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Life history and biological parameters of two *Orius* predators on flower and leaf-inhabiting thrips, with notes on the reproductive biology of *Thrips setosus* Moulton

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Abstract

The flower thrips *Frankliniella occidentalis* (Pergande), the leaf-dwelling thrips *Echinothrips americanus* Morgan and the most recent thrips invader *Thrips setosus* Moulton are currently amongst the most concerning pests in Dutch greenhouses. Although *F. occidentalis* is now successfully controlled by generalist predators of the genus *Orius* in vegetable crops, *E. americanus* and *T. setosus* still lack an efficient biological control agent, especially in ornamental crops where these pests are particularly problematic. In the first part of this study, we evaluated the predation capacity, predation behaviour, prey preference and life history traits of the two commercial anthocorid predators, *Orius laevigatus* (Fieber) and *Orius majusculus* (Reuter) on these key pests, in laboratory trials at 25 °C.

Female adults of *O. laevigatus* and O. *majusculus* fed on 18 and 20 *F. occidentalis* adults, 30 and 42 *E. americanus* adults and 36 and 38 *T. setosus* adults per day, respectively. Complementarily, we observed that *F. occidentalis* showed a very agile behaviour in contrast to the low mobility levels observed for *T. setosus* and *E. americanus*. This suggests that prey's mobility was one of the factors influencing the predation capacity of *Orius*.

All development and reproductive parameters were more favourable on the prey *E. americanus,* including intrinsic rates of increase, averaging 0.1662 and 0.1538 females/female/day for *O. laevigatus* and *O. majusculus*, respectively. Additionally, slightly superior fecundities and longevities were scored by *O. laevigatus* in comparison to *O. majusculus*. Overall *O. laevigatus* reared on *E. americanus* had the best performance with values of fecundity (169 eggs/female), longevity (23.6 days for females) and intrinsic rate of increase, approaching what has previously been reported on the high-quality prey *Ephestia kuehniella* Zeller eggs. Contrastingly, *O. majusculus* reared on *F. occidentalis* registered an inferior performance with the lowest intrinsic rate of increase (0.1320).

Regarding the second part of the study, we investigated the effects of supplemental foods on life history traits of *T. setosus*. This species has been recently introduced in Europe and little is known about its biology. We found that the addition of *E. kuehniella* eggs, *Artemia franciscana* Kellogg cysts, pollen or honey to a bean leaf did not influence the oviposition rate or longevity of *T. setosus* females. We thus conclude that these common commercial foods can be used to benefit populations of natural enemies without the risk of at the same time increasing densities of *T. setosus*. So far, this species have not shown omnivorous habits. Additionally, based on progeny production of unmated adult females we conclude that *T. setosus* reproduces through haplodiploid arrhenotoky (unfertilized eggs produce haploid males and fertilized eggs produce diploid females).

Altogether our findings add to the understanding of the reproductive biology and feeding habits of this unstudied species. Furthermore, we show evidence of the potential of *O. laevigatus* and *O. majusculus* as biological control agents of *F. occidentalis, E. americanus* and *T. setosus*. Particularly, we discuss *E. americanus* as a high-quality prey for *Orius*.

Keywords: Biological control, Anthocoridae, Thripidae, predation, life table, food supplementation

Resumo

Tripes (Thysanoptera: Thripidae) são uma das pragas mais importantes para a agricultura e horticultura a nível global. Particularmente *Frankliniella occidentalis* (Pergande) é uma das espécies de tripes mais prejudiciais e que acabou por se espalhar por todo o mundo nos últimos 30 anos. Com uma distribuição menos cosmopolita, a espécie *Echinothrips americanus* Morgan foi detetada pela primeira vez na Holanda em 1993, tendo sido desde então um problema crescente para os agricultores, especialmente na cultura do pimentão e em culturas ornamentais. Mais recentemente, em 2014, a espécie *Thrips setosus* Moulton oriunda do Japão foi detetada em estufas holandesas na cultura de *Hydrangea.* Para além de tipicamente se alimentar da folhagem da planta alvo, este novo invasor é também um vetor eficiente do destrutível vírus do tomate manchado (Tomato spotted wilt virus, TSWV) que afeta diversas culturas vegetais e ornamentais e, portanto, tem o potencial de se tornar numa importante praga da horticultura holandesa, especialmente porque muito pouco se sabe sobre a sua biologia.

Embora o controlo biológico de *F. occidentalis* com predadores generalistas seja atualmente uma opção disponível comercialmente e que mostra resultados bem sucedidos, esta praga é ainda difícil de controlar em culturas ornamentais. É também nestas culturas, para as quais o mercado exige zero tolerância a danos cosméticos, onde as espécies *E. americanus* e *T. setosus* são particularmente problemáticas e para as quais ainda se procura um agente de controlo biológico eficiente, em alternativa à aplicação de pesticidas. Além disso, o estabelecimento de novas espécies invasoras frequentemente interfere com as práticas de controlo integrado de pragas em vigor. Na primeira parte deste projeto o objetivo foi avaliar a capacidade, comportamento e preferência predatória assim como o desenvolvimento, reprodução e taxas de crescimento intrínseco de duas espécies comerciais de predadores generalistas, *Orius laevigatus* (Fieber) e *Orius majusculus* (Reuter), nestas três pragas chave. Todos os ensaios foram realizados em condições laboratoriais a 25 °C.

A capacidade predatória foi testada individualmente para cada predador do sexo feminino em arenas artificiais contendo uma folha de pimentão como substrato e 60 tripes adultas de acordo com cada espécie testada. Fêmeas de *O. laevigatus* e *O. majusculus* predaram 18 e 20 *F. occidentalis*, 30 e 42 *E. americanus* e 36 e 38 *T. setosus* por dia, respetivamente. O comportamento predatório dos antocorídeos assim como o comportamento de cada espécie de tripes foram analisados em ensaios com a duração de 1 h. Foi possível observar diferenças acentuadas particularmente entre o comportamento ágil de *F. occidentalis* e o comportamento sedentário de *E. americanus,* sendo que *T. setosus* apresentou um nível de mobilidade intermédio. O comportamento das tripes, particularmente o seu nível de mobilidade, é sugerido como um dos fatores que afetam a capacidade predatória dos antocorídeos.

Características da história de vida dos predadores *Orius* foram estudadas em arenas contendo uma folha de feijão como substrato durante os estádios larvais sendo posteriormente substituída por um pedaço de vagem de feijão, com a função de substrato de oviposição. Todos os parâmetros de desenvolvimento e de reprodução registaram resultados mais favoráveis com *E. americanus* como presa, resultando em taxas de crescimento intrínseco relativamente elevadas com os valores 0,1662 e 0,1538 fêmeas produzidas por fêmea por dia para *O. laevigatus* e *O. majusculus,* respetivamente. Fecundidade e longevidade ligeiramente superiores foram registadas pela espécie *O. laevigatus* comparativamente a *O. majusculus*. No geral, indivíduos de *O. laevigatus* alimentados com a presa *E. americanus* obtiveram os melhores resultados, registando valores de fecundidade (169 ovos/fêmea), longevidade (23,6 dias para fêmeas) e taxa de crescimento intrínseco bastante próximos ou até superiores ao reportado numa dieta de alta qualidade constituída por ovos do lepidóptero *Ephestia kuehniella* Zeller. Pelo contrário, indivíduos de *O. majusculus* alimentados com *F. occidentalis* registaram um desempenho inferior e consequentemente a menor taxa de crescimento intrínseco (0,1320).

A segunda parte desta dissertação focou-se no estudo da espécie *T. setosus* sobre a qual a literatura disponível é ainda limitada. Em particular, foram estudados os efeitos de vários suplementos alimentares no desempenho de *T. setosus*. A suplementação alimentar é uma técnica cada vez mais utilizada em programas de controlo biológico com a finalidade de melhorar o estabelecimento de populações de inimigos naturais em culturas e acelerar o seu crescimento populacional. No entanto, determinados suplementos podem simultaneamente promover o crescimento populacional de pragas e desta forma perturbar os sistemas de controlo biológico.

A qualidade nutricional de quatro tipos de suplementos alimentares comummente utilizados para beneficiar agentes de controlo biológico foi testada em *T. setosus* através da medição da taxa de oviposição e da longevidade de fêmeas alimentadas nestas dietas. Fêmeas recém-formadas (0-24 h) foram individualizadas em pequenos recipientes contendo uma folha de feijão apenas ou adicionando um dos suplementos testados: pólen (Nutrimite$^{\text{TM}}$), ovos do lepidóptero *E. kuehniella*, cistos do crustáceo *Artemia franciscana* Kellogg ou mel adquirido numa superfície comercial. A taxa de oviposição das fêmeas foi medida diariamente durante 4 dias contando o número de ovos postos por dia na folha de feijão. Todos os tratamentos obtiveram resultados semelhantes para a taxa de oviposição e longevidade das fêmeas. Assim, foi possível concluir que estes suplementos alimentares podem ser utilizados para beneficiar populações de inimigos naturais sem o risco de ao mesmo tempo promoverem o crescimento populacional de *T. setosus*. Com base nos conhecimentos adquiridos até à data, esta espécie não exibe hábitos alimentares omnívoros como se verifica, por exemplo, em *F. occidentalis*.

Por último, o objetivo foi determinar o modo de reprodução em *T. setosus*. Os insetos da ordem Thysanoptera são haplodiplóides, o que significa que ovos haplóides originam machos e ovos diplóides originam fêmeas, e podem exibir diferentes modos reprodutivos: arrenotoquia (ovos não fertilizados produzem machos e ovos fertilizados produzem fêmeas), telitoquia (ovos não fertilizados produzem exclusivamente fêmeas) e deuterotoquia (ovos não fertilizados originam fêmeas e machos). Foi testada a produção de prole por fêmeas adultas não fertilizadas observando-se a emergência de 100% machos. Com base neste resultado sugere-se que a espécie *T. setosus* se reproduz através de arrenotoquia haplodiplóide. Contudo, análises citológicas e genéticas devem complementar este resultado.

Para concluir, a informação obtida nesta dissertação contribuiu para um aumento do conhecimento sobre a biologia reprodutiva e hábitos alimentares do mais recente invasor *T. setosus*. Além disso, este estudo evidencia o potencial de *O. laevigatus* e *O. majusculus* como agentes de controlo biológico das tripes *F. occidentalis*, *E. americanus* e *T. setosus*. Em particular a espécie *E. americanus* é discutida como uma dieta de alta qualidade para antocorídeos.

Palavras chave: Controlo biológico, Anthocoridae, Thripidae, predação, tabela de vida, suplementação alimentar

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1 Introduction

1.1 Biological control

With the world’s human population estimated to reach 10 billion people by the year of 2050, agriculture has never been under more pressure. There is an urge to increase the levels of food production starting by maximizing the crop yield through an effective control of pests and diseases (FAO, 2017). Chemical control - the use of pesticides to reduce or eliminate a crop pest - is the major tool used to manage insect pests in agricultural crops. However, the negative impacts of an intensive use of chemical control methods include public health problems resulting from pesticide residues on food and exposure to chemicals; development of insecticide resistance in pests; loss of wildlife and biodiversity; ground water contamination; amongst others (Pimentel, 2005). Alternatively, a substitute for chemical control has become increasingly adopted since the end of the nineteenth century: biological control (DeBach, 1964). Biological control is defined as the use of living organisms to reduce the population density of a pest. Three types of biological control can be distinguished: classical, augmentative and conservation biological control.

