. They

suggested the use of this pollen diet in Tier-I toxicity assays to evaluate potential adverse effects of transgenic plants on non-target heteropteran predators. Females of *O. thripoborus* and *O. naivashae* showed a reduction in fecundity of 54 and 74%, respectively, when fed on pollen, as compared to when fed on *E. kuehniella* eggs. Other studies reported a strong reduction in fecundity for *Orius* spp. fed pollen instead of prey (Fauvel 1974; Salas-Aguilar & Ehler 1977; Kiman & Yeargan 1985; Cocuzza et al. 1997b). Females of *Orius tantillus* (Motschulsky) failed to lay any eggs when reared on maize pollen (Venkatesan et al. 2008). Differences in developmental and reproductive performance of *Orius* spp. feeding on pollen could be due to differences in nutritional quality, particularly pertaining amino acid and lipid content, and defensive properties of the pollen (Stanley & Liskens 1974; Richards & Schmidt 1996a). Besides defensive structural traits of pollen grains, their antinutritive or even toxic qualities may have a negative impact on the biological performance of pollinivores (Lundgren 2009).

Despite that animal prey is required for optimal development and reproduction, numerous workers have observed *Orius* spp. feeding on pollen in the field (e.g., Salas-Aguilar & Ehler 1977; Cocuzza et al. 1997b; Corey et al. 1998). Pollinivory is considered to be an adaptive strategy to sustain populations of these predators when prey numbers are low, which may eventually lead to a more effective pest control. Feeding on pollen may also play an important role in a preventive release strategy (Cocuzza et al. 1997b). Populations of *Orius* spp. may be supported by pollen-producing wild or cultivated plants in the vicinity of the crop. Alternatively, the pollen itself can be applied to the crop (e.g., van Rijn et al. 2002). However, the outcome of conservation measures based on increasing pollen input may not be unequivocal. Skirvin et al. (2007) found that the presence of pollen reduced predation by *O. laevigatus* of thrips by 40%, leading to higher pest populations. More on the negative side, the plant feeding habit may expose *Orius* bugs to systemic insecticides (Cocuzza et al. 1997b).

Like in *O. laevigatus* (Bonte & De Clercq 2008), lifetime oviposition data and oocyte counts were strongly correlated in *O. thripoborus* and *O. naivashae* females reared on different diets. Some caution is warranted in the case of *O. naivashae*, for which the correlation was only marginally significant, due to the high variability of the oviposition data. This linear relationship may thus be used to more cost effectively predict the reproductive capacity of these predators as a function of their diet (Bonte & De Clercq 2008).

Whereas reported sex ratios in other *Orius* spp. are essentially 1:1, sex ratios of *O. naivashae* were female biased, particularly in the second experiment where 2 to 4.5 times more females emerged than males. A similar trend was observed in the stock culture of *O. naivashae* and in previous experiments (**see Chapters 3 and 4**). As nymphal survival in our experiments was very high, skewed sex ratios are unlikely the result of differential survival of males and females. It is hypothesised that endosymbionts are involved in this sex ratio distortion, but this requires further study (**see Chapter 9**).

Developmental and reproductive performance of *O. thripoborus* was superior to that of *O. naivashae*, with a faster nymphal development, shorter preoviposition period and overall better fecundity. *Orius thripoborus* also performed better on pollen than *O. naivashae*. Body weights of both species in our study were generally similar, except when the predators were offered *E. kuehniella* eggs and green beans, resulting in heavier body weights for *O. naivashae*. In field collections, adults of *O. naivashae* are mostly larger than those of *O. thripoborus* (Hernández & Stonedahl 1999). However, in **Chapter 6** it was shown that the larger size of *O. naivashae* is not beneficial in terms of predation capacity as compared with *O. thripoborus*. All these findings may lead to the conclusion that *O. thripoborus* has greater potential than *O. naivashae* for use in biological control programmes. However, it deserves emphasis that in the current study the predators were reared individually and only so for one generation under (optimal) laboratory conditions. Other factors like diapause (**see Chapter 5**), temperature preferences (**see Chapter 4**), searching behaviour, predation capacity (**see Chapter 6**) and habitat and prey preference (**see Chapter 6**) may determine the effectiveness of these predators in the field (Chambers et al. 1993; van den Meiracker 1994; Coll & Ridgeway 1995; Honda et al. 1998).

Chapter 8

Mite and insect materials as factitious foods for *Orius* spp.

Redrafter after:

Bonte, J., Van de Walle, A., Conlong, D. and De Clercq, P. 2015. Eggs of *Ephestia kuehniella* and *Ceratitis capitata*, and motile stages of the astigmatid mites *Tyrophagus putrescentiae* and *Carpoglyphus lactis* as factitious foods for *Orius* spp*. Insect Science* (in press).

8.1 Introduction

Orius species are omnivores that can also exploit plant resources, such as plant juices and pollen (**section 2.4.7.2**), and can be raised on unnatural/factitious and artificial foods (**section 2.5.2**). In the current mass production of these and other predatory heteropterans, mainly factitious foods are being used. Such alternative foods can reduce the cost of mass-rearing these natural enemies (**section 2.5.2.1**).

Factitious foods are live or dead organisms that are not normally attacked by the predator, mostly because they do not occur in its natural habitat, but do sustain its development in a laboratory environment (**section 2.5.2.1**). Several studies have shown that eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller constitute a nutritionally superior food for *Orius* bugs (e.g., Cocuzza et al. 1997b; Ferkovich & Shapiro 2004; Bonte & De Clercq 2008; Tan et al. 2011; **Chapter 7**). However, the continuous use of lepidopteran eggs as a factitious food in mass rearing systems does have some drawbacks, the most important of which is their high cost (e.g., ca. US$500 for 1 kg of *E. kuehniella* eggs) (**section 2.5.2.1**). This has resulted in a search for cheaper alternative foods, like cysts of brine shrimps (*Artemia* sp.) (Arijs & De Clercq 2001b; De Clercq et al. 2005). In **Chapter 7**, we demonstrated that hydrated decapsulated cysts of *Artemia franciscana* Kellogg sustained development and reproduction of *O. thripoborus* and *O. naivashae*, with similar or slightly inferior results as compared with *E. kuehniella* eggs. However, prolonged rearing on cysts as a sole food has been associated with fitness losses in *Orius* bugs (**section 2.5.2.1**). Moreover, as hydrated *Artemia* cysts need to be daily refreshed – or at least rehydrated – for optimal *Orius* feeding (Arijs & De Clercq 2001b), using these cysts in rearing systems is labor intensive. The availability of an economically viable and nutritionally adequate food as an alternative to flour moth eggs and *Artemia* cysts could be a crucial asset for rationalising the large-scale production of *Orius* bugs and other insect predators (De Clercq et al. 2005).

Eggs of fruit flies (Tephritidae) have been proposed as a potential source of factitious food for predatory insects (De Clercq et al. 2013). Eggs of the oriental fruit fly, *Dacus (Bactrocera) dorsalis* (Hendel) have been used to rear nymphs of the predaceous mirid *Tytthus mundulus* (Breddin) (Takara & Nishida 1981). Furthermore, Liquido and Nishida (1985) suggested that fresh eggs of the medfly, *Ceratitis capitata* Wiedemann, could be used as prey in the mass rearing of the mirid *Cyrtorhinus*

lividipennis Reuter. Previous studies have also indicated the potential of fruit fly eggs for the culturing of anthocorid bugs. Whereas *Orius insidosus* (Say) could be reared for at least a single generation on *D. dorsalis* eggs (Takara & Nishida 1981), Steinberg and Cayol (2009) reported that the overall performance of *Orius laevigatus* Fieber maintained on processed Mediterranean fruit fly eggs was comparable to that on *E. kuehniella* eggs. As fruit flies are produced by the billions in mass-rearing facilities for Sterile Insect Technique (SIT) purposes, market prices of their eggs are competitive with those of *E. kuehniella*.

Commercial suppliers routinely use astigmatid mites such as *Thyreophagus entomophagus* (Laboulbene), *Tyrophagus putrescentiae* (Schrank) and *Carpoglyphus lactis* (L.) as inexpensive prey for culturing a number of predatory mites of the Phytoseiidae family (Bolckmans & Van Houten 2006; Fidgett & Stinson 2014; Huang et al. 2013). Several *Orius* species have been reared on *T. putrescentiae*, albeit with varying degrees of success (Husseini et al. 1993; Nagai et al. 1998; Gomaa & Agamy 2002; Yang et al. 2009).

In the present paper, we compared the developmental and reproductive parameters of *O. thripoborus* and *O. naivashae* on different factitious foods: eggs of *E. kuehniella* and *C. capitata*, and mixed motile stages of the astigmatid mites *T. putrescentiae* and *C. lactis*. Given promising results in a first generation, developmental and reproductive performance of *O. thripoborus* was assessed over four generations of continuous rearing on *C. capitata* eggs.

8.2 Materials and Methods

8.2.1 *Orius thripoborus* and *O. naivashae* stock cultures

Stock colonies of *O. naivashae* and *O. thripoborus* were reared as described in **section 4.2.1**.