Classical biological control was the first type of biological control deliberately and widely practiced (DeBach, 1964). It consists in the introduction of a natural enemy of non-native origin to control an invasive pest. The goal is to permanently establish populations of natural enemies that will suppress a pest population or reduce its rate of spread. Control is permanent - the pest and natural enemy continue to exist at very low densities without disruptions or outbreaks - and self-sustainable - once established, natural enemies of targeted pests should require no further intervention - thus providing long-term control at minimal ongoing cost (van Lenteren, 2012a).

Augmentative biological control consists in the production of native or non-native biological control agents to be released in large numbers into crops obtaining control of pests in the short-term. While classical biological control seeks to be permanent, in this method the biological control agents are expected to suppress the pest but not to persist from one cropping cycle to the next (van Lenteren, 2012a). This type of biological control has been applied with success in the last century in several cropping systems and today is used in many areas of agriculture, such as fruit and vegetable crops, especially in greenhouse production regimes (van Lenteren, 2012a). Most importantly, augmentative biological control consists of a crucial element: the mass production of the biological control agent in biofactories and its economics. During the past years, the commercial biological control industry has made progresses in developing efficient mass production, shipment and release methods as well as practical guidelines for farmers (van Lenteren & Tommasini, 2003; Morales-Ramos *et al.*, 2014; Leppla & De Clercq, 2019). These advances in the industry have made possible for biological control to become more cost-effective and a strong competitor against chemical control.

Conservation biological control has only recently received more attention even though it has been suggested in Europe as early as 1827 (van Lenteren, 2012a). In this type of biological control the aim is to protect, maintain and increase the naturally occurring population of biological control agents. Therefore, and in order to improve the effectiveness of that population, the environment is often manipulated or modified, for example through provision of natural vegetation patches that increase biodiversity, alternative hosts, supplementary food, oviposition sites or elimination of indiscriminate applications of insecticides (Landis *et al.*, 2000; El-Wakeil *et al.*, 2017).

Overall, when applying biological control methods there is no risk of pesticide resistance and a very low risk of food, water, and environment pollution. Also, there is a strong reduction of growers' exposure to toxic pesticides. Moreover, products free of pesticides meet the increasing demands by modern societies for high quality products as well as for environmental awareness (Pimentel, 2005). To conclude, biological control is undoubtedly the most sustainable, economically profitable and environmentally safest pest management method and is expected to make up 35-40% of all crop protection methods in the year 2050 (van Lenteren, 2012a).

1.2 Greenhouse crops

While open-field production systems are exposed to natural conditions, greenhouse production systems offer a controlled environment adapted to the needs of each crop and a precise nutritional control that results in higher yield and longer crop seasons (Vox *et al.*, 2010). Regardless of their structural complexity (varying from simple plastic film-covered tunnels to highly technological glass covered structures), greenhouses are in general characterized by a rain-free, warm, humid, and windless environment, ideal conditions for crop growing but unfortunately also favourable for the development of pests and diseases (Hanafi, 2003). Conditions like high plant density, year-round production and continuous heating may increase pest outbreaks.

On the other hand, the characteristics of the greenhouse environment may encourage the use of biological control agents. Firstly, the climate control management techniques available enable the optimization of the conditions for the release and implementation of natural enemies (van Lenteren, 2000; Messelink, 2017). Besides, the control of temperature or humidity allows to better predict how the natural enemy and pest populations will development (van Roermund *et al.*, 1997). Secondly, all pest management measures can be applied per greenhouse as they are isolated units and therefore there is a limited risk of interference with pest management in neighbouring greenhouses (van Lenteren, 1988).

Greenhouse production requires the use of large amounts of energy to control a variety of monitoring systems; agro-chemicals to control pests and diseases; water consumption and the generation of huge quantities of waste which form an enormous production cost that is only justified by the cultivation of economically important crops (Bakker *et al.*, 1995; Vox *et al.*, 2010). Those protected crops are typically vegetables like tomato, cucumber, sweet pepper, strawberry, aubergine and ornamental crops where extremely low levels of pest infestation are tolerated (van Lenteren, 1988; Bakker *et al.*, 1995). The main crops of the cut-flowers sector are rose, chrysanthemum, carnation, freesia, gerbera and orchids (Bakker *et al.*, 1995).

But even in protected environments like greenhouses, the diversity of pests in a single crop can be surprisingly high. Whiteflies, thrips, spider mites, aphids, leafminers and caterpillars are amongst the most important insect and mite pests of greenhouse crops cultivated worldwide (Messelink, 2017; Knapp *et al.*, 2020). Dealing with these invasions can be particularly challenging in organic greenhouse crops where no chemical tools are allowed. Moreover, each crop has particular characteristics that makes them differently vulnerable to pests but also affects the performance of natural enemies. As a result, every crop has its own food web of pests and natural enemies and thus the development of biological control programmes needs to be crop specific (Messelink, 2017).

1.3 Thrips as important pests in greenhouses

Thrips (Thysanoptera) are small (averaging about 2 mm), opportunistic, vagile and often invasive insects colonizing a wide range of niches and habitats. The order Thysanoptera consists of more than 5500 species of thrips, encompassing two suborders, eight families and hundreds of genera. The life cycle of these insects consists of an egg stage, two active larval instars (L1 and L2), a nonfeeding prepupal stage and either one (suborder Terebrantia) or two (suborder Tubulifera) sedentary pupal stages followed by the adult stage. Tubulifera species lay eggs outside of host material and are all placed in the family Phlaeothripidae. Terebrantia species have a well-developed ovipositor allowing them to lay eggs inside plant tissue and are organized in the remaining seven families (Lewis, 1997).

Amongst all families we can find an astonishingly broad range of lifestyles that exploit a variety of resources. These comprise obligate predators used as control agents against other thrips (e.g. *Franklinothrips* spp.), ectoparasitic thrips (e.g. *Aulacothrips dictyotus* Hood), fungus-feeding thrips (e.g. *Idolothrips spectrum* Haliday) and many phytophagous thrips such as leaf feeders like gall inducing species [e.g. *Gynaikothrips ficorum* (Marchal)] or flower feeders including a few species with important role as pollinators (e.g. pollination of cycads by *Cycadothrips* in Australia) (Kirk, 1996).

Thrips feed by using its single mandible to pierce a hole in the plant surface through which its stylet is inserted to draw the food (Hunter & Ullman, 1992). This piercing-sucking feeding technique causes a range of symptoms in plant tissue. On leaves, extensive feeding leads to the characteristic silvering damage due to air entering cells from which the contents have been removed. A depletion in the density of foliage can cause a reduction in the photosynthesis levels and consequently in the crop production. On fruits, the effect is similar leading to scarring and corky tissue development. Such scarring and deformation injuries resulting not only from feeding but also from thrips oviposition drastically reduce the crop yield and the aesthetic quality and marketability of the products. The latter is especially severe in the ornamental sector in which the threshold for cosmetic damage is extremely low (Childers & Achor, 1995; Childers, 1997).

Adding to a wide resource utilization, several species of Thysanoptera are preadapted to an invasive lifestyle that allows them to successfully establish and proliferate when introduced in a new uncolonized region. Lack of obligate diapause, multivoltinism and polyphagy are biological attributes that often predispose the invasiveness of these insects. Besides, they exhibit high fecundity, short generation times and a predisposition for parthenogenesis that allows small founding populations to rapidly increase and establish independently of male density (Morse & Hoddle, 2006; Reitz, 2009).

Thrips are known for their haplodiploidy sex-determination system, which means that males usually develop from haploid eggs and females from diploid eggs. As to their mode of reproduction all three types of parthenogenesis can be distinguished in thrips: arrhenotoky, thelytoky and deuterotoky (Krüger, 2016). Arrhenotoky is the most common form of reproduction where unfertilized (haploid) eggs develop parthenogenetically into males and fertilized (diploid) eggs develop into females. Amongst many others, *Frankliniella occidentalis*, *Echinothrips americanus* and *Gynaikothrips ficorum* (Marchal) reproduce by this mode (Lewis, 1973). In thelytoky, unfertilized eggs develop exclusively into females and the process of fertilization is absent. Only a few species like for example *Chaetanaphothrips orchidii* (Moulton) reproduce by thelytoky (Lewis, 1973). Furthermore, a different form of thelytoky can be induced by the infection with parthenogenesis-inducing bacteria in the genus *Wolbachia* or *Cardinium* causing the revertible or microbe-associated thelytoky (Kumm & Moritz, 2008). Some species, such as *Thrips tabaci* Lindeman, are able to reproduce either by arrhenotoky or thelytoky depending on the geographical area of the population (Lewis, 1973) or even adopt both reproductive modes in the same geographical area (Nault *et al.*, 2006). It was also in *T. tabaci* that deuterotoky was reported for the first time occurring in thrips (Nault *et al.*, 2006). Deuterotoky, also known as amphitoky, is an uncommon parthenogenetic mode of reproduction in thrips where both sexes are produced from unfertilized eggs.

Regarding their role as pests in horticulture, several species of the family Thripidae are known as important plant pests in ornamental and vegetable crops worldwide (Lewis, 1997). This project focuses on three of the most important thrips pests recognized in Dutch greenhouses: *Frankliniella occidentalis*, *Echinothrips americanus* and *Thrips setosus*.

1.3.1 *Frankliniella occidentalis*

The western flower thrips *Frankliniella occidentalis* (Pergande), originally from the western North America, has become a major worldwide pest of agricultural and horticultural crops (Lewis, 1997). It was unintentionally introduced in the Netherlands in 1983 and in the last decades it has spread almost worldwide via transport of infected horticultural material (Kirk & Terry, 2003). This cosmopolitan and highly polyphagous invader is abundant in many field and greenhouse crops where it is a particularly significant pest in cucumber, sweet pepper, aubergine and many ornamental crops (Reitz *et al.*, 2020).

Frankliniella occidentalis causes direct damage resulting from feeding and oviposition on leaves, flowers, and fruits, as well as indirect damage caused by the transmission of destructive plant viruses in the genus *Orthotospovirus* (Bunyaviridae). In fact, the western flower thrips is the most efficient thrips vector of several Tospoviruses, including Alstroemeria necrotic streak virus (ANSV), Chrysanthemum stem necrosis virus (CSNV), Groundnut ringspot virus (GRSV), Impatiens necrotic spot virus (INSV), Tomato chlorotic spot virus (TCSV), Tomato zonate spot virus (TZSV), and Tomato spotted wilt virus (TSWV), economically the most important (Rotenberg & Whitfield, 2018).

Frankliniella occidentalis is also a model organism for studying omnivory since this species feeds on pollen, small arthropods and leaf cell contents. Alternatively to being a pest of agricultural crops and wild plants it can prey on eggs of its natural enemies, the predatory mite *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) and *Amblyseius cucumeris* Oudemans (Acari: Phytoseiidae) (Faraji *et al.*, 2001). Moreover, *F. occidentalis* predates on eggs of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Trichilo & Leigh, 1986) and crawlers of the whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) which are both herbivores and its competitors (van Maanen *et al.*, 2012). Thus, *F. occidentalis* is an intraguild predator, competing with and feeding on spider mites and whiteflies. Overall, the omnivorous habits of *F. occidentalis* may provide it a competitive advantageous over other thrips when the food sources available are limited.

After many years of research, the management of this damaging pest is still a challenge for the growers. Although in vegetables the implementation of biological control programmes has been successful for numerous years (Messelink *et al.*, 2006, 2008), in ornamental crops the control of *F. occidentalis* still relies on the frequent use of insecticides which combined with its biological attributes results in a great propensity for developing resistance to chemical insecticides (Bielza, 2008; Gao *et al.*, 2012). More effective biological control solutions are particularly required in ornamental crops as an alternative to chemical control methods.

1.3.2 *Echinothrips americanus*

The poinsettia thrips *Echinothrips americanus* (Morgan) is an increasing problem in greenhouse cultivation. This leaf-dwelling thrips is native to eastern North America, but in 1993 it was reported for the first time in The Netherlands on *Araceae* cultivations, probably imported from USA through cuttings of *Syngonium* (Vierbergen, 1998). In recent years *E. americanus* has spread into several European and Asian countries being found in a wide spectrum of host plants including ornamentals and vegetables like sweet pepper and cucumber (Vierbergen *et al.*, 2006). Currently this pest is particularly problematic in roses, gerberas, bouvardias and potted plants (Pijnakker *et al.*, 2017a).

This highly polyphagous pest causes extensive foliage damage characterized by black spots that reduce photosynthesis and the economic value of the crop. Nevertheless, the pest status of *E. americanus* is generally not as important as, for example, of *F. occidentalis* (Trdan *et al.*, 2003). Unlike this flower thrips that preferably reside in narrow spaces in the apical buds, the leaf-dwelling *E. americanus* is exposed onto older leaves of the plant canopy, thus can be easily controlled using chemical insecticides (Vierbergen, 1998; Shipp *et al.*, 2001). Nevertheless, cases of resistance to the common insecticides applied have started to be detected in some local populations (Ivanova *et al.*, 2017). Moreover, with growers still heavily relying on pesticides, the success of biological control practices is limited as non-selective pesticides disrupt the already established biological control agents of other pests (Hoogerbrugge *et al.*, 2014).

1.3.3 *Thrips setosus*

The Japanese flower thrips *Thrips setosus* Moulton is native to Japan where is one of the most widespread leaf-living thrips on herbaceous crops (Kobatake & Nakazawa, 1988). *Thrips setosus* was primarily detected in The Netherlands in 2014, causing a serious infestation in *Hydrangea* culture in Kudelstaart (Vierbergen & Loomans, 2016). Remarkably, *Hydrangea* has not previously been recorded as a host plant of this species, which in its native range is known as a pest of ornamentals like *Dianthus*, *Chrysanthemum* and *Dahlia*, and vegetables like pepper, tobacco (Koizumi, 1985) and tomato (Kobatake, 1984). After its introduction in the Netherlands, *T. setosus* has been recorded in many other European countries including Croatia, France, Germany and the United Kingdom, threatening a variety of hosts such as *Begonia*, *Chrysanthemum*, *Origanum*, *Saintpaulia*, *Aster*, *Poinsettia*, *Sparmannia* and *Streptocarpus* (Pijnakker *et al.*, 2019).

Contrary to what its common name suggests, the Japanese Flower thrips are not regarded as true flower thrips because even though they feed on sterile *Hydrangea* flowers (Vierbergen & Loomans, 2016) they are not known to feed on pollen (Murai, 1991). This polyphagous species is primarily a leaf-feeder where it typically causes silvering damage and excrements (Vierbergen & Loomans, 2016). Consequently, *T. setosus* extensive flower and leaf damage have especially high impacts in ornamental crops as the market exhibits zero tolerance to cosmetic damage. Additionally, *T. setosus* has the potential to cause severe indirect damage as a vector of Tomato spotted wilt virus (TSWV), one of the most important thrips transmitted virus that affects several vegetable and ornamental crops (Tsuda *et al.*, 1996).

Besides its virus transmission, little is known about the ecology, phenology, reproductive biology, and host range of *T. setosus*. Studies so far demonstrate an ecological performance characterized by a fast development (shorter than *E. americanus* but slightly longer than *F. occidentalis*), high fecundity and high potential of increase (Murai, 2011) predisposing *T. setosus* to become a potential major pest in many crops. Although the mode of reproduction in *T. setosus* is still unclear Vierbergen and Loomans (2016) suggested haploid facultative arrhenotoky based on the sex ratio found. Its reproductive diapause induced by a short-day photoperiod (Nakao, 1998) is probably the reason why this species is able to survive relatively severe winters in the north of Japan (Murai, 2001) being likely to survive Dutch winters as well, even in the northern parts of the country (Vierbergen & Loomans, 2016). Currently, control measures are mainly based on insecticides applications, with still a large spectrum of insecticides available (Pijnakker *et al.*, 2019). Nevertheless, chemical control should not be relied as a primary and single method since the risk of development insecticide resistance is significant.

1.4 Biological control of thrips

Intensive research on the biology and ecology of thrips have been fundamental for the development of the variety of tactics currently available in integrated pest management programmes of this pest. For a more efficient thrips management, growers can make a combinational use of different strategies: detection and monitoring, physical and mechanical measures, host plant resistance, cultural control, behavioural control, chemical control, and biological control (Mouden *et al.*, 2017; Reitz *et al.*, 2020). As an alternative to conventional insecticides, in the last decades many natural enemies have been introduced in the augmentative biological control market, providing adequate control for the needs of each pest, crop or level of infestation (Gurr & Wratten, 2000). Predatory bugs, predatory mites, soil-dwelling predators and entomopathogens are the major groups of biocontrol agents commercially used against thrips (Mouden *et al.*, 2017), yet other natural enemies have also been studied for this purpose.

1.4.1 Predatory mites

Predatory mites are undoubtfully the most important group of biological control agents in commercial augmentative biological control (Knapp *et al.*, 2018). They are mainly used in protected crops to control phytophagous mites, whiteflies and thrips. Regarding thrips control, some species of predatory mites prey exclusively on first instar larvae whereas others are capable of preying upon both larval stages (Mouden *et al.*, 2017), yet they never prey on adult thrips.

Decades of research have provided growers with a wide range of commercial mite species. At present, *Amblyseius swirskii* Athias-Henriot and *Neoseiulus cucumeris* (Oudemans) are the two major species used in thrips control (Knapp *et al.*, 2018). Nevertheless, many other species such as *Amblydromalus limonicus* (Garman and McGregor), *Iphiseius degenerans* and *Transeius montdorensis* (Schicha) proved to be efficient thrips predators and are thus also commercialized (Cock *et al.*, 2010; van Lenteren, 2012b; Mouden *et al.*, 2017).

Distinctly, *A. swirskii* has quickly become the main predator used for biological control of *F. occidentalis* in protected crops worldwide and a top seller in the commercial market since its introduction in 2005 (Buitenhuis *et al.*, 2015; Calvo *et al.*, 2015; Knapp *et al.*, 2018). Other than being a good generalist predator, several factors contribute to the success of this phytoseiid mite: (1) it can be easily mass reared allowing economic mass production; (2) it can feed on non-prey food sources such as pollen to persist in the crop in periods of prey scarcity; and (3) it can control several pests simultaneously including *Frankliniella occidentalis*, the whiteflies *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Messelink *et al.*, 2008) and the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (Buitenhuis *et al.*, 2015; Calvo *et al.*, 2015).

Following the success of several predatory mite species against pollinivorous thrips such as *F. occidentalis*, the commercially available predatory mites *Neoseiulus cucumeris* (Oudemans) and *Iphiseius degenerans* were evaluated against *E. americanus* but neither of them was successful (Opit *et al.*, 1997). The predatory mites *Amblyseius swirskii* (Athias-Henriot), *Amblydromalus limonicus* (Garman and McGregor), *Euseius gallicus* Kreiter and Tixier and *Euseius ovalis* (Evans) proved to be good candidates for controlling poinsettia thrips in initial studies (Ghasemzadeh *et al.*, 2017). However, long-term greenhouse trials showed that predatory mites do not provide an efficient control of *E.*

americanus, even when in high population densities (Pijnakker *et al.*, 2017a). The reason for this remains unclear.

Regarding *T. setosus* control, the species *N. cucumeris, E. gallicus, Amblyseius andersoni* (Chant) and *A. swirskii,* have also been tested either with or without the addiction of pollen as supplemental food. Unfortunately, even when the predatory mites were able to establish in the crop none of the tested strategies was successful in controlling *T. setosus* (Pijnakker *et al.*, 2019).

Soil-dwelling predators compliment the work of biocontrol agents that predate on larvae and adults by providing predation on thrips pupae. Importantly, about one-third of *F. occidentalis* life is spent in the soil after pupae drop from host plant to complete pupation in higher relative humidity conditions (Steiner *et al.*, 2011). Therefore, soil-dwelling predators may enhance the biological control of thrips like *F. occidentalis* but are expected to be ineffective against thrips that do not pupate in the soil. In Europe, three soil-dwelling predatory mites are available for commercial use, *Macrocheles robustulus* (Berlese) (Messelink & van Holstein-Saj, 2008), *Stratiolaelaps scimitus* (Berlese) and *Gaeolaelaps aculeifer* (Canestrini) (Mouden *et al.*, 2017).

Summarizing, during the last decade predatory mites have been successfully and widespread used as biological control agents of *F. occidentalis* in greenhouse vegetable and ornamental crops (Messelink *et al.*, 2006, 2008; Buitenhuis *et al.*, 2015) but contrastingly unsuccessful against *E. americanus* (Pijnakker *et al.*, 2017a) and *T. setosus* (Pijnakker *et al.*, 2019). As a consequence, growers' reliance on the alternative chemical approach is still particularly concerning in high-value ornamental crops where the perceived risks of insect damage often encourages intensive insecticide applications.

1.4.2 Entomopathogens

Thrips biological control agents based on entomopathogenic microorganisms consist of nematodes and fungi. Entomopathogenic nematodes are insect-specific parasites that enter the host insect through its natural apertures (oral cavity, anus and spiracles) or through the cuticle and penetrate the insect's hemocoel where they release their symbiotic bacteria, thus causing the host death (Dowds & Peters, 2002). Several species and strains in the nematode genera *Steinernema*, *Heterorhabditis* and *Thripinema* have been evaluated to control thrips, however *Steinernema feltiae* (Filipjev) is currently the only commercial entomopathogenic nematode against *F. occidentalis* (Trdan *et al.*, 2007). This lack of species variety available in the market is likely due to increased costs of mass producing these agents and often high concentrations required for sufficient control, making its commercialization economically not viable (Ebssa *et al.*, 2004).

Furthermore, entomopathogenic fungi also belong in the category of entomopathogenic microorganisms used as biological control agents. The fungal conidia infect thrips directly by penetrating their cuticles to obtain nutrients for growth and reproduction, thus killing the host. Although with inconsistent efficiency, several formulations of entomopathogenic fungi, like for example *Lecanicillium muscarium* (Petch) Zare & W. Gams (Hypocreales: Cordycipitaceae), are now available to control thrips populations in greenhouse vegetable and ornamental crops (Jacobson *et al.*, 2001; Ugine *et al.*, 2007; Mouden *et al.*, 2017; van Lenteren *et al.*, 2018). However, important constrains such as difficulties in mass production, storage, and formulation haven't allowed entomopathogenic fungi to prosper as biocontrol agents (Gonzalez *et al.*, 2016).