8.2.2 Factitious foods

Four factitious foods were tested for *O. thripoborus* and *O. naivashae*: frozen eggs of *E. kuehniella* and *C. capitata*, and two types of live prey.

Frozen eggs of *E. kuehniella* were supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands). A second factitious food consisted of frozen inactivated *C. capitata* eggs and were supplied by Andermatt Biocontrol AG (Grossdietwil, Switserland). The inactivation of medfly eggs, after having been harvested

in water, is a patented process that includes removal of excess water. Eggs are then put into sachets and subjected to Individual Quick Freezing (IQF), which ensures that they can be stored in a standard freezer without loss of quality for a minimum period of 9 months (Shouster-Dagan et al. 2011). Thawed eggs of *E. kuehniella* and *C. capitata* were stored in a refrigerator for no longer than two days.

Live prey comprised a mixture of all active stages (larvae, nymphs and adults) of the dried fruit mite, *C. lactis*, and the mold mite, *T. putrescentiae.* Colonies of these astigmatid mites were initiated from individuals supplied by Koppert B.V. and were kept in a climatic cabinet at 25°C. The mites were reared in insect breeding dishes (10 cm diameter, 4 cm high) (SPL Life Sciences, Republic of Korea) with a mesh hole (4 cm in diameter) in the lid. The dishes were placed in Styrofoam boxes (in complete darkness) with 2 cm of water. As predators can be affected by the nutrient composition of the prey's food (Mayntz & Toft 2000; Huang et al. 2013), both astigmatid mites were cultured on the same diet. The diet of the astigmatid mites was modified from Zdarkova et al. (1999) and Nguyen et al. (2013), and consisted of a ground mixture of hulled buckwheat (55%), brewer's yeast (10%), Pond Food Balance Sticks (10%) (Vitakraft, Bremen, Germany), sucrose (5%), and tap water (20%). The diet was refreshed every week.

8.2.3 Experiments

All experiments were performed at Ghent University in climatic cabinets set at 25 ±1°C, 65 ± 5% RH and a 16:8 (L:D) h photoperiod. In each experiment, similar plastic containers were used (4.5 cm diameter, 2 cm high), the lids of which had a ventilation hole screened with fine-mesh gauze. In each treatment, a flat green bean pod (*Phaseolus vulgaris* L.) was provided as a water source and substrate. All foods were supplied to the predators at libitum. Flour moth and medfly eggs were refreshed every three days, astigmatid mites were replenished every other day.

8.2.3.1 Nymphal development

In the experiment assessing the development of both *Orius* spp. on the different diets, 67 to 95 first instars (< 24 h old) were caged in individual plastic containers. Development and survival of the nymphs were monitored daily, and newly emerged adults were sexed and weighed using a Sartorius Genius ME215P balance (Sartorius, Goettingen, Germany).

8.2.3.2 Reproduction

On each tested prey, newly emerged adults (< 24 h old) from the development experiment were paired and transferred to individual plastic containers. The adults were offered the same food as during their nymphal life. Bean pods were checked daily for eggs to determine the preoviposition period. When the first egg was laid, bean pods were replaced every other day until the female died. Dead males were not replaced. Lifetime oviposition, egg hatch, and adult longevity were monitored.

8.2.3.3 Multigeneration study with O. thripoborus on C. capitata eggs

Given promising results in a first generation, developmental and reproductive performance of *O. thripoborus* was assessed over four generations of continuous rearing on *C. capitata* eggs. For this purpose, about 80 adults from the stock culture were placed in a Plexiglas cage (9 cm diameter, 3.5 cm high) containing a small sharp pepper plant and cultured in the same way as described for the stock colony, but now *E. kuehniella* eggs were replaced by *C. capitata* eggs as food for the adults and their progeny (founder generation, G0). Eggs were collected and subsequent nymphs and adults were reared in groups as for the stock colony. This procedure was followed for four successive generations. Seventy-two newly hatched nymphs from the third generation were individually caged to monitor their development (G3) and reproduction (G4) on medfly eggs as described above.

8.2.4 Statistical analysis

Data analysis was carried out using IBM SPSS Statistics 21 (IBM Corp. 2012).

For all studied parameters, a two-factor analysis with food and species as factors was applied using the appropriate model (2-way ANOVA or generalised linear model). In case the factor food was found to be significant (i.e., for the parameters male and female adult weight, pre-oviposition period, oviposition period, egg hatch and male longevity), a post-hoc analysis was performed to separate means. A Tukey post-hoc test was used for male and female adult weight, whereas generalised linear model based post-hoc tests were applied for all other of the above listed parameters. When a significant interaction between the factors was found, means were compared pairwise. If the data were continuous and a Kolmogorov-Smirnov test indicated that the values were normally distributed, the parameter was analysed using analysis of variance (ANOVA). When continuous data were not normally distributed, a non-parametric Kruskal-Wallis H test was used. In the latter case, means were

separated using a Mann-Whitney U test. In case of non-continuous data, a generalised linear model was used with a link function and error distribution depending on the nature of the data. Each analysis started with a saturated model and interactions and non-significant main factors were dropped at a significance level of 0.05. Countable data were analysed using a generalised linear model, with a Poisson distribution if applicable or a negative binomial distribution in case of overdispersion, as determined by the deviance and Pearson goodness-of-fit statistics (Hilbe 2011). If none of the generalised linear models were applicable, a non-parametric model was applied (Kruskal-Wallis H test, followed by Mann-Whitney U test). Parameters expressed as percentages (binary) were compared by means of a logistic regression. This regression is a generalised linear model using a probit (log odds) link and a binomial error function (McCullagh & Nelder 1989). Sex ratios were tested versus an equal female:male distribution (1:1 ratio) by means of Chi-square tests.

Non-continuous data from the multigeneration experiment with *O. thripoborus* (G0 vs. G3 for nymphs; G1 vs. G4 for adults) were compared pairwise using a generalised linear model (see above), whereas an independent sample t-test was applied for analysing normally distributed continuous data.

8.3 Results

Table 8.1 presents the results of a two-factor analysis assessing the effect of species and factitious food on developmental and reproductive parameters of *O. thripoborus* and *O. naivashae*. Parameter values and significant differences as a function of species and food are shown in **Tables 8.2 and 8.3** for development and reproduction, respectively.

8.3.1 Nymphal development

Nymphal survival on the different foods ($\chi^2 = 217.443$; df = 7; $P < 0.001$) ranged from 53.7 to 93.5% for *O. thripoborus* and from 1.0 to 95.2% for *O. naivashae* (**Table 8.2**). Whereas survival of *O. naivashae* nymphs reared on either type of factitious eggs was similar, nymphal survival of *O. thripoborus* was higher on eggs of *E. kuehniella* than on medfly eggs. Survival rate of the latter species fed on *T. putrescentiae* was as high as on *C. capitata* eggs. However, when reared on *C. lactis*, only about half of the *O. thripoborus* nymphs survived. Less than 7% of *O. naivashae* nymphs reached adulthood when astigmatid mites were offered. As a result, the number of replicates for *O. naivashae* fed on astigmatid mites was low and statistical analysis of their parameters was uninformative.

Table 8.1 Results of a logistic regression or a two-way ANOVA indicating the effect of species (O. thripoborus and O. naivashae) and factitious foods (E. kuehniella eggs, C. capitata eggs, C. lactis and T. putrescentiae) on developmental and reproductive parameters of the predators at 25°C

Parameter	Species			Food			Species × food			Error term
	F/χ^2	df	*P*	F/χ^2	df	*P*	F/χ^2	df	*P*	df
Nymphal survival[a]	36.796	1	<0.001	158.686	3	<0.001	90.116	3	<0.001	-
Female developmental time[b]	2.111	1	0.148	87.471	3	<0.001	19.118	2	<0.001	199
Male developmental time[b]	3.385	1	0.068	49.286	3	<0.001	41.543	2	<0.001	160
Female adult weight[b]	46.372	1	<0.001	110.753	3	<0.001	2.827	1	0.094	199
Male adult weight[b]	17.836	1	<0.001	32.870	3	<0.001	0.088	1	0.767	161
Proportion of ovipositing females[a]	<0.001	1	1.000	2.297	3	0.513	6.857	2	0.032	-
Preoviposition period[c]	0.203	1	0.653	13.996	3	0.003	0.217	1	0.641	-
Oviposition period[c]	3.238	1	0.072	70.500	3	<0.001	1.492	1	0.222	-
Lifetime oviposition[c]	47.935	1	<0.001	115.015	3	<0.001	4.403	1	0.036	-
Egg hatch[a]	23.369	1	<0.001	262.047	3	<0.001	2.974	1	0.085	-
Female longevity[c]	9.280	1	0.002	71.987	3	<0.001	8.750	2	0.013	-
Male longevity[c]	6.136	1	0.013	45.836	3	<0.001	0.256	1	0.613	-

[a] Binary probit test (Wald Chi-square); [b] two-way ANOVA; [c] generalised linear model with negative binomial distribution (Wald Chi-square

Orius females (χ^2 = 149.395; df = 6; *P* < 0.001) developed faster on factitious eggs (11.5 to 13.1 days) than on astigmatid mites (14.9 to 16.5 days) (**Table 8.2**). Whereas developmental times of *O. thripoborus* females were similar on eggs of *C. capitata* and *E. kuehniella*, *O. naivashae* females developed slower on medfly eggs than on flour moth eggs. However, females of *O. naivashae* developed faster on *E. kuehniella* eggs than those of *O. thripoborus*. Females of the latter species developed faster on mould mites than on dried fruit mites. Developmental times of males showed similar trends to those of females and similar effects of species and factitious food on developmental time as observed for females were found in males of both species (χ^2 = 129.413; df = 6; *P* < 0.001).

Within each food, *O. thripoborus* adults were generally heavier than those of *O. naivashae* (**Tables 8.1 and 2**). Further, in both anthocorids, body weights were higher on *E. kuehniella* eggs than on *C. capitata* eggs. Lightest *O. thripoborus* females were observed when nymphs were fed *C. lactis* or *T. putrescentiae* ($F = 81.737$; df = 3, 201; $P < 0.001$). For males, similar effects of species and factitious food on adult weight as mentioned for females were noted ($F = 27.258$; df = 3, 163; $P < 0.001$).

Sex ratios of both species did not deviate from a 1:1 ratio on any of the tested foods (**Table 8.2**).

8.3.2 Reproduction

Proportions of ovipositing females ($\chi^2 = 8.559$; df = 6; $P = 0.200$) fluctuated between 81.0 and 97.0% for *O. thripoborus*, but this parameter was not affected by food within this species, whereas it was in *O. naivashae* (**Tables 8.1 and 8.3**). Proportionally more *O. thripoborus* than *O. naivashae* females oviposited when fed medfly eggs, but on flour moth eggs similar numbers of females produced eggs in the two species. Proportions of egg producing *O. naivashae* females were higher on eggs of *E. kuehniella* than on those of *C. capitata*. None of the three *O. naivashae* females that had reached adulthood on *T. putrescentiae* mites were able to produce eggs.

No effect of species on preoviposition period was observed and first oviposition was only delayed when predators were fed dried fruit mites. Likewise, oviposition periods did not differ between species and were longer on insect eggs than on astigmatid mites. Nonetheless, the duration of egg production was double as long on *C. lactis* than on *T. putrescentiae* (**Table 8.3**).

Lifetime oviposition ranged from 23.2 to 129.3 eggs for *O. thripoborus* and from 32.4 to 65.2 eggs for *O. naivashae* (**Table 8.3**). *Orius thripoborus* females produced a higher number of eggs than those of *O. naivashae* ($\chi^2 = 126.520$; df = 5; $P < 0.001$). Whereas lifetime oviposition of *O. thripoborus* did not differ between eggs of *E. kuehniella* and *C. capitata*, *O. naivashae* females were twice as fecund on the former than on the latter. The number of eggs produced by *O. thripoborus* on *C. lactis* and *T. putrescentiae* was similar and lower than on both egg diets. Egg hatch was overall higher in *O. naivashae* than in *O. thripoborus*, and in both species it was lower on medfly eggs than on the other foods (**Table 8.3**).

Table 8.2 Developmental parameters (mean ± SE) of Orius thripoborus and Orius naivashae on four factitious foods (25°C, 16:8 (L:D) h and 65 ± 5% RH; bean pod)

Species	Food	n[a]	Nymphal survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio (male:female)[c]
				Females	Males	Females	Males	
O. thripoborus	*E. kuehniella* eggs	77	93.5 ± 2.8a	12.6 ± 0.10b	13.1 ± 0.10b	0.41 ± 0.01A	0.30 ± 0.01A	1:1.12
	C. capitata eggs	74	75.7 ± 5.0c	13.1 ± 0.23b	13.0 ± 0.20b	0.36 ± 0.01C	0.29 ± 0.01C	1:1.55
	C. lactis	67	53.7 ± 6.1d	16.5 ± 0.37e	16.3 ± 0.24e	0.25 ± 0.01E	0.22 ± 0.01E	1:1.57
	T. putrescentiae	77	81.1 ± 4.4bc	14.9 ± 0.18d	15.1 ± 0.20d	0.24 ± 0.01E	0.22 ± 0.01E	1:1.25
O. naivashae	*E. kuehniella* eggs	83	95.2 ± 5.4a	11.5 ± 0.18a	11.4 ± 0.12a	0.40 ± 0.01B	0.31 ± 0.01B	1:0.93
	C. capitata eggs	67	92.5 ± 3.2ab	14.3 ± 0.16c	14.2 ± 0.19c	0.31 ± 0.01D	0.25 ± 0.01D	1:1.30
	C. lactis	95	1.0 ± 1.0e	-	15.0[b]	-	0.16[b]	-
	T. putrescentiae[d]	79	6.3 ± 2.8e	15.8 ± 0.95cde	-	0.21 ± 0.02F	-	-

Means within a column followed by the same letter (lowercase for pairwise comparisons; uppercase for two-factor analysis) are not significantly different: $P > 0.05$; Mann-Whitney U test (developmental times); or binary probit test (nymphal survival)

[a] Initial number of first instars tested

[b] No SE could be determined as only a single male successfully developed

[c] None of the values differ significantly from a 1:1 ratio; χ^2 and P values were 0.222, 0.637; 2.571, 0.109; 1.778, 0.182; 0.778, 0.378; 0.114, 0.736; and 1.032, 0.310, respectively (Chi-square test, df = 1)

[d] Only 5 females and no males reached adulthood

Table 8.3 Reproductive parameters and longevity (mean ± SE) of Orius thripoborus and Orius naivashae on four factitious foods (25°C, 16:8 (L:D) h and 65 ± 5% RH; bean pod)

Species	Food	Proportion of ovipositing females (%)[a]	Preoviposition (d)	Oviposition period (d)	Lifetime oviposition	Egg hatch (%)	Longevity (d)	
							Females	Males
O. thripoborus	*E. kuehniella* eggs	84.4 ± 6.5ab (37)	6.2 ± 0.4A	22.9 ± 2.0A	129.3 ± 13.5a	66.8 ± 0.8B	35.7 ± 1.8a	32.6 ± 2.5B
	C. capitata eggs	97.0 ± 3.0a (33)	6.4 ± 0.5A	24.4 ± 2.4A	116.1 ± 12.6a	52.2 ± 0.9C	33.9 ± 2.5ab	29.6 ± 3.2BC
	C. lactis	81.0 ± 8.8ab (21)[b]	8.9 ± 1.9B	15.3 ± 2.6B	32.4 ± 5.5c	70.2 ± 2.1B	26.3 ± 2.5b	21.8 ± 4.7C
	T. putrescentiae	91.4 ± 4.8ab (35)	5.3 ± 0.4A	7.4 ± 0.6C	23.2 ± 2.2c	70.9 ± 1.7B	14.1 ± 0.8c	15.6 ± 1.1D
O. naivashae	*E. kuehniella* eggs	96.7 ± 3.3a (30)	5.7 ± 0.3A	21.5 ± 1.7A	65.2 ± 7.6b	73.6 ± 1.1A	36.6 ± 2.2a	44.2 ± 2.9A
	C. capitata eggs	79.4 ± 7.0b (35)	6.4 ± 1.0A	17.6 ± 1.9A	32.4 ± 5.5c	55.6 ± 1.7B	28.9 ± 2.0b	36.7 ± 2.8A
	C. lactis	-	-	-	-	-	-	-
	T. putrescentiae	0.0 ± 0.0c (3)[c]	-	-	-	-	5.7 ± 1.3d	-

Means within a column followed by the same letter (lowercase for pairwise comparisons; uppercase for two-factor analysis) are not significantly different: $P > 0.05$; binary probit test (proportion of females; egg hatch); or generalised linear model with negative binomial distribution (preoviposition period; oviposition period; lifetime oviposition; longevities)

[a] the number of adult pairs tested is placed in parentheses

[b] 6 out of 21 females were paired with 1- to 2-day-old males from the stock colony

[c] as no males developed, the 3 females were paired with 1- to 2-day-old males from the stock colony

Female adults of *O. thripoborus* lived as long as those of *O. naivashae*, except on *T. putrescentiae* which allowed *O. thripoborus* females to live twice as long as those of *O. naivashae*. Female longevities were longest on the egg diets, intermediate on dried fruit mites and shortest on mould mites. In *O. naivashae,* female longevity was shorter on *C. capitata* eggs than on *E. kuehniella* eggs (χ^2 = 153.330; df = 6; $P < 0.001$) (**Table 8.3**).

Longevities of male *O. naivashae* adults were longer than those of *O. thripoborus* and a similar effect of diet on longevity as observed for females was seen in males (**Table 8.1**).

8.3.3 Multigeneration study with *O. thripoborus* on *C. capitata* eggs

Developmental parameters of *O. thripoborus* reared for four successive generations on *C. capitata* eggs were 83.3 ± 4.4% for nymphal survival; 13.3 ± 0.19 and 13.5 ± 0.14 days for female and male developmental time, respectively; 0.33 ± 0.01 and 0.26 ± 0.01 mg for female and male adult weight, respectively; and a 1:1.40 (male:female) sex ratio. No differences in developmental traits were observed between G0 and G3, yet the first-generation adults were heavier than those from G4 (nymphal survival: χ^2 = 1.310, df = 1, P = 0.252; female developmental time: P = 0.108; male developmental time: P = 0.068; female adult weight: t = 2.714, df = 56, P = 0.009; and male adult weight: t = 2.248, df = 55, P = 0.033).