In general, single treatments with entomopathogens may often result in low and inconsistent control levels when compared to other agents. On the other hand, the use of microbials in combination with biological control agents such as foliar-dwelling mites and soil-dwelling mites has shown more encouraging results promoting the incorporation of these combinatorial strategies in integrated pest management programmes (Pozzebon *et al.*, 2015; Gonzalez *et al.*, 2016; Mouden *et al.*, 2017).

1.4.3 Predatory thrips

Although predatory thrips are not amongst the most important biological control agents, the obligate predator *Franklinothrips vespiformis* Crawford (Thysanoptera: Aelothripidae) is known to be efficient against thrips like *F. occidentalis* and *T. tabaci* (Pizzol *et al.*, 2008). This aggressive predator was tested for its potential as a natural enemy of *E. americanus* (Pijnakker *et al.*, 2017a) and *T. setosus* (Pijnakker *et al.*, 2019). In both studies, *F. vespiformis* effectively predated the two thrips species yet its establishment in commercial crops was problematic and the costs of inundative releases became unviable.

1.4.4 Predatory bugs

1.4.4.1 Mirids

Despite the success of other agents in controlling thrips, mirid generalist predators (Hemiptera: Miridae) have been particularly evaluated for their simultaneous management of both thrips and whiteflies. In a recent study, *Nesidiocoris tenuis* (Reuter) and *Macrolophus pygmaeus* (Rambur) were capable of establishing and significantly reducing the numbers of both pests in sweet pepper, whereas *Dicyphus maroccanus* Wagner was not effective in reducing the infestations in the same crop (Bouagga *et al.*, 2018). Furthermore, Messelink and Janssen (2014) demonstrated that the combined release of the generalist predators *M. pygmaeus* and *O. laevigatus* enhances the biological control of *F. occidentalis* and of the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in sweet pepper crops.

Following the trend, this group of biological control agents have been investigated for the management of *E. americanus*. In a preliminary study, the predation capacity of the omnivorous predator *Dicyphus errans* (Wolff) on *E. americanus* was assessed confirming its suitability as a biological control agent (Ingegno *et al.*, 2017). More recently, *Macrolophus pygmaeus* (Rambur) was also described as effectively suppressing *E. americanus* on single gerbera plants (Leman *et al.*, 2020). Although these agents may be a promising biocontrol solution for the pest, special attention should be given to its possible implementation considering the fact that the plant-feeding behaviour of these predators can potentially cause flower malformations (Castañé *et al.*, 2011).

1.4.4.2 Anthocorids

Among many natural enemies known to attack thrips, anthocorid bugs (Hemiptera: Anthocoridae) are probably the most important as biological control agents in protected crops. Many species in the genus *Orius* have proved to be particularly successful predators against thrips in vegetable and ornamental crops (Mouden *et al.*, 2017; Reitz *et al.*, 2020). Anthocorid bugs will be the target biological control agents in this dissertation and for this reason they will be introduced with more detail in the next subchapter.

1.5 *Orius* as biological control agents against thrips

Orius, commonly known as minute pirate bugs, is a genus of omnivorous bugs in the family Anthocoridae. Anthocorid bugs reproduce sexually, and their life cycle consist of an egg stage followed by five nymphal stages in which the nymphs increase in size until reaching the adult stage (Leon-Beck & Coll, 2009). As omnivorous arthropods they feed both on prey and plant resources while having a generalist feeding spectrum, meaning that they predate on a wide range of insects such as aphids, whiteflies, thrips, and spider mites. However, their main prey are thrips of which they predate on larvae and adults, giving them a potential advantage over other natural enemies that cannot subdue adult thrips.

The habitat preference of *Orius* is linked to their pollinivorous feeding habits. Adults are highly attracted to pollen producing plants where they preferably colonize the flower parts (van den Meiracker & Ramakers, 1991; Hansen *et al.*, 2003). Importantly, the fact that flower-inhabiting thrips and most anthocorid predators are anthophilous facilitates pest suppression due to habitat overlap (Northfield *et al.*, 2017). Furthermore, the consumption of plant provided-foods, such as pollen, sustains *Orius* populations in periods of prey scarcity thus enhancing pest control throughout the crop season (Coll & Guershon, 2002). For example, pepper growers are encouraged to release *Orius* when the plant has started flowering, thus ensuring that the population will establish feeding on pollen, even in the absence of the preferred prey. However, many crops do not provide the additional food resources (e.g. cucumber) required for the natural enemies to establish without prey and therefore strategies like the provision of supplemental food should be considered (Oveja *et al.*, 2016).

The choice of plant host is important not only for its nutritional value but also because the plant characteristics can influence *Orius* oviposition and reproductive success, and consequently its establishment in the crop. As anthocorids exhibit endophytic oviposition (Lundgren, 2011), they prefer host plants with thin external tissues (Lundgren *et al.*, 2008). Additionally, in ornamental crops the success of *Orius* depends on the structural complexity of the flowers that may provide a refuge for thrips (Pozzebon *et al.*, 2015). Since the potential of *Orius* species was discovered, intensive research programmes have tested the ability of different species in controlling thrips (Tommasini, 2003; Blaeser *et al.*, 2004; Bonte *et al.*, 2015). To date, about a dozen of *Orius* species are commercially available in the world, differing in the market value and region where they are used (van Lenteren, 2012b; Mouden *et al.*, 2017).

1.5.1 *Orius insidiosus*

Orius insidiosus (Say) was the first species of *Orius* commercially available for augmentative biological control (van Lenteren, 2012b). Although in sweet pepper *O. insidiosus* was able to suppress western flower thrips almost to extinction, the success of this natural enemy is partial during autumn and winter when they enter reproductive diapause induced by photoperiod (Funderburk *et al.*, 2000). As *F. occidentalis* and several other thrips species do not undergo diapause it was essential to find a non-diapausing species of *Orius* suitable for winter and early spring seasons when inoculative releases are necessary. Furthermore, in Europe the exotic species *O. insidiosus* was later replaced by the indigenous *O. laevigatus* which has become the main *Orius* species for thrips control (van Lenteren, 2012a).

1.5.2 *Orius laevigatus*

Orius laevigatus (Fieber) has met great success worldwide as a biological control agent as it provides good control under various conditions and it is easily mass-produced (van Lenteren, 2012a). The fact that this species does not enter reproductive diapause (Tommasini & van Lenteren, 2003) allows it to successfully suppress thrips populations all-year-round in vegetables and ornamentals (Weintraub *et al.*, 2011; van Lenteren, 2012b). Whereas other biological control agents have failed in controlling *E. americanus*, anthocorid predators on the other hand have shown more encouraging results. In short-term trials *O. laevigatus* was observed to effectively predate all stages of *E. americanus* on gerbera (Pijnakker *et al.*, 2017a) as well as the above stated species *O. insidiosus* in pepper plants (Opit *et al.*, 1997). However, the long-term control provided by these predators against *E. americanus* was found to be insufficient.

1.5.3 *Orius majusculus*

The predatory bug *Orius majusculus* (Reuter) is commercially available in Europe and USA (Mouden *et al.*, 2017) being more commonly used in northern European regions. Unlike *O. laevigatus,* this species shows reproductive diapause induced by photoperiod (Meiracker, 1994) which may limit its success to specific seasons.

Oveja *et al.* (2012) studied the effect of pollen on some predators proving that *O. laevigatus* shows a substantial increase in its performance when supplied with pollen in contrary to *O. majusculus* for which only a slightly improvement in the longevity is observed. Furthermore, van Schelt *et al.* (2002) considers *O. majusculus* a more promising biological control agent against *E. americanus* based on its greater spatial overlap and superior long-term impact on the population of *E. americanus* in sweet pepper plants. While *O. laevigatus* is highly attracted to the flower parts of the plant (Hansen *et al.*, 2003), *O. majusculus* is suggested as a less pollen-dependent (Ramakers & O'Neill, 1999) and for this reason its presence is expected to prevail on leaves where leaf-dwelling thrips most often occur. Moreover, in greenhouse cucumber (most commercial varieties are ginoic hybrids – they only produce female flowers and do not have pollen) *O. majusculus* is particularly effective because it can successfully control the flower thrips *F. occidentalis* and establish in the crop (Oveja *et al.*, 2016) while other *Orius* species cannot (Chambers *et al.*, 1993; Rajabpour *et al.*, 2011).

1.6 "Standing army" of beneficials

During the last decade, the concept of building a "standing army" of beneficials in greenhouse crops has received increasing attention in biological control (Messelink, 2017; Pijnakker *et al.*, 2017b). A "standing army" is a population of biological control agents which is maintained through the provision of supplemental food sources such as pollen, harmless arthropods, or factitious preys like *Ephestia kuehniella* eggs or *Artemia franciscana* cysts. The presence of alternative foods enables generalist predators to reproduce and to establish in the crop even before pests occur and allows its subsistence in periods of low prey densities, therefore contributing to a more suitable pest management strategy. Several strategies can be implemented to support the build-up of populations of beneficials (Messelink *et al.*, 2014).

1.6.1 Pollen

Pollen is an important food source for several predatory mites, flower-dwelling anthocorids and some mirid species (Ramakers & O'Neill, 1999). When not present naturally in the crop, pollen can be supplemented to improve the performance of these natural enemies. This strategy is particularly effective with predatory mites. In 2013, *Typha angustifolia* Linneaus pollen became commercially available and the use of this new product started being implemented with predatory mites. The application of commercial pollen became a routine practice of growers in many crops like rose and cucumber, allowing predatory mites to establish in the crop before the pest arrival with only a couple of introductions (Pijnakker *et al.*, 2017b).

As for anthocorids and mirids the effect of artificial pollen is often less beneficial when applied alone, it is often applied in combination with other food supplements. For instance, when fed only on pollen both *O. laevigatus* and *O. albidipennis* strongly reduced egg production whereas the latter also suffered a reduction in longevity (Cocuzza *et al.*, 1997b). On the other hand, when pollen is supplemented in combination with *E. kuehniella* eggs the result is an increase in the fecundity of *O. albidipennis* (Cocuzza *et al.*, 1997b). Similarly, this combination of supplemental foods can have a positive effect on

O. insidiosus establishment in crop plants (Labbé *et al.*, 2018). Overall, the contrasting results of several studies on this subject suggest that the response to pollen can strongly vary depending on the predator species but also on the plant origin of pollen (Chitturi *et al.*, 2006), thus requiring a careful evaluation of its use in biological control programmes.

1.6.2 *Ephestia kuehniella* eggs

Sterilized eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (henceforth *Ephestia*) are a highly nutritional factitious prey that has been widely used in the mass rearing of generalist predators and parasitoids such as *Trichogramma* (Hymenoptera: Trichogrammatidae). This type of food has invariably been found to be the most suitable for generalist predatory bugs, sustaining the greatest fecundity and longevity of several predators [*O. laevigatus* (Cocuzza *et al.*, 1997b), *O. insidiosus* (Calixto *et al.*, 2013; Labbé *et al.*, 2018), *O. albidipennis* (Cocuzza *et al.*, 1997b), *M. pygmaeus* (Labbé *et al.*, 2018), *O. majusculus* (Montoro *et al.*, 2020a)], when compared to several other diets. This improvement on the predator's life history traits is a result of the high protein and high fatty acids contents present on *E. kuehniella* eggs (De Clercq *et al.*, 2005; Montoro *et al.*, 2020a). Unfortunately, this type of food requires a complex production system (Doğramaci *et al.*, 2011) that increases its market cost therefore making it unsuitable for wide scale application in crop food supplementation. The need of finding a less costly food source is encouraging the investigation of alternative foods with similar potential value.

1.6.3 *Artemia franciscana* cysts

The cysts of the brine shrimp *Artemia franciscana* (Leach) (henceforth *Artemia*) are known to be suitable for mirids, anthocorids and phytoseiids for many years. Despite of it, *Artemia* has exclusively been used in commercial greenhouses to enhance the predatory bug *Macrolophus pygmaeus* in tomato and sweet pepper crops (Messelink *et al.*, 2014). Diet supplementation with *Artemia* cysts has been reported to increase the fitness of *Orius* but often to a smaller extent than *Ephestia* eggs (De Clercq *et al.*, 2005; Oveja *et al.*, 2012). In contrast, Labbé *et al.* (2018) found that *O. insidiosus* could not complete its development when fed exclusively on commercial *Artemia* cysts. When supplemented to predatory mites, cysts of *Artemia* have shown to be suitable for several species including *Iphiseius degenerans* (Vantornhout *et al.*, 2004), *Amblydromalus limonicus* (Vangansbeke *et al.*, 2014) and *Amblyseius swirskii* (Nguyen *et al.*, 2014) yet contrasting results have been reported in similar studies (Leman & Messelink, 2015; Vangansbeke *et al.*, 2016; Sade *et al.*, 2019). This lack of consensus is likely due to differences in the intrinsic quality of the *Artemia* strains used in the experiments and consequently its nutritional value or differences in the dehydration and decapsulation process.

Even though the results of food supplementation with *Artemia* cysts do not approach what has been found with *Ephestia* eggs, the use of this factitious prey shows important advantages. The biochemical analyses of *Artemia* cysts revealed that this food contains equal or greater amounts of protein than *Ephestia* eggs but almost three times less fatty acid content (De Clercq *et al.*, 2005). Despite some sporadic reports of improved establishment of predators due to the supplementation with *Artemia* cysts (Oveja *et al.*, 2016), *Orius* predators do not perform well on the available *Artemia* products. In terms of the economic feasibility however, it is estimated that a commercial product based on standard quality *Artemia* cysts is about 3% of the cost of pure *Ephestia* eggs (Biobest; Beneficial Insectary, Redding, CA), which confers an important advantage for this food. Recently, Sade *et al.* (2018) tested a new commercial form of *Artemia* and found that the application of this premium quality *Artemia* cysts allowed *O. laevigatus* to early establish in a commercial pepper crop providing a better control of thrips.

Nevertheless, the production of this nutritional superior *Artemia* product may increase its commercial cost.

1.6.4 Risks of food supplementation

The standing army approach can involve risks to pest control due to the fact that alternative food can also be consumed by omnivorous pest species such as thrips. Whereas leaf-inhabiting thrips like *E. americanus* do not feed on pollen, the pollinivorous *F. occidentalis* shows an increase in population growth when fed on several types of pollen, including cattail and corn pollen, as well as on the eggs of *E. kuehniella* (Leman & Messelink, 2015; Vangansbeke *et al.*, 2016). However, studies with predatory mites support that possible short-term positive effects on prey densities through predator satiation (functional response) or increased thrips reproduction (numerical response) were compensated by the long-term predator's numerical response, in the end resulting in a better thrips control (van Rijn *et al.*, 2002; Leman & Messelink, 2015). Compared to pollen or *E. kuehniella* eggs, *A. franciscana* cysts represent a smaller risk of enhancing thrips population build up as they were found to have no effect or only a limited promoted effect on the development and reproduction of *F. occidentalis* (Leman & Messelink, 2015; Vangansbeke *et al.*, 2016).

Overall, when building a "standing army" of beneficials it is important to select types of food that are more suitable for predators than for thrips, thus minimizing the potential risk of a pest outbreak. Consequently, studying the response of predator and prey on different types of alternative foods is crucial for a more effective use of food supplementation in biological control programmes.

1.7 Aims and research objectives

As highlighted above, *F. occidentalis* is now successfully controlled by predatory mites and bugs in vegetable crops (Mouden *et al.*, 2017; Reitz *et al.*, 2020). However, predatory mites demonstrate limited success in the control of *E. americanus* (Hoogerbrugge *et al.*, 2014; Ghasemzadeh *et al.*, 2017; Pijnakker *et al.*, 2017) and the few *Orius* species evaluated show promising but not yet satisfying results (Opit *et al.*, 1997; Pijnakker *et al.*, 2017a). Further research is needed to find a suitable biological control agent for *E. americanus* specially in ornamental crops where this pest is particularly problematic. Evaluating a less pollen dependent predatory bug, such as *O. majusculus*, might lead to better results by decreasing habitat mismatch observed between highly anthophilous *Orius* species, that colonize the flower parts of the plant (Hansen *et al.*, 2003) and leaf-inhabiting thrips (Ramakers *et al.*, 2000). Overall, few studies have evaluated anthocorid predators as biological control agents of *E. americanus* and *T. setosus*.

Particular attention was given to the recent invader *T. setosus*, as very little is known about its reproductive biology. We intend to improve knowledge on the reproductive biology of this species by showing evidence of *T. setosus* mode of reproduction, previously suggested as arrhenotoky (Vierbergen & Loomans, 2016). Furthermore, we examined whether *T. setosus* shows omnivorous feeding habits like *F. occidentalis* does by testing its performance on plant provided resources (pollen and honey) as well as on factitious preys (*E. kuehniella* eggs and *A. franciscana* cysts). Research on *T. setosus* is crucial to assess its economic and ecological significance and whether this justifies the development of control programmes for this species. In search for an effective biological control agent for the Japanese flower thrips, *Orius* species are undoubtfully a promising solution (Nagai, 1990).

Altogether, the aim of this research project was to:

1. Evaluate the predation capacity, predation behaviour, prey preference, juvenile development and reproduction of two commercial anthocorid predators (*O. laevigatus* and *O. majusculus*) on flower (*F. occidentalis*) and leaf-inhabiting thrips (*E. americanus* and *T. setosus*) in laboratory trials.
2. Study the effects of different types of supplemental food on *T. setosus* oviposition and longevity, and the mode of reproduction of this species.

Data obtained in this study contribute to understand more about the biology and behaviour of *O. laevigatus* and *O. majusculus* on important thrips species diets. Additionally, studying the effects of alternative foods on *T. setosus* life history traits will help understand how the provision of supplemental food for the natural enemies will influence the population growth of this pest. Overall, we aspire to provide the scientific community with important results that can further contribute to biological control solutions for the greenhouse sector.

2 Materials and Methods

2.1 Insect cultures

2.1.1 *Orius laevigatus* and *Orius majusculus*

Female adults of *O. laevigatus* and *O. majusculus* were obtained from Koppert Biological Systems (Thripor-L, Berkel en Rodenrijs, The Netherlands) and EWH BioProduction (Tripsrovtæge, Tappernøje, Denmark), respectively. They were further reared in plastic jars (Ø 11 cm x 13 cm) with lids covered with fine-mesh gauze (size 80 µm) for ventilation.

Inside each jar, one organic bean pod was placed as a water source and oviposition substrate, as well as a piece of tissue paper and buckwheat covering the bottom of the jar to provide hiding places and reduce cannibalism. Nymphs and adults were fed with a mixture of frozen *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs (Koppert B.V.) and *Artemia franciscana* (Anostraca: Artemiidae) decapsulated cysts (BioBee Biological Systems Ltd., Sde Eliyahu, Israel). Food was offered on sticky note cards that were previously dipped in a container with food adhering to the sticky surface of the card. Biweekly, food sources were replenished, decaying bean pods were replaced and pods carrying predator eggs were placed into a new jar. The rearings were maintained in climatic cabinets at 25 ± 1 °C for *O. laevigatus* and 22 ± 1°C for *O. majusculus*, 70 ± 10% relative humidity (RH) and a photoperiod of 16:8 (L:D).

2.1.2 *Frankliniella occidentalis*, *Echinothrips americanus* and *Thrips setosus*

All thrips were reared continuously on plants placed inside insect rearing cages (BugDorm-2400F, MegaView Science Co. Ltd.; Taichung, Taiwan, China) in small greenhouse compartments. Sweet pepper plants (*Capsicum annuum* L. Maranello F1; Enza Zaden Beheer, Enkhuizen, The Netherlands), green bean plants (*Phaseolus vulgaris* L.) and flowering *Chrysanthemum* plants (cv. Miramar) were replenished weekly for the rearings of *E. americanus*, *T. setosus* and *F. occidentalis*, respectively. In order to produce same age larvae for the different experiments, two methods were used.

For the *F. occidentalis*, an extra population was reared in the laboratory on bean pods. Around 200 thrips were collected from the greenhouse rearing and transferred to plastic jars (Ø 11 cm x 13 cm) containing bean pods serving as food and oviposition substrate for the thrips and supplemented with honeybee pollen offered on sticky note cards as described above, to enhance oviposition. Three times per week pollen cards and decayed bean pods were replaced and the beans containing eggs were transferred into a new jar, starting a new synchronised rearing unit.

For *E. americanus* and *T. setosus* the leaf disc method was used. Adult thrips were collected from the rearings and transferred to leaf discs (sweet pepper for *E. americanus* and bean for *T. setosus*) in groups of approximately 40 thrips. After 24 h the adults were removed, and the leaf disc was kept until the larvae that emerged from the eggs reached the life stage needed for the experiments. The leaf discs were placed in 280-ml plastic cups (Ø 8 cm x 5 cm) (Paardekooper Verpakkingen, Oud-Beijerland, The Netherlands), the lids of which had a ventilation hole covered with a fine-mesh gauze (size 80 µm). Circular leaf discs covering the whole diameter of the container were embedded with the abaxial side up in a layer of water agar (1% agar). All rearing units and experiments were kept in climatic cabinets at 25 ± 1 °C, 70 ± 10% relative humidity (RH) and a photoperiod of 16:8 (L:D), unless stated otherwise.

2.2 Experiments

All experiments were performed in climatic cabinets at 25 ± 1 °C, 70 ± 10% relative humidity (RH) and a photoperiod of 16:8 (L:D) h.

2.2.1 Predator behaviour, capacity and life tables of two *Orius* species on different thrips

Throughout the experiments different combinations of two predator species, *O. laevigatus* and *O. majusculus*, on three prey species, *F. occidentalis*, *E. americanus* and *T. setosus* were tested. Exceptionally, due to problems with regular infestations in the *T. setosus* rearing it was not possible to perform the development and reproduction experiment on this thrips species. All insect species required in this study were collected from stock colonies established as stated above.