Preoviposition period was the only reproductive trait that was influenced by multigeneration rearing, being shorter in G4 (4.0 ± 0.2 days) than in G1 ($P < 0.001$). For the remaining reproductive parameters, results were 91.7 ± 5.8% for proportion of ovipositing females (χ^2 = 0.750, df = 1, P = 0.386); 19.1 ± 2.5 days for oviposition period (χ^2 = 1.839, df = 1, P = 0.175); 105.8 ± 17.8 eggs for total fecundity (χ^2 = 0.173, df = 1, P = 0.677); 50.3 ± 1.0% for egg hatch (χ^2 = 2.059, df = 1, P = 0.151); and 28.1 ± 2.8 and 25.8 ± 1.5 days for female (χ^2 = 2.126, df = 1, P = 0.145) and male longevity (χ^2 = 1.352, df = 1, P = 0.245), respectively.

8.4 Discussion

The present study demonstrates that frozen processed eggs of *C. capitata* sustained development and reproduction of *O. thripoborus* and *O. naivashae*, with similar or slightly inferior results as compared with *E. kuehniella* eggs. Only nymphal survival, adult weight and egg hatch of *O. thripoborus* were,

albeit to a limited extent, less favourable on medfly eggs than on flour moth eggs. Whereas survival of *O. naivashae* nymphs was similar on both egg diets, it was not as successful on *C. capitata* eggs as on *E. kuehniella* eggs in terms of the other developmental and reproductive parameters. Fecundity was even halved when *O. naivashae* was offered medfly eggs as compared to flour moth eggs. Nannini et al. (2009) reported similar reductions in nymphal survival and female fertility in the mirid bug *Macrolophus pygmaeus* Rambur when reared on fresh medfly eggs as compared with *E. kuehniella* eggs, but its developmental rate was similar on both factitious foods. In contrast, in *O. laevigatus* juvenile mortality and fecundity remained unaltered when processed medfly eggs were used as food instead of *E. kuehniella* eggs in the mass production (Shouster-Dagan et al. 2011). A study carried out by Liquido and Nishida (1985) showed that fruit fly eggs are also suitable for feeding the mirid *C. lividipennis*. Besides the eggs, also the larval stages of *C. capitata* have been shown to be suitable for rearing heteropteran predators, such as *O. laevigatus* (Steinberg & Cayol 2009) and *Macrolophus caliginosus* Wagner (Nannini et al. 2008a, b).

Nutrient balances in a diet may be expressed and lead to impaired fitness of the insect only after several generations of rearing (De Clercq et al. 2005). For instance, in the third generation on brine shrimp cysts, *O. laevigatus* nymphs took 18% longer to develop, and adults were shorter-lived and about 60% less fecund than those maintained on *E. kuehniella* eggs (De Clercq et al. 2005). However, in the present study, developmental and reproductive performance of *O. thripoborus* did not deteriorate after being reared for four successive generations on processed medfly eggs, but remained slightly inferior as compared with *E. kuehniella* eggs. Based on these results, we suggest that *C. capitata* eggs may be an adequate food to at least partially replace *E. kuehniella* eggs for long-term culturing of *Orius* bugs, although there may be differences in performance on medfly eggs among *Orius* species, as observed in the present study. To reduce inputs of expensive lepidopteran eggs, *A. franciscana* cysts are currently being used as a supplemental food in commercial mass cultures of *Orius* spp. and other predatory bugs (Bonte & De Clercq 2008; **Chapter 7**). Likewise, medfly eggs can be offered to these predators either in part of their life cycle or in a mixture with lepidopteran eggs or *Artemia* cysts.

It deserves emphasis that in the present study the predators were reared individually. In mass rearing systems of anthocorids and other arthropod predators, several stages coexist at the same time which

has been noted to lead to cannibalism (De Clercq & Degheele 1992; Chambers et al. 1993; Tommasini et al. 2002; Schausberger 2003; Baniameri et al. 2005). The rate of cannibalism may depend on predator density and on the nutritional capacity of the food offered (Grundy et al. 2000; Tommasini et al. 2002; Schausberger 2003; Leon-Beck & Coll 2007; Bonte & De Clercq 2011). When maintained on processed *C. capitata* eggs, the phytoseiid mite *Amblydromalus limonicus* Garman & McGregor displayed cannibalistic behavior, resulting in a negative population growth (Vangansbeke et al. 2014). However, in our multigeneration study with *O. thripoborus,* in which the predator was maintained for four consecutive generations on medfly eggs, no such problem was observed.

An important consideration for the commercial rearing of biocontrol agents on medfly eggs is that this factitious food is a magnitude cheaper as compared with *E. kuehniella* eggs. Millions of *C. capitata* and other tephritid fruit flies are currently being produced every week in SIT facilities. The great majority of the *C. capitata* flies are employed in sterile insect releases, but the fly is also used as a host for tephritid parasitoids (Nannini et al. 2008a). High numbers of *C. capitata* eggs can be produced at very low cost (Mitchell et al. 1965; Tanaka et al. 1969; Hendrichs et al. 1995; Steinberg & Cayol 2009). Shouster-Dagan et al. (2011) stated that processed medfly eggs are 35 to 50% cheaper than a diet based on *E. kuehniella* eggs for the mass production of *O. laevigatus*. Another asset of using medfly eggs in insect rearing systems is that this food allows a low frequency of replenishment, e.g. every 3 days in this study, resulting in reduced labor costs.

Due to its broad range of suitable diets, high fecundity and low rearing costs, *T. putrescentiae* is the most popular astigmatid mite being used as an alternative prey for arthropod predators (Huang et al. 2013). Currently, there are more than 10 predator species that can be mass reared using *T. putrescentiae*, mainly predatory mites (Li et al. 2000; Xia et al. 2003; Yang et al. 2009; Huang et al. 2013). Also *C. lactis* is routinely used by commercial biocontrol suppliers in the production of phytoseiid mites (Bolckmans & Van Houten 2006; Fidgett & Stinson 2008; Huang et al. 2013). To our knowledge, only a few studies have focused on the use of astigmatid mites for rearing *Orius* species, and only *T. putrescentiae* was tested. The latter mite was deemed suitable for mass rearing *O. sauteri* (Yang et al. 2009) and *O. laevigatus* (Gomaa & Agamy 2002), but resulted in poor nymphal survival and low egg production in *O. tantillus* (Nagai et al. 1998). In our study, astigmatid mites were not a suitable

food for *O. thripoborus* and *O. naivashae*. Nymphal survival of *O. naivashae* offered *C. lactis* or *T. putrescentiae* was below 7%, and none of the resulting females produced eggs. *O. thripoborus* did develop and reproduce when fed on astigmatid mites, albeit at significantly lower rates than on the flour moth and medfly egg diets. These suboptimal to poor results indicate that *C. lactis* and *T. putrescentiae* are a nutritionally inferior or difficult to handle prey for *O. thripoborus* and *O. naivashae*. Furthermore, chemicals produced by astigmatid mites that act as alarm pheromones and allomones may make them less suitable prey for insect predators (Kuwahara 2004; Raspotnig 2006). Our results suggest that *C. lactis* is a more suitable prey, as compared with *T. putrescentiae,* during the adult life of *O. thripoborus* than for its nymphal development. This may be due to the different nutritional requirements of the immature versus adult stages of *O. thripoborus*, or to differences in their capacity to handle the astigmatid prey. Previous experiments (**Chapters 6 and 7**) demonstrated a higher level of nutritional plasticity in *O. thripoborus* as compared with *O. naivashae*. This difference was confirmed on all tested foods in the present study, and was more pronounced on a less optimal prey, like the astigmatid mites.

In conclusion, this and previous studies indicate that processed medfly eggs are a suitable and relatively cheap alternative to *E. kuehniella* eggs for the rearing of *Orius* spp. and other heteropteran predators. More research is warranted to optimise the application of medfly eggs in large-scale mass cultures and to assess their value for the production of other predatory arthropods.

Chapter 9

General discussion, conclusion and future perspectives

Since the end of the 19th century, biological control has been increasingly used and is considered the most environmentally safe pest management system, that can be at the same time economically profitable. Biological control agents have no or only little side-effects on non-target organisms and the environment, and pest organisms cannot develop resistance against arthropod natural enemies. In contrast to chemical control, the benefit-cost ratio and the success of finding new biological control agents is higher whereas development costs are lower (Bale et al. 2008; van Lenteren 2012). As a result, more than 170 arthropod species, of which seven of the genus *Orius* (Hemiptera: Anthocoridae), are commercially used in biocontrol programmes in different parts of the world (Cock et al. 2010; van Lenteren 2012). *Orius* species have been applied successfully in biological control programmes in greenhouse and open-field cropping systems against various thrips pests worldwide (van den Meiracker & Ramakers 1991; Riudavets 1995; Cranshaw et al. 1996; Frescata and Mexica 1996; van Lenteren et al. 1997; Funderburk et al. 2000; Ohta 2001; Wang et al. 2001).

Sugarcane is one of South Africa's most important crops and is susceptible to a range of pests, with *Fulmekiola serrata* Kobus (Thysanoptera: Thripidae), the sugarcane thrips, being one of the major emerging pests in young sugarcane. Because thrips exhibit a cryptic lifestyle and rapidly develop resistance to pesticides, they are difficult to control. Therefore, an alternative pest management strategy using an effective biological control agent would be beneficial for local growers. During surveys performed in and around South African sugarcane fields in 2008 and 2009, *Orius thripoborus* (Hesse) and *Orius naivashae* (Poppius) were selected as candidate biocontrol agents of *F. serrata*. To establish whether these two anthocorids can be successful biological control agents, field observations and laboratory experiments were performed during this study in order to achieve a comprehensive understanding of their biology and ecology as natural enemies against thrips and other pests. The focus was placed on the use of these predatory bugs in augmentative biological control, either in open field crops or in protected cultivation. Furthermore, several alternative foods were tested with the aim of reducing production costs for these predators.

Based on laboratory experiments, we suggested the complementarity of both predators in terms of their temperature adaptations: *O. thripoborus* was found to be adapted to a slightly cooler

temperature range as compared with *O. naivashae*. Yet, this complementarity could not be confirmed based on our field observations. At the low end of the temperature range, lower threshold temperatures for nymphal development were estimated to be 10.2°C for *O. thripoborus* and 11.8°C for *O. naivashae*, whereas at 15°C, reproductive parameters of the former species were better than those of the latter. Moreover, eggs of both species did not develop successfully at a constant temperature of 12°C. However, average diurnal temperatures of 12°C or lower were not recorded in the regions of survey. The temperature above which the rate of nymphal development started decreasing was between 33 and 35°C for *O. naivashae*, whereas the upper threshold for nymphal development of *O. thripoborus* was between 29 and 33°C. At this high end of the tested temperature range, also reproduction of *O. thripoborus* began to drop at a lower temperature as compared with *O. naivashae*. During field surveys, though, both anthocorid species occurred in the hottest regions that were monitored, and *O. thripoborus* was also widespread in less hot and/or dryer regions. As only a part of South Africa was surveyed, the geographical distribution of these predators may be much wider than that observed in this study. Furthermore, laboratory tests were performed at constant temperatures, which represent a simplified approach of the fluctuating outdoor temperatures.

To estimate the relationship between temperature and development, linear models are only reliable within a limited temperature range. Still, they can be applied for estimating the thermal budgets and number of generations per year. Based on our estimated linear model parameters, the number of generations under Durban climate conditions (South African Weather Service 2011) was calculated to be 10 for both *O. thripoborus* and *O. naivashae*. In the field, the number of observed generations may be different from that calculated based on the model, but deviations are believed to be limited.

Further, our laboratory studies showed that a 12h photoperiod and 18°C, representing the average autumn conditions in the southwestern part of South Africa, induced reproductive diapause in 84% and 42% of *O. naivashae* and *O. thripoborus* females, respectively. Nonetheless, based on field observations in autumn and winter in South Africa, winter diapause is not likely to be a critical trait in populations of *O. thripoborus* and *O. naivashae* occurring in warm temperate climate regions. Still, diapause may be induced in both anthocorids during autumn in cooler regions or in regions with more pronounced temperature extremes. Such conditions may occur at higher elevations in the interior part

of South Africa; unfortunately, in the framework of the present study no surveys for *Orius* species could be performed in this part of South Africa. During winter nights, temperatures in this region can occasionally drop below the freezing point. When it comes to such cold temperatures, laboratory tests showed that *O. naivashae* was killed faster at 0°C or 5°C as compared with *O. thripoborus*. Measured values of the supercooling points of both *Orius* species were similar, but these data are not believed to be sufficiently reliable and comprehensive indicators of cold tolerance (Bale 1996).

The release of an exotic polyphagous predator can result in the establishment of the species and this may have negative side effects. Although there are no plans for considering the use of these southern African predators in northwestern Europe, it may be interesting to estimate their potential for outdoor establishment in this region based on the observed cold hardiness in the laboratory. When exposed to 5°C, all adults in our study had died by day 8 and day 12, for *O. naivashae* and *O. thripoborus*, respectively, indicating their susceptibility to chilling injury due to above-zero cold temperatures. Hatherly et al. (2005) reported a strong positive correlation between maximum field survival and survival at 5°C in the laboratory for several arthropod biological control agents and this trend has been confirmed by subsequent studies (Hatherly et al. 2008; Hughes et al. 2009; Hughes et al. 2011). When applying the relationship between LT50 at 5°C and field survival calculated by Hatherly and coworkers (2005; updated by Bale et al. 2009) to our dataset, it can be predicted that *O. thripoborus* and *O. naivashae* would not persist longer than 30 and 24 days, respectively, in the field during western European winters and that both *Orius* predators can be classified in the low risk category based on their low likelihood of establishment. It is noteworthy that more cold tolerant populations of *O. thripoborus* and *O. naivashae* may appear in the cooler geographic regions of South Africa which may have bearing on the risk they may pose when imported into cooler regions of other continents for biocontrol purposes.

Both *Orius* spp. may hold promise as augmentative biological control agents in southern Africa provided that they can be reared cost effectively. This study showed that optimal rearing conditions for the tested anthocorids were 25°C and 16:8h L:D, with *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs as a food source. Yet, when using these lepidopteran eggs as a food source, production costs of *Orius* species can be high. Alternative factitious foods proposed in this study were cysts of the

brine shrimp, *Artemia franciscana* Kellogg (Crustaceae: Artemiidae) and eggs of the medfly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). These foods fully supported development and reproduction of both predators and are a magnitude cheaper than *E. kuehniella* eggs. Besides, performance of *O. thripoborus* on both alternative factitious foods was only slightly inferior to that on *E. kuehniella* eggs. However, medfly eggs seemed to be more promising for mass production of this predator than *A. franciscana* cysts, as they were more easy to use and were suitable for prolonged rearing of *O. thripoborus*. Moreover, as *C. capitata* is produced in South Africa in large numbers for sterile insect technique (SIT) purposes (Fruit Fly Africa 2015), medfly eggs are easily available in this country and could be a suitable diet to support local mass production of *Orius* bugs.

In order to further rationalise the mass production of heteropteran predators, plant material may be omitted from the rearing system (Riddick 2009; De Clercq et al. 2013). In cultures of *Orius* bugs, water is supplied via plant materials such as pods of green bean (*Phaseolus vulgaris* L.). Our findings suggested that *O. naivashae* and *O. thripoborus* could develop and reproduce successfully on *E. kuehniella* eggs supplemented with free water encapsulated in Parafilm. However, the presence of a bean pod as a moisture source yielded better nymphal survival and faster development of *O. thripoborus* and *O. naivashae*, suggesting that the predators may extract extra nutrients from the bean pod. As a consequence, when omitting plant material from the rearing system, it may be advisable to compensate for these extra nutrients when only free water is provided. Bonte and De Clercq (2010a) showed that adding sucrose (5%) to a water dome did not benefit the overall fitness of *O. laevigatus*, suggesting that other nutrients are more beneficial in its diet. Further research is warranted to identify the supplemental nutrients that the anthocorids extract from plant materials. These nutrients can then be added to the water source of the predators and offered in Parafilm domes or microcapsules (Tan et al. 2010). Besides being a source a moisture (Grenier et al. 1989; Richards & Schmidt 1996a), plants also serve as an oviposition substrate for *Orius* bugs (Castañé & Zalom 1994; Coll 1996; Richards & Schmidt 1996b; Lundgren & Fergen 2006) and provide hiding places, thus reducing cannibalism (van de Veire 1995; Cocuzza et al. 1997b). Wax paper has been proven to be a suitable artificial living substrate for *Orius* species (Bonte & De Clercq 2010a, 2011), but an artificial oviposition substrate with suitable physical qualities allowing proper insertion of the eggs by *Orius* females and easy hatching of the nymphs has not been developed. More research on functional artificial oviposition substrates is

needed in order to eliminate the need for plant material in commercial *Orius* rearing systems. Also the attractiveness of the oviposition substrate for the females could be improved by incorporating plant extracts as cues to elicit oviposition behaviour (De Puysseleyr et al. 2014).

Pollen appear to be an important alternative food in the life of the studied *Orius* species. In the laboratory, a pollen diet alone allowed 66 and 78% of the nymphs of *O. thripoborus* and *O. naivashae*, respectively, to reach adulthood. Moreover, 67 and 75% of the *O. thripoborus* and *O. naivashae* females, respectively, were able to produce some viable eggs. During field surveys, nearly all of the predatory bugs were collected from the flowers of their host plants, feeding on flower thrips or on the pollen itself. Hence, populations of *Orius* spp. may be supported by pollen-producing wild or cultivated plants in the vicinity of crops. Habitats with different plant communities and phenologies attract alternative prey and can, whether or not in combination with pollen, support populations of omnivorous predators when target prey becomes scarce in a given crop system (Coll 1998; Lundgren 2009). Providing pollen-producing plants in/around crops which may support natural or augmented populations of *O. thripoborus* and/or *O. naivashae* may be a valuable conservation measure in South African cropping systems.