In the performance of the experiments two main types of containers can be distinguished. In the majority of them, 280-ml plastic cups (Ø 8 cm x 5 cm) (Paardekooper Verpakkingen, Oud-Beijerland, The Netherlands) were used, the lids of which had a ventilation hole covered with a fine-mesh gauze (size 80 µm). Circular leaf discs covering the whole diameter of the container were embedded with the abaxial side up in a layer of water agar (1% agar).

When referring to small cups, 30-ml transparent plastic medicine cups (Ø 2.5 cm at the bottom and 4 cm) (Vandeputte Medical Nederland, Nieuwegein, The Netherlands) with the same ventilated lids as above were used. Circular leaf discs were placed upside down in a layer of gypsum soaked with water every other day to maintain the moisture.

2.2.1.1 Predator behaviour

For the behaviour trial, 1-week-old female predators (i.e., reproductively active) were collected from the rearings and starved individually in small plastic cups for 24 hours (at 25°C) having only access to a piece of bean pod. Forty adult thrips were added in the plastic cup arena (Ø 8 cm x 5 cm) consisting of a disc of sweet pepper leaf serving as food for the prey and a moisture source for the predator. As explained above, the leaf disc covered the whole diameter of the arena ensuring no hiding spaces for the insects and thus improving the accuracy of the observations. Thrips were allowed to establish and start feeding in the experimental arena for 1 h and subsequently the starved predator was introduced, and its behaviour was recorded for 1 h. Each predator was used for only one observation and then discarded.

Based on preliminary observations, the predator’s behaviours were recorded and grouped in 3 different categories:

i) ‘base’, which includes walking, resting (remaining motionless), grooming (using legs to clean antennae or other body parts) and ovipositing;

ii) ‘host-handling’, including mainly feeding (mouthparts inserted into prey) but also walking with the prey on the rostrum.

iii) ‘encounter’ characterized as the predator showing a reaction to the presence of the prey by extending its rostrum towards it. An encounter was successful when it resulted to the capture of prey or unsuccessful when prey escaped. The number of total encounters as well as the number of successful and unsuccessful encounters were scored.

Predation rate was measured as the number of preys killed per hour. The success ratio for each prey species was calculated as total number of feeding events divided by the total number of encounters. In total 6 replicates of each predator/prey combination were carried out. Observations were aided using a stereomicroscope (Leica MZ9$_5$ Binocular Stereo Microscope).

2.2.1.2 Predation capacity

Predation capacity of the female adults of both *Orius* species was assessed on the three different thrips species. One-week-old female adults of *O. laevigatus* and *O. majusculus* were collected randomly from stock cultures. Adults were individually starved for 24 h in small plastic cups containing a piece of green bean pod as a water source. After the starvation period, each predator was transferred to a plastic cup arena (Ø 8 cm x 5 cm) containing an excess of prey and a circular sweet pepper leaf disc serving as food for the prey and a moisture source for the predator. Based on pilot experiments, 60 adult thrips were offered in each predator/prey combination, ensuring an excess of prey available for the timeframe of the assay. 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 experiment was replicated 15 times per treatment. To account for the natural mortality of prey, a control treatment for all prey species was included using the same prey density in the absence of the predator. In the case of control mortality exceeding 5%, the Abbott's formula (Abbott, 1925) was used to correct the number of preys consumed by the predator in the experimental period.

2.2.1.3 Prey preference

In this trial, both predator species were tested for their prey preference between *F. occidentalis* and *E. americanus* offered in equal numbers. Once more, 1-week-old female adults of *O. laevigatus* and *O. majusculus* were collected and starved for 24h as stated above. Maintaining the same total prey density of 60 thrips as in the previous trial, 30 adult *F. occidentalis* and 30 adult *E. americanus* were released in the experimental arena (Ø 8 cm x 5 cm plastic cup containing a sweet pepper leaf disc) followed by the introduction of a single predator. The experiment was terminated 8 h later to ensure that none of the two prey species was completely depleted and the number of dead and live thrips of each species was counted. For each *Orius* species the experiment was replicated at least 10 times. A control treatment was set-up with the same prey density but in the absence of the predator to account for mortality due to natural causes or competition effects.

Prey preference index (α) of each *Orius* species for *E. americanus* was estimated using the following equation modified from Chesson (1978):

$$\alpha_1 = \left(\frac{r_1}{n_1}\right) / \left[\left(\frac{r_1}{n_1}\right) + \left(\frac{r_2}{n_2}\right)\right] \tag{2.1}$$

where r_1 is the number of dead *E. americanus*, n_1 is the total number of *E. americanus*, r_2 is the number of dead *F. occidentalis*, n_2 is the total number of *F. occidentalis*. Afterwards, the prey preference for *F. occidentalis* was calculated by subtracting the *E. americanus* preference from 1.0.

2.2.1.4 Life history traits

2.2.1.4.1 Development

In each treatment, first nymphal instars (<24 h old) of the two predator species were placed in individual small cups (Ø 2.5 x 4 cm) containing a sweet pepper leaf disc in gypsum serving as food for the prey and a moisture source for the predator. Juvenile stages of each thrips species were offered as food for the *Orius* during their nymphal stage and all prey were supplied *ad libitum* and replenished every other day. Development and survival of nymphs were monitored and recorded every other day for the first seven days, and subsequently daily. Newly emerged adults (<24 h old) were sexed and weighed using a balance (Sartorius BP 211 D).

2.2.1.4.2 Reproduction

Newly emerged adults were paired and transferred to individual plastic cups (Ø 8 cm x 5 cm) where a flat piece of green bean pod (around 6 cm) was provided as water source and substrate for oviposition. This bean pod was placed in suspension in the centre of the container using an insect pin size 3 in order to avoid hiding spaces for the thrips. Adult predatory bugs were fed with adult thrips of the same species as in their juvenile development *ad libitum*.

Bean pods were checked daily for eggs under a stereoscope to determine the pre-oviposition period. When the first egg was recorded, the bean pods were scored for eggs and replaced by fresh ones every other day, until the female died. Dead experimental individuals were paired with 1-week-old old males or females from the stock culture, to ensure that every experimental individual was paired throughout its lifetime.

2.2.1.4.3 Egg hatch

To monitor egg hatch rate, a total of more than 50 eggs from different females were selected during the reproduction experiments and incubated in a Petri dish at 25 °C. The developmental time and hatch rate of the eggs were obtained by examining the status of the eggs daily, until all eggs hatched. Newly hatched nymphs were removed from the Petri dish to avoid cannibalism.

2.2.1.5 Intrinsic rate of increase

Intrinsic rates of increase and their pseudo-values were calculated with the R software using the *lifetable.r* computer programme developed by Maia *et al.*, (2000, 2014) . Specifically, line name, female identity, age, and number of eggs laid per female at each oviposition date, proportion of female offspring and nymphal survival were used as input. A sex-ratio of 1:1 was assumed in all treatments as found previously (Tommasini *et al.*, 2004).

The intrinsic rate of increase (*Rm*) of the two *Orius* species, expressed as the number of females per female per day, was obtained by iteration of the equation (2.2 using the *uniroot* function based on the Brent's iterative method (Brent, 1973):

$$\sum_{x=x_0}^{\Omega g} e^{-Rm} l_x m_x = 1 \tag{2.2}$$

where l_x is the proportion of females surviving to age x and m_x is the mean number of female progeny per adult female at age x. The jackknife procedure was used to calculate the standard error of *Rm* and further perform multiple comparisons between treatments based on these jackknife variance estimates (Meyer *et al.*, 1986).

2.2.2 Biology of *Thrips setosus*

2.2.2.1 Effects of supplemental food on *T. setosus*

The nutritional quality of four types of supplemental food for *T. setosus* was assessed in the laboratory by measuring oviposition rates and longevities on these diets.

2.2.2.1.1 Longevity

Thrips were collected from the rearing and transferred to bean leaf discs (plastic cups of Ø 8 cm x 5 cm) in groups of approximately 20 allowing oviposition for 24 h after which they were removed. Leaf discs were kept until the eggs hatched and the larvae reached the adult stage.

Newly emerged females were placed for 24 h on a new leaf disc where enough male thrips were added to ensure mating. Less than 48 h old female mated thrips were individualized in small cups (Ø 2.5 x 4 cm) containing a bean leaf disc on a gypsum substrate and the supplemental food. A control treatment with no addition of supplemental food was included.

Four types of supplemental food were tested: (1) frozen *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs (Koppert B.V.); (2) *Artemia franciscana* (Anostraca: Artemiidae) decapsulated cysts (BioBee Biological Systems Ltd.); (3) cattail pollen obtained from Biobest (Nutrimite^{TM}, Westerlo, Belgium); and (4) commercial Acacia honey purchased from a supermarket. All supplemental foods, with the exception of the honey, were offered in excess through sticky note cards as explained in section 2.1.1. The honey was offered by greasing the walls of the cup with it. Bean leaves and food were refreshed 3 times per week until the female died. Each of the 5 treatments was repeated at least 10 times.

2.2.2.1.2 Reproduction

When the thrips used in the previous experiment were 8-9 days old, their oviposition was recorded daily for 4 days. During this period, bean leaves were changed daily after which they were placed in a 6-well plate covered with water-saturated cotton serving as a moisture substrate. The well dishes were then incubated for 2 days in the same conditions as the experiment. After this incubation period more than 72 h old eggs were obtained (based on preliminary observations more developed eggs were easier to detect) and the number of eggs laid in each leaf disc was counted using a stereomicroscope by illuminating the leaf by transmitting light (Leica M80). Thrips that died during this period of oviposition measurement were not included. The mean number of eggs laid per female per day was analysed. At least 7 replicates per treatment were carried out.

2.2.2.2 Determination of the reproductive mode in *T. setosus*

In order to test for arrhenotoky, the most common form of reproduction in Thysanoptera, it was examined whether unmated females produced exclusively male progeny. Small larvae of *T. setosus* were collected from the rearing and transferred individually to bean leaf discs where they were reared until becoming adults. The thrips were kept isolated to avoid matting. In groups of 5 performing a total of 20 thrips, the virgin females were transferred to new bean leaf discs to allow oviposition during 24 h. Afterwards the thrips were removed, and the leaf discs were kept in climatic cabinets until the eggs hatched and the larvae reached the adult stage. The adult thrips were then sexed.

2.3 Statistical analysis

All the analysis with two independent variables started with a two-factor saturated model and at a significance level of 0.05 non-significant main factors and interactions were dropped. In case a factor with 2 degrees of freedom was found to be significant a *post-hoc* Tukey's test was performed to separate means. Once the interaction term was not significant in the tested models the analysis proceeded with a one-factor analysis using the appropriate model (1-way ANOVA or generalized linear model) and a *post-hoc* Tukey's test.

Generalized linear models (GLMs) varied in their error distribution and link function depending on the nature of the data. Count data were analysed using a GLM with Poisson error distribution and binary data were analysed with a GLM model with binomial error distribution. When we detected overdispersion, the distribution of the error was changed to quasipoisson and quasibinomial, for count and binary data, respectively (McCullagh & Nelder, 1989). In case of underdispersed data where none of the GLMs were applicable, a non-parametric Kruskal-Wallis H test was used. Sex-ratios were compared to an expected equal male: female distribution (1:1) by means of Chi-square tests. The preference indexes for two prey species were compared with paired t-tests. All statistical analyses were performed using the program R version 3.5.3 (R Development Core Team 2019). Microsoft Excel (2009) was used to handle the data and construct all the graphs presented.