Given the high predation capacity against the western flower thrips, *Frankliniella occidentalis,* shown in our laboratory tests, *O. thripoborus* and *O. naivashae* are believed to have good potential as biocontrol agents of thrips. However, in the absence of thrips prey, the feeding behaviour of both *Orius* species may be very diverse. Based on the theory that an omnivorous insect chooses its food in order to maximise reproductive success (Coll & Guershon 2002), *O. naivashae* may select pollen over non-thrips prey when thrips prey is scarce. This food preference of *O. naivashae* was suggested by the high numbers of this species collected from pollen producing grassland weedy forbs during our surveys in South Africa. In contrast with *O. thripoborus*, *O. naivashae* has never been observed in the pollen-free spindle of young sugarcane, in association with *F. serrata*. Although laboratory tests showed that *O. naivashae* had a similar predation capacity on adult *F. serrata* thrips compared with *O. thripoborus*, the habitat preferences of the former species may make it a less suitable predator for use against the sugarcane thrips. The interaction between *Orius* species and *F. serrata* as a key pest in South African sugarcane production was only touched upon briefly in this dissertation, mainly because of

experimental limitations related to the rearing of this thrips species. In the future, more work needs to be done on the predator performance of *O. thripoborus* towards *F. serrata* on individual sugarcane plants in cage experiments and eventually in the field.

Compared with *O. naivashae*, *O. thripoborus* showed a faster nymphal development, shorter preoviposition period, and overall higher fecundity on both animal prey and pollen. Moreover, when thrips prey is scarce, our study showed that *O. thripoborus* may build up its populations on pollen and/or on other prey. Further, our findings indicate that *O. thripoborus* appears to hold better promise than *O. naivashae* for the suppression of non-thrips prey such as aphids and spider mites. Since our field observations showed that *O. thripoborus* was more prevalent in taller vegetation than in pollen producing weeds, this predator could be a candidate biocontrol agent for use against thrips and other arthropod pests in cash crops such as sugarcane, maize, sunflower, mango, peach, avocado, citrus and grape vine. In sugarcane, control of *F. serrata* is most critical in the young cane stage, so either augmentation or conservation of *O. thripoborus* should focus on this crop stage. In the management of *Orius* spp. populations for integrated pest control (e.g., against *F. serrata*), the temporal resources provided by pollen-producing neighbouring plants can be appropriately synchronised with the predator and pest population build-up in the crop (e.g., sugarcane). The flowers of adult sugarcane could provide alternative food for *O. thripoborus* when no sufficient prey is available in the crop, though these *Orius* populations run the risk of being reduced during harvesting of the cane. Given that the sugarcane industry in South Africa burns 90% of its crop while 10% is harvested green (Hurly et al. 2003), it is important that there is a refuge for the mobile stages of natural enemies of sugarcane pests during harvesting of the adult cane. Management practices in sugarcane should consider the conservation of plants which support populations of natural enemies in the vicinity of the crop when pest populations in the cane are still low. When pest populations in the crop start to build up again, cutting of these neighbouring plants may force the predators to move into the crop (Coll 1998). However, as *O. thripoborus* also may hold promise as a predator of eggs and larvae of the stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), management practices could focus on keeping *O. thripoborus* in the crop when this pest has been noted in the cane. *Eldana saccharina* attacks cane of all ages, although it prefers physiologically mature cane (Atkinson 1980). Further, current management practices in young sugarcane include the application of insecticides, with imidacloprid (plant cane) or

acetamiprid (ratoon cane) as the main active substances (Mike Way, personal communication). Both insecticides have been reported to be harmful for *Orius* predators (Delbeke et al. 1997; Naranjo & Akey 2005; Funderburk et al. 2013). In the context of the integrated pest management in sugarcane, the use of these insecticides should be adjusted considering the role of natural enemies such as *O. thripoborus*.

In the laboratory, both in the stock colony and during the experiments, a female biased sex ratio was repeatedly observed in *O. naivashae*, ranging between 1:1.8 and 1:11 (male:female). Treatment of the adult population with an antibiotic (0.05% rifampicin) (Stouthamer et al. 1990) resulted in an equally distributed sex ratio of their offspring, implying that endosymbionts are involved in this sex-determining mechanism (J. Bonte, unpublished data). Molecular studies showed that *Wolbachia* (Rickettsiaceae, class α-Proteobacteria) was present in infected adults, but a co-infection with another endosymbiont, such as *Spiroplasma* (Spiroplasmataceae, class Mollicutes, phylum Firmicutes), cannot be excluded. Bacterial symbionts of arthropods can have different modes of action in distorting the sex ratio in their host (Stouthamer et al. 1990; Werren 1997) and, to date, the sex-determining mechanism in *O. naivashae* has not been clarified. More research is needed on the effect of the endosymbiont(s) on the overall performance of *O. naivashae*. These results can have important implications for the potential use of this predator in biocontrol programmes (Werren 1997; Stouthamer et al. 2004; Machtelinckx et al. 2009). On the condition that the development, reproduction and predation of infected *O. naivashae* populations is not inferior to that of non-infected ones, the excess of females in the infected populations could lead to a more effective biological control. More females result in a faster population growth, and as females have to invest more energy in their progeny, they consume more prey than males. However, if the relative rarity of males lowers female mating success, then endosymbiont infection may indirectly reduce growth of the host's population (Floate et al. 2006).

In conclusion, *O. thripoborus* appeared to be, in many aspects, a more suitable biocontrol agent than *O. naivashae* for use against arthropod pests in South Africa. Compared with *O. naivashae, O. thripoborus* showed a less restricted climate preference, a more favourable cold tolerance and an overall better predation. Further, a better nutritional plasticity was recorded in *O. thripoborus* on both animal prey and pollen. Besides, *O. thripoborus* also adapted better to factitious foods, compared with

O. naivashae. Thus, the better nutritional plasticity of *O. thripoborus* does not only make it a more easy and cheaper to rear this predator compared with *O. naivashae*, but may also have a positive influence on its performance as a biocontrol agent. Therefore, it makes sense that, based on our findings, a South African company has recently started up the mass production of *O. thripoborus* for commercial biological control purposes in South Africa.

Further research should focus on optimising the application of *O. thripoborus* for the suppression of thrips pests in economically important protected and open field crops in South Africa, including sugarcane. The currently used management practices in many of these crops, which are now primarily based on frequent pesticide applications, need to be adapted in order to maximise the effect of this natural enemy on the target pests. Training programmes and other extension tools should be developed in order to convince local farmers of this integrated approach. Besides, within the scope of optimising the effectiveness of *O. thripoborus* as a biocontrol agent, its prey preference and interactions between this predator and non-target prey, including intraguild interactions, should be assessed in the laboratory, as well as in the field. Further, it remains to be studied how combined effects of abiotic factors and resources could lead to trade-offs and compensatory effects in the overall performance of *O. thripoborus*.

The efficacy of augmentative biological control programmes in an open field crop like sugarcane relies on the successful establishment of the natural enemy in the crop. First, the phenology of *O. thripoborus* and its main prey in cropping systems at different locations in South Africa warrants further study. Given the moderate dispersal capacity of *Orius* species, it is also important to identify the factors that determine its movements in and out of the crop and to evaluate habitat management measures that can assist in keeping this predatory bug in or near the crop. Furthermore, populations of *O. thripoborus* from different geographic locations may show divergent climate and habitat preferences, which is an aspect deserving further study, as these populations can extend the areas and cropping systems in southern Africa in which this biocontrol agent can be applied.

Summary

Biological control of arthropod pests is gaining the interest of growers all over the world. Species of the genus *Orius* are important natural enemies of thrips and other harmful arthropods in a variety of agricultural and horticultural crops and have been widely used in biological control programmes in Europe, the Americas and Asia (**Chapter 2**). In South Africa, thrips are key pests in major crops, with the sugarcane thrips, *Fulmekiola serrata* Kobus, as a model example. As thrips are notably difficult to control with pesticides, an effective indigenous natural enemy could provide local growers with an alternative management strategy against this pest. During surveys performed in and around South African sugarcane fields, the two little studied anthocorid predators, *Orius thripoborus* (Hesse) and *Orius naivashae* (Poppius), were selected as candidate biocontrol agents. In this dissertation, the autecology of these flower bugs was studied and their potential as biocontrol agents of thrips and other arthropod pests in South Africa was assessed based on field observations and laboratory tests.

Chapter 3 deals with the natural enemy surveys, especially for indigenous anthocorid predators of sugarcane thrips, being done between 2008 and 2013 in and around sugarcane fields in the Mpumalanga and KwaZulu-Natal Provinces of South Africa. Four *Orius* species were recorded during the surveys. First records for South Africa of *Orius tantillus* (Motchulsky) and *O. naivashae* were made, and the presence of *O. thripoborus* and *Orius brunnescens* (Poppius) in the country was confirmed. For each species, habitat and climate preferences were described. *Orius thripoborus* was the only anthocorid natural enemy which was observed preying on the sugarcane thrips *F. serrata* in the field.

In **Chapter 4**, the developmental and reproductive traits of *O. thripoborus* and *O. naivashae* were examined at several constant temperatures in the laboratory. Development was studied at 12, 15, 19, 23, 25, 29, 33 and 35°C. Eggs of both species did not hatch at 12°C. Nymphal survival was poor at 15°C for *O. naivashae*, and at 33°C and 35°C for *O. thripoborus*. Total development time of males and females decreased with increasing temperature. Based on a linear degree-day (DD) model, lower threshold temperatures for egg and nymphal development were estimated to be 9.4 and 10.2°C for *O. thripoborus*, and 11.3 and 11.8°C for *O. naivashae*. Thermal requirements for these stages were 73.8

and 191.1 DD, and 65.2 and 168.2 DD, respectively. Adult reproduction was studied at 15, 19, 25 and 33°C. Highest lifetime fecundities for *O. thripoborus* and *O. naivashae* were found at 25°C. At 15°C, half of the *O. thripoborus* females oviposited, whereas *O. naivashae* females only produced infertile eggs. At 33°C, on the other hand, most of the *O. naivashae* females produced eggs, while *O. thripoborus* females did not oviposit. Our observations suggest that *O. thripoborus* is adapted to a slightly cooler temperature range as compared with *O. naivashae*. The complementarity of both predators in terms of their temperature adaptation opens possibilities for their use in biological control programmes at different times of the season.

In **Chapter 5**, the cold hardiness and overwintering potential of *O. thripoborus* and *O. naivashae* were assessed in the laboratory. Diapause traits were studied by observing nymphal development and reproductive performance of adults at 18°C and three photoperiods (10:14, 12:12 and 14:10 (L:D) h); a 12 h light regime was also tested at 23°C. A 12 h photoperiod and 18°C induced reproductive diapause in 84% and 42% of *O. naivashae* and *O. thripoborus* females, respectively. Cold tolerance of adults was measured by determining the supercooling point (SCP, the temperature at which the insect's body fluids freeze) and lethal time (LT_{50}, the time required to kill 50% of the population) at 0 and 5°C. All observed SCPs ranged from -21 and -17°C. Significantly lower SCP values were observed for acclimated (7 days at 10°C) *O. naivashae* females. LT_{50}-values averaged 6.4 and 4.4 days at 0°C and 11.6 and 7.8 days at 5°C, for adults of *O. thripoborus* and *O. naivashae*, respectively. The findings indicate that *O. naivashae* is less cold tolerant and has a higher diapause incidence compared with *O. thripoborus*. Therefore, the latter species may have better potential for use in biological control programmes in the cooler regions of southern Africa or elsewhere.

Orius thripoborus and *O. naivashae* have potential as biological control agents of thrips pests in southern Africa, but may also hold promise for the control of other harmful arthropods. In **Chapter 6**, the predation capacity, development, reproduction and growth rates of both predatory species on the key pests *Frankliniella occidentalis* (Pergande) (western flower thrips), *Tetranychus urticae* Koch (two-spotted spider mite) and *Myzus persicae nicotianae* Blackman (tobacco aphid) were examined under laboratory conditions. Female adults of *O. thripoborus* and *O. naivashae* killed 24 and 18 *F. occidentalis* 2nd instars, and 15 and 21 *T. urticae* eggs per day, respectively. Developmental and reproductive

parameters of both *Orius* species were most favourable on *F. occidentalis*. Their intrinsic rates of increase (r_m) were highest when fed on *F. occidentalis*, averaging 0.123 and 0.131 females/female/day for *O. thripoborus* and *O. naivashae*, respectively. On the other prey, *O. thripoborus* showed significantly higher r_m-values than *O. naivashae*. Overall, r_m-values on *M. persicae nicotianae* were higher than on *T. urticae*, although differences were only significant for *O. thripoborus*. For *O. naivashae*, the estimated intrinsic rates of increase on the tested non-thrips prey were slightly negative. Our findings indicate the potential of both *Orius* spp. as biocontrol agents of thrips, whereas only *O. thripoborus* appears to hold promise for the suppression of aphids and spider mites as well.

In **Chapter 7**, the effect of moisture source and diet on the development and reproduction of *O. thripoborus* and *O. naivashae* was examined in the laboratory. Supplementing eggs of the flour moth *Ephestia kuehniella* (Zeller) with a green bean pod as a moisture source yielded better nymphal survival and faster development, as compared with free water encapsulated in Parafilm, suggesting that the predators may extract extra nutrients from the bean pod. The impact of two factitious foods and moist honey bee pollen on developmental and reproductive parameters of both predators was also investigated. The overall performance of both *Orius* species on *E. kuehniella* eggs and cysts of the brine shrimp *Artemia franciscana* Kellogg was better than on pollen. Nonetheless, a pollen diet alone allowed 66 and 70% of the nymphs of *O. thripoborus* and *O. naivashae*, respectively, to reach adulthood. Overall, developmental and reproductive performance of *O. thripoborus* on the tested diets was superior to that of *O. naivashae*.

As a follow up to the experiments with *A. franciscana* cysts in Chapter 7, several other factitious foods were tested for rearing *O. thripoborus* and *O. naivashae* in **Chapter 8**. Developmental and reproductive traits of both *Orius* species were examined when offered frozen eggs of the Mediterranean flour moth, *E. kuehniella*, frozen processed eggs of the medfly, *Ceratitis capitata* Wiedemann, or mixed motile stages of the astigmatid mites *Tyrophagus putrescentiae* (Schrank) or *Carpoglyphus lactis* (L). Whereas *C. lactis* and *T. putresecentiae* proved to be an inferior food for rearing *O. thripoborus* and *O. naivashae*, eggs of *C. capitata* fully supported development and reproduction of both predators. Results on medfly eggs were similar or slightly inferior to those on *E. kuehniella* eggs, which is the standard food for culturing these anthocorid bugs. *Orius thripoborus* could be maintained for four consecutive

generations on *C. capitata* eggs, indicating that processed medfly eggs can be a suitable and cheaper alternative to *E. kuehniella* eggs for prolonged rearing of these *Orius* spp.

In **Chapter 9**, general conclusions and future research perspectives are presented. In summary, *O. thripoborus* appeared to be, in many aspects, a more suitable biocontrol agent than *O. naivashae* for use against arthropod pests in South Africa. Due to its better nutritional plasticity, *O. thripoborus* is a more easy and cheaper to rear natural enemy with an overall better predator performance compared with *O. naivashae*. Based on these findings, a South African company has recently started up the mass production of *O. thripoborus* for commercial biological control purposes in South Africa. Further research is needed to optimise the augmentative application and conservation of *O. thripoborus* as a biocontrol agent of thrips in sugarcane and other major crops in South Africa.

Samenvatting

De interesse bij land- en tuinbouwers voor de biologische bestrijding van geleedpotige plaagorganismen groeit wereldwijd. Het genus *Orius* omvat belangrijke natuurlijke vijanden van trips en andere schadelijke geleedpotigen in verscheidene land- en tuinbouwgewassen. *Orius* roofwantsen worden dan ook vaak aangewend in biologische bestrijdingsprogramma's in zowel Europa, Amerika als Azië (**Hoofdstuk 2**). Tripsen vormen een sleutelplaag in economisch belangrijke gewassen in Zuid-Afrika, met de suikerriettrips, *Fulmekiola serrata* Kobus, als typevoorbeeld. Daar de onderdrukking van trips met pesticiden moeilijkheden kent, zou de beschikbaarheid van een doeltreffende inheemse natuurlijke vijand van deze plaag interessant zijn voor de lokale suikerrietproducenten. Tijdens veldonderzoek dat werd uitgevoerd in (de omgeving van) suikerrietplantages in Zuid-Afrika, werden twee relatief onbekende predatoren van de Anthocoridae familie, namelijk *Orius thripoborus* (Hesse) en *Orius naivashae* (Poppius), naar voor geschoven als kandidaat-biologische bestrijders. Op basis van observaties in het veld en in het labo werd in dit proefschrift de autecologie van deze bloemenwantsen bestudeerd en nagegaan of zij geschikt zouden zijn als biologische bestrijders van trips en andere geleedpotige plaagorganismen in Zuid-Afrika.

Hoofdstuk 3 behandelt de zoektocht naar natuurlijke vijanden en inheemse roofwantsen van de suikerriettrips in het bijzonder, uitgevoerd tussen 2008 en 2013 in en rondom suikerrietplantages in de Zuid-Afrikaanse provincies Mpumalanga en KwaZulu-Natal. Er werden vier *Orius* soorten waargenomen tijdens dit veldonderzoek. De eerste waarnemingen van *Orius tantillus* (Motchulsky) en *O. naivashae* in Zuid-Afrika werden opgetekend. Verder werd de aanwezigheid van *O. thripoborus* en *Orius brunnescens* (Poppius) in dit land bevestigd. De preferenties op vlak van habitat en klimaat werden voor elke soort beschreven. *Orius thripoborus* was de enige waargenomen natuurlijke vijand die zich in het veld effectief met de suikerriettrips voedde.