3 Results

3.1 Predator behaviour, capacity and life tables of two *Orius* species on different thrips

3.1.1 Predator behaviour

The total number of encounters, its success ratio and the predation rate of both *Orius* species with the tested prey species are presented in Table 3.1. Predator and prey identity influenced the percentage of successful encounters (χ^2 = 8.513, df = 1, 32, P = 0.048 and χ^2 = 50.881, df = 2, 30, $P < 0.001$, respectively) whereas interaction was not significant (χ^2 = 5.924, df = 2, 28, P = 0.275). Both *Orius* species successfully subdued less *F. occidentalis* comparing to the other thrips species. *O. laevigatus* was more efficient in subduing *F. occidentalis* (29.1%) than was *O. majusculus* (14.7%) (χ^2 = 4.6855, df = 1, 9, P = 0.014) (Table 3.1).

Although predator identity was not significant (χ^2 = 3.747, df = 1, 30, P = 0.053), prey identity (χ^2 = 37.158, df = 2, 31, $P < 0.001$) and the interaction term (χ^2 = 6.729, df = 2, 28, P = 0.035) affected the total number of encounters. In general, the results of the total number of encounters with thrips were consistent with the results of success ratio meaning that higher number of encounters corresponded to lower success ratios. Predators of *O. majusculus* had significantly more encounters with individuals of *F. occidentalis* than did *O. laevigatus* (χ^2 = 7.0367, df = 1, 9, P = 0.008) (Table 3.1). *O. laevigatus* and *O. majusculus* predated a similar number of thrips, ranging between 1.67 and 3.67, in the 1 h experimental period (χ^2 = 0.8794, df = 1, 30, P = 0.348, for predator species; χ^2 = 5.2508, df = 2, 31, P = 0.072, for thrips species; χ^2 = 0.2213, df = 2, 28, P = 0.895, for the interaction) (Table 3.1).

Table 3.1 Number of encounters, success ratio and predation rate (means ± SE) of *O. laevigatus* and *O. majusculus* on different thrips species.

Parameters	Prey species	Predator species	
		O. laevigatus	*O. majusculus*
Number of encounters[1]	*F. occidentalis*	9.0 ± 2.2 bA	14.5 ± 2.3 Bc
	E. americanus	5.7 ± 0.9 aA	4.0 ± 0.6 Aa
	T. setosus	6.2 ± 1.7 abA	8.3 ± 2.8 Ab
Success ratio (%)[2]	*F. occidentalis*	29.1 ± 3.5 aB	14.7 ± 4.5 aA
	E. americanus	69.6 ± 11.1 bA	80.6 ± 16.3 bA
	T. setosus	66.7 ± 12.0 bA	62.3 ± 15.5 bA
Predation rate[3]	*F. occidentalis*	2.4 ± 0.4 aA	1.7 ± 0.3 aA
	E. americanus	3.7 ± 0.6 aA	3.2 ± 0.8 aA
	T. setosus	3.6 ± 0.5 aA	3.2 ± 0.4 aA

[1] Number of total encounters per hour.

[2] Number of feeding events ÷ numbers of encounters × 100.

[3] Number of prey killed per hour.

Means with different lowercase letters within a column and a parameter and means with different uppercase letters within rows are significantly different ($P < 0.05$): generalized linear model with quasibinomial distribution for success ratio or Poisson distribution for predation rate and total number of encounters. Between 5 and 6 individuals were tested for each treatment.

3.1.2 Predation capacity

The number of thrips killed in 24 h by adults of *O. laevigatus* and *O. majusculus* is shown in Table 3.2. As the thrips natural mortality did not exceed 5% in any treatment, the number of thrips predated was not corrected with the Abbott's formula.

Predator identity influenced the predation capacity (F = 7.6326, df = 1, 88, P = 0.007), with *O. majusculus* consuming overall more prey than *O. laevigatus*. The influence of prey identity was also significant (F = 37.6648, df = 2, 86, P < 0.001), showing that anthocorids killed significantly less *F. occidentalis* than *E. americanus* and *T. setosus*. The interaction term was not significant (F = 2.0306, df = 2, 84, P = 0.138). Predation capacities only differed between *Orius* species when *E. americanus* was offered as food. When fed on this prey, *O. majusculus* was more voracious than *O. laevigatus* (Table 3.2).

Table 3.2 Predation capacities (means ± SE) of *O. laevigatus* and *O. majusculus* on different thrips species, expressed as the number of preys killed in 24 h.

Prey species	Predator species	
	O. laevigatus	*O. majusculus*
F. occidentalis	17.7 ± 1.93 aA	20.2 ± 1.59 aA
E. americanus	30.1 ± 2.39 bA	42.2 ± 2.82 bB
T. setosus	35.9 ± 3.02 bA	37.7 ± 2.43 bA

Means with different lowercase letters within columns and means with different uppercase letters within rows are significantly different (P < 0.05): generalized linear model with quasipoisson distribution. For each treatment 15 individuals were tested.

3.1.3 Prey preference

Prey preferences of both *Orius* species are shown in Table 3.3. *O. laevigatus* and *O. majusculus* showed a significant preference for *E. americanus* over *F. occidentalis* (t = 10.072, df = 9, P < 0.001; t = 10.513, df = 10, P < 0.001; respectively). Preference was similar between *O. laevigatus* and *O. majusculus* (t = -1.3938, df = 18.827, P = 0.180 for *E. americanus*; t = 1.3938, df = 18.827, P = 0.180 for *F. occidentalis*).

Table 3.3 Number of thrips of each species (*F. occidentalis* and *E. americanus*) predated in 24 h and respective prey preference indexes (mean ± SE) (varying from 0 to 1, with 1 indicating 100% prey preference) of *O. laevigatus* and *O. majusculus*.

Predator	Prey			
	F. occidentalis		*E. americanus*	
	Number of thrips predated	Preference index	Number of thrips predated	Preference index
O. laevigatus	3.8 ± 0.8	0.201 ± 0.030 aA	13.8 ± 2.2	0.799 ± 0.030 aB
O. majusculus	2.8 ± 0.7	0.138 ± 0.034 aA	14.5 ± 1.9	0.862 ± 0.034 aB

Means of preference indexes with different lowercase letters within columns and means with different uppercase letters within rows are significantly different (P < 0.05): unpaired t-test and paired t-test, respectively. Between 10 and 11 individuals were tested for each treatment.

3.1.4 Life history traits

3.1.4.1 Development

Developmental parameters of *O. laevigatus* and *O. majusculus* reared on two different species of thrips are shown in Table 3.4. Nymphal survival was overall higher on *E. americanus* (χ^2 = 5.6510, df = 1, 1, P = 0.017), and was similar for both *Orius* species (χ^2 = 0.0037, df = 1, 2, P = 0.951). The interaction between factors was not significant (χ^2 = 2.1559, df = 1, 0, P = 0.142). When comparing within predator species, *O. majusculus* showed similar survivals on a diet of *F. occidentalis* and *E. americanus*, contrary to *O. laevigatus* which had a significantly higher survival rate when fed on *E. americanus* (Table 3.4). Nymphal developmental times were similar for all combinations of predator-prey species (H = 2.0882, df = 3, P = 0.554, for males; H = 0.95653, df = 3, P = 0.812, for females).

Table 3.4 Developmental parameters (means ± SE) of *O. laevigatus* and *O. majusculus* on different thrips species at 25 °C.

Predator species	Prey species	Nymphal survival (%)	Developmental time (days)		Adult weight (mg)		Sex ratio (male: female)
			Males	Females	Males	Females	
O. laevigatus	*F. occidentalis*	67.2 ± 6.0b (61)	10.9 ± 0.2a (15)	10.8 ± 0.1a (26)	0.325 ± 0.019a (15)	0.386 ± 0.019a (26)	1:1.73
	E. americanus	88.9 ± 4.7a (45)	10.8 ± 0.2a (12)	10.7 ± 0.1a (27)	0.391 ± 0.017a (12)	0.448 ± 0.011b (27)	1:2.25*
O. majusculus	*F. occidentalis*	73.5 ± 5.4ab (68)	10.6 ± 0.2a (28)	10.8 ± 0.2a (22)	0.465 ± 0.013b (28)	0.609 ± 0.024c (22)	1:0.78
	E. americanus	79.6 ± 5.8ab (49)	10.7 ± 0.3a (12)	10.8 ± 0.1a (25)	0.531 ± 0.028b (12)	0.723 ± 0.065d (25)	1:2.08*

Means within a column followed by the same letter are not significantly different (P > 0.05): generalized linear model with binomial distribution (nymphal survival); Kruskal-Wallis *H* test (developmental times); ANOVA (weights).

The number of individuals tested in each parameter is placed in parenthesis.

Values followed by an asterisk differ significantly from a 1:1 sex ratio by means of a Chi-square test.

The influence of predator and prey species on the longevity of adults was similar for males and females. Independently of the prey they fed on, *O. majusculus* individuals were always heavier than those of *O. laevigatus* (F = 50.633, df = 1, 64, P < 0.001 and F = 275.904, df = 1, 97, P < 0.001, for males and females respectively). Feeding on *E. americanus* always resulted in higher body weight for both predators (F = 11.947, df = 1, 64, P < 0.001 for *O. laevigatus*; F = 32.628, df = 1, 97, P < 0.001 for *O. majusculus*). Interaction between factors was not significant (F = 0.0003, df = 1, 63, P = 0.986 and F = 3.0196, df = 1, 96, P = 0.085, respectively). Females of both *Orius* species gained significantly more weight when feeding on *E. americanus* (F = 105.99, df = 3, 96, P < 0.001), however this effect was not observed in the males (F = 20.535, df = 3, 63, P < 0.001).

Effects on the sex ratio of the studied populations were observed dependently on the prey species. When *O. laevigatus* and *O. majusculus* were offered *E. americanus* it was observed a female biased sex ratio (χ^2 = 5.7692, df = 1, P = 0.016; χ^2 = 4.5676, df = 1, P = 0.033, respectively). On the other hand, no significant deviations from a 1:1 sex ratio were observed when a diet of *F. occidentalis* was offered (χ^2 = 2.9512, df = 1, *P* = 0.086; χ^2 = 0.72, df = 1, *P* = 0.396, respectively).

3.1.4.2 Reproduction

Reproductive parameters, longevity and intrinsic rate of increase of *O. laevigatus* and *O. majusculus* as a function of prey species are presented in Table 3.5. Pre-oviposition period of the studied *Orius* was not significantly different between treatments (χ^2 = 0.57675, df = 1, 70, *P* = 0.448, for predator species; χ^2 = 0.00900, df = 1, 69, *P* = 0.924, for prey species; χ^2 = 1.66212, df = 1, 68, *P* = 0.197, for the interaction).

Table 3.5 Reproductive parameters, longevity and intrinsic rate of increase (*Rm*) (means ± SE) of *O. laevigatus* and *O. majusculus* on different thrips species at 25 °C.

Predator species	Prey species	Pre-oviposition period (days)	Lifetime oviposition	Egg hatch (%)	Longevity (days)		*Rm*
					Males	Females	
O. laevigatus	*F. occidentalis*	3.53 ± 0.15a (17)	109.0 ± 8.4b (17)	88.0 ± 3.8a (75)	17.0 ± 1.4c (12)	16.6 ± 0.8b (18)	0.1504 ± 0.0028b
	E. americanus	4.17 ± 0.41a (18)	169.0 ± 14.6c (18)	97.2 ± 1.9a (72)	22.7 ± 1.4d (10)	23.6 ± 1.4c (18)	0.1662 ± 0.0029c
O. majusculus	*F. occidentalis*	3.86 ± 0.21a (14)	49.8 ± 5.4a (14)	87.1 ± 6.0a (31)	8.1 ± 0.7a (19)	10.8 ± 1.0a (17)	0.1320 ± 0.0048a
	E. americanus	3.30 ± 0.15a (23)	99.7 ± 13.8b (23)	89.5 ± 4.1a (57)	12.8 ± 1.7b (11)	18.5 ± 1.4b (24)	0.1538 ± 0.0040bc

Means within a column followed by the same letter are not significantly different (P > 0.05): generalized linear model with Poisson distribution (pre-oviposition period and longevity); generalized linear model with quasipoisson distribution (lifetime oviposition); generalized linear model with binomial distribution (egg hatch); Tukey's test after jackknife estimates (*Rm*).

The number of individuals tested in each parameter is placed in parenthesis.

Lifetime oviposition was affected by predator species (χ^2 = 578.36, df = 1, 70, *P* < 0.001) with *O. laevigatus* laying more eggs than *O. majusculus;* and by prey species (χ^2 = 498.15, df = 1, 69, *P* < 0.001) so that consuming *E. americanus* always resulted in a higher lifetime fecundity. The interaction between the factors was not significant (χ^2 = 23.13, df = 1, 68, *P* = 0.328). *O. majusculus* feeding on *F. occidentalis* registered the lowest fecundity (49.8 eggs), less than half the number of eggs *O. laevigatus* laid when feeding on the same prey (109 eggs) (χ^2 = 1099.6, df = 3, 68, *P* < 0.001). A significantly higher number of eggs was recorded for *O. laevigatus* on a diet of *E. americanus* (169 eggs) which was almost twice the fecundity of *O. majusculus* on the same prey (99.7 eggs) (Table 3.5).

Oviposition patterns of *Orius* females are presented in Figure 3.1. The pattern of oviposition is comparable for both predators and preys in terms of pre-oviposition period and peak oviposition. Overall, females started to oviposit 3 days after emergence and reached the oviposition peak 3 days later. Only when a diet of *E. americanus* was offered to *O. laevigatus* the highest oviposition peak was recorded on the 10th day of adulthood. Sporadic cases of non-ovipositing females were found. In general, it is possible to see that the decline in the oviposition rate is more marked for the *Orius* fed on *F. occidentalis* prey and the same females have a shorter oviposition period.

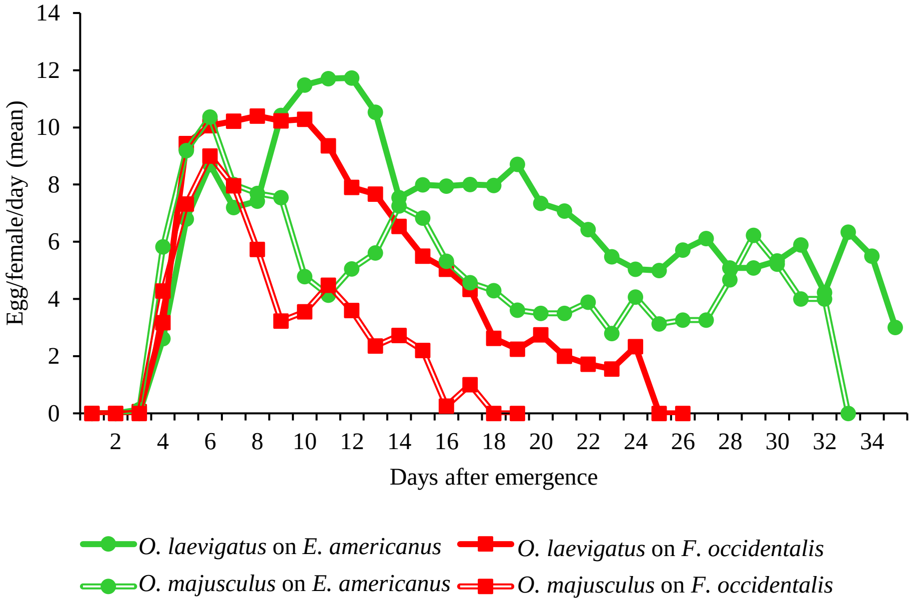

Figure 3.1 Lifetime oviposition curve of *O. laevigatus* and *O. majusculus* reared on *F. occidentalis* and *E. americanus* at 25 °C.

Egg hatch rate of the two predators was never lower than 87% and did not significantly differ between treatments (χ^2 = 0.9932, df = 1, 21, P = 0.319, for predator species; χ^2 = 3.2561, df = 1, 20, P = 0.071, for prey species; χ^2 = 1.7293, df = 1, 19, P = 0.188, for the interaction).

Male and female adults of *O. majusculus* lived significantly shorter than the adults of *O. laevigatus* (χ^2 = 85.902, df = 1, 50, P < 0.001 and χ^2 = 24.479, df = 1, 75, P < 0.001, respectively). The prey identity also influenced the longevity of the *Orius* predators, with a diet on *E. americanus* resulting in longer longevities for both sexes (χ^2 = 23.273, df = 1, 49, P < 0.001 and χ^2 = 60.149, df = 1, 74, P < 0.001, respectively for males and females). The interaction between factors was not significant (χ^2 = 1.344, df = 1, 48, P = 0.2463 and χ^2 = 2.524, df = 1, 73, P = 0.112, for males and females respectively). For males, the shortest longevity time was recorded for *O. majusculus* fed on *F. occidentalis* whereas *O. laevigatus* on a diet of *E. americanus* lived the longest (χ^2 = 110.52, df = 3, 48, P < 0.001). The similar pattern was observed for females (χ^2 = 87.152, df = 3, 73, P < 0.001) (Table 3.5).

The intrinsic rate of increase (*Rm*) of both *Orius* species reared on *E. americanus* was higher than when reared on *F. occidentalis*. The population of *O. majusculus* fed on *F. occidentalis* obtained the lowest value of intrinsic rate of increase whereas *O. laevigatus* fed on *E. americanus* obtained the highest value (Table 3.5).

3.1 Biology of *Thrips setosus*

3.1.1 Effects of supplemental food on *T. setosus*

The oviposition rates and longevities of *T. setosus* females fed on different diets are presented in Figure 3.2 and Figure 3.3, respectively. The daily oviposition rate of *T. setosus* was not influenced by the diet (*F* = 0.9177, df = 4, 160, *P* = 0.46), time (*F* = 0.2541, df = 1, 160, *P* = 0.61) or by their interaction (*F* = 0.7055, df= 4, 156, *P* = 0.5893). Likewise, the longevity of females was similar in all diet treatments (*F* = 0.2364, df = 4, 42, *P* = 0.916), varying between 23.2 and 28.3 days (Figure 3.3).

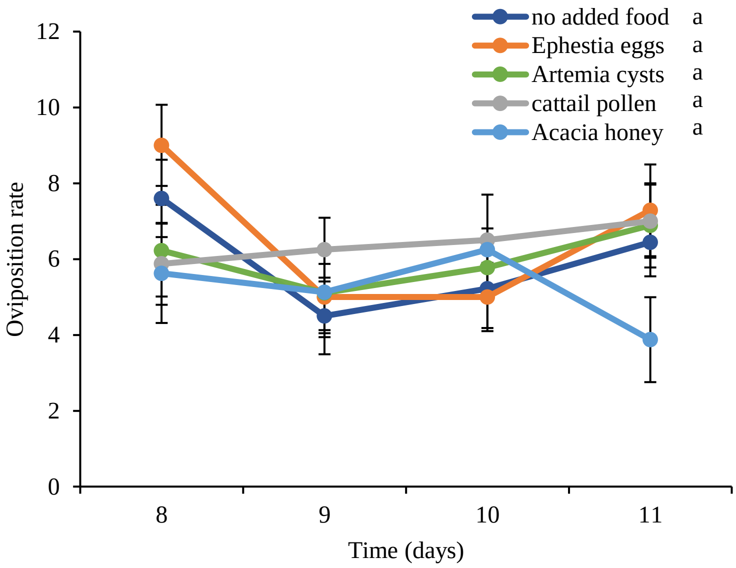

Figure 3.2 Effects of added food sources to bean leaves on oviposition rates of young females of *T. setosus*. The food supplements were sterilized eggs of *E. kuehniella*, decapsulated cysts of *A. franciscana*, cattail pollen (Nutrimite^{TM}) and Acacia honey. Average (± SE) number of eggs produced per female through time are presented. Same letters among treatments through time indicate no significant differences (2-way ANOVA: P > 0.05).

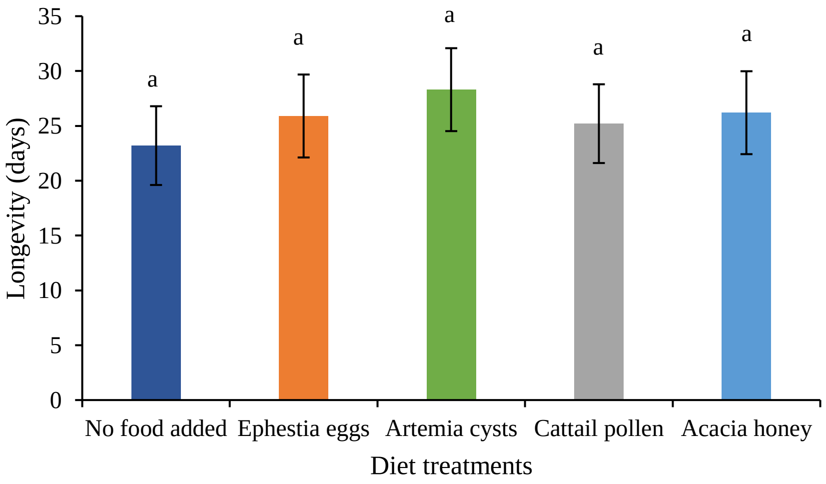

Figure 3.3 Longevity (days) of young *T. setosus* females (means ± SE) on different treatments of supplemental food. The food supplements were sterilized eggs of *E. kuehniella*, decapsulated cysts of *A. franciscana*, cattail pollen (Nutrimite^{TM}) and Acacia honey. Same letters among treatments indicate no significant different (P > 0.05): generalized linear model with quasipoisson distribution.

3.1.2 Determination of the reproductive mode in *T. setosus*

The progeny of unmated *T. setosus* females was observed. Once the eggs laid in the bean leave discs reached the adult stage a sex-ratio of 100% males was obtained, thus unmated *T. setosus* females produced exclusively male progeny.

4 Discussion

4.1 Predator behaviour, capacity and life tables of two *Orius* species on different thrips

Although *Orius* species are considered generalist predators that attack a wide range of arthropod preys, it has been consistently shown that the type of prey can strongly