In **Hoofdstuk 4** werd de ontwikkeling en voortplanting van *O. thripoborus* en *O. naivashae* bij verscheidene constante temperaturen in het laboratorium onderzocht. De ontwikkeling werd bestudeerd bij 12, 15, 19, 23, 25, 29, 33 en 35°C. De ontluiking van eitjes bleef uit bij 12°C. Bij een

temperatuur van 15°C voor *O. naivashae*, en bij 33 en 35°C in het geval van *O. thripoborus*, was de overleving van de nimfen ondermaats. De totale ontwikkelingsduur van zowel mannetjes als wijfjes nam af bij stijgende temperaturen. Op basis van een lineair daggradenmodel werd het ontwikkelingsnulpunt voor eitjes en nimfen geschat op 9.4 en 10.2°C voor *O. thripoborus*, en op 11.3 en 11.8°C voor *O. naivashae*. De warmtebehoeftes voor deze ontwikkelingsstadia waren respectievelijk 73.8 en 191.1 daggraden, en 65.2 en 168.2 daggraden. De voortplanting werd bestudeerd bij 15, 19, 25 en 33°C. De hoogste eiproducties bij *O. thripoborus* en *O. naivashae* werden waargenomen bij 25°C. Bij 15°C slaagde de helft van de *O. thripoborus* wijfjes erin om eitjes af te leggen, maar die van *O. naivashae* produceerden enkel onvruchtbare eitjes. Bij 33°C daarentegen produceerde het merendeel van de wijfjes van *O. naivashae* eitjes, terwijl *O. thripoborus* wijfjes geen eileg vertoonden. Onze waarnemingen geven aan dat het temperatuurbereik van *O. thripoborus* een lichte verschuiving naar koelere temperaturen vertoont t.o.v. dat van *O. naivashae*. Het feit dat beide roofwantsen elkaar aanvullen op vlak van temperatuur biedt mogelijkheden voor het gespreide gebruik van deze predatoren in biologische bestrijdingsprogramma's in de loop van het seizoen.

De winterhardheid en overwinteringsmogelijkheden van *O. thripoborus* en *O. naivashae* werden in **Hoofdstuk 5** onderzocht in het laboratorium. De diapauzekenmerken van deze roofwantsen werden bestudeerd door observatie van hun nimfale overleving en voortplanting bij 18°C in combinatie met drie lichtregimes (10:14, 12:12 en 14:10 (L:D) u); bijkomend werd een lichtperiode van 12 u bij 23°C getest. Een lichtperiode van 12 u bij 18°C induceerde reproductieve diapauze bij respectievelijk 84 en 42% van de wijfjes van *O. naivashae* en *O. thripoborus*. De koudetolerantie van volwassen roofwantsen werd achterhaald door het superkoelingspunt (de temperatuur waarbij de lichaamsvloeistoffen van een insect bevriezen) en de letale tijd (LT_{50}, de tijd vereist om 50% van de populatie af te doden) bij 0 en 5°C te bepalen. De waargenomen superkoelingspunten varieerden alle tussen -21 en -17°C, maar waren lager voor geacclimatiseerde (7 dagen bij 10°C) wijfjes van *O. naivashae*. LT_{50}-waarden voor volwassen individuen van *O. thripoborus* en *O. naivashae* bedroegen gemiddeld respectievelijk 6.4 en 4.4 dagen bij 0°C en 11.6 en 7.8 dagen bij 5°C. Deze bevindingen geven aan dat *O. naivashae* minder koudetolerant en meer diapauzegevoelig is dan *O. thripoborus*. De laatst vermelde soort heeft dan ook meer potentieel om toegepast te worden in biologische bestrijdingsprogramma's in de koelere regio's van zuidelijk Afrika.

Orius thripoborus en *O. naivashae* bieden mogelijkheden als biologische bestrijders van tripsplagen in zuidelijk Afrika, maar zijn mogelijk ook veelbelovend voor de onderdrukking van andere schadelijke geleedpotigen. De predatiecapaciteit, ontwikkeling, voortplanting en populatiegroei van beide roofwantssoorten op de sleutelplagen *Frankliniella occidentalis* (Pergande) (de Californische trips), *Tetranychus urticae* Koch (de bonenspintmijt) en *Myzus persicae nicotianae* Blackman (de tabaksluis) werden onderzocht onder laboratoriumomstandigheden in **Hoofdstuk 6**. Volwassen wijfjes van *O. thripoborus* en *O. naivashae* voedden zich per dag met respectievelijk 24 en 18 tweedestadiumlarven van *F. occidentalis*, en op 15 en 21 *T. urticae* eitjes per dag. De ontwikkeling en voortplanting van beide *Orius* soorten waren het meest gunstig op *F. occidentalis*. Hun intrinsieke groeisnelheden (r_m) lagen het hoogst wanneer ze zich voedden met *F. occidentalis*, met gemiddelde waarden van 0.123 en 0.131 wijfjes/wijfje/dag voor respectievelijk *O. thripoborus* en *O. naivashae*. Op andere prooien vertoonde *O. thripoborus* beduidend hogere r_m-waarden dan *O. naivashae*. Globaal gezien lagen de intrinsieke groeisnelheden op *M. persicae nicotianae* hoger dan op *T. urticae*, maar beduidende verschillen kwamen enkel voor bij *O. thripoborus*. De berekende r_m-waarden voor *O. naivashae* gevoed op de niet-trips prooien waren lichtjes negatief. Onze bevindingen tonen aan dat beide *Orius* soorten potentieel hebben als biologische bestrijders van trips, maar dat enkel *O. thripoborus* eveneens geschikt lijkt voor de onderdrukking van bladluizen en spintmijten.

In **Hoofdstuk 7** werden laboratoriumproeven uitgevoerd om de invloed van de vochtbron en het dieet op de ontwikkeling en de voortplanting van *O. thripoborus* en *O. naivashae* na te gaan. Indien eitjes van de meelmot, *Ephestia kuehniella* (Zeller), werden aangevuld met een stukje snijboon als vochtbron leverde dit een hogere nimfale overleving en een snellere ontwikkeling op in vergelijking met een vochtbron bestaande uit water, ingekapseld in Parafilm. Dit geeft aan dat deze roofwantsen bijkomende nutriënten uit de snijboon kunnen halen. Verder werd het belang van twee onnatuurlijke voedingsbronnen en verse bijenpollen op de ontwikkeling en voortplanting van beide roofwantsen nagegaan. De totale prestaties van beide *Orius* soorten op *E. kuehniella* eitjes en op cysten van het pekelkreeftje *Artemia franciscana* Kellogg waren beter dan op bijenpollen. Desalniettemin maakte een dieet van enkel bijenpollen het mogelijk dat respectievelijk 66 en 70% van de nimfen van *O. thripoborus* en *O. naivashae* ontwikkelden tot volwassen wantsen. Globaal beschouwd was de ontwikkeling en voortplanting van *O. thripoborus* op de onderzochte diëten superieur aan deze van *O. naivashae*.

In navolging van de experimenten met *A. franciscana* cysten in Hoofdstuk 7, werden in **Hoofdstuk 8** bijkomende onnatuurlijke diëten in het laboratorium onderzocht voor de kweek van *O. thripoborus* en *O. naivashae*. De ontwikkeling en voortplanting van beide *Orius* soorten werden nagegaan op volgende voedingsmedia: diepgevroren eitjes van de Mediterrane meelmot, *E. kuehniella*; diepgevroren, behandelde eitjes van de Mediterrane fruitvlieg, *Ceratitis capitata* Wiedemann; en gemengde, mobiele stadia van de voedermijten *Tyrophagus putrescentiae* (Schrank) of *Carpoglyphus lactis* (L). Er werd aangetoond dat *C. lactis* en *T. putrescentiae* een weinig geschikte voedingsbron vormden voor de kweek van *O. thripoborus* en *O. naivashae*, terwijl *C. capitata* eitjes de ontwikkeling en voortplanting van beide roofwantsen volledig ondersteunden. De resultaten op eitjes van *C. capitata* waren gelijkaardig of licht minderwaardig aan deze op *E. kuehniella* eitjes, welke de gebruikelijke voedingsbron is om deze roofwantsen te produceren. Het was mogelijk om *O. thripoborus* gedurende vier opeenvolgende generaties op eitjes van *C. capitata* te handhaven. Dit geeft aan dat behandelde eitjes van de Mediterrane fruitvlieg een geschikt en goedkoper alternatief kunnen zijn voor *E. kuehniella* eitjes bij de langdurige kweek van deze *Orius* soorten.

In **Hoofdstuk 9** worden algemene conclusies getrokken en verdere onderzoeksvragen geformuleerd. *Orius thripoborus* lijkt op vele vlakken een betere biologische bestrijder van geleedpotige plaagorganismen in Zuid-Afrika dan *O. naivashae*. Omwille van zijn groter aanpassingsvermogen op vlak van voeding valt *O. thripoborus* eenvoudiger en goedkoper te kweken en presteert deze soort over de hele lijn beter als predator dan *O. naivashae*. Op basis van de resultaten uit dit proefschrift begon een Zuid-Afrikaans bedrijf onlangs met de massaproductie van *O. thripoborus* voor de commerciële toepassing van deze biologische bestrijder in Zuid-Afrika. Om de vermeerdering en conservatie van *O. thripoborus* als biologische bestrijder van trips in suikerriet en andere belangrijke gwassen in Zuid-Afrika in praktijk te brengen, is verder onderzoek nodig.

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Curriculum vitae

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Publications

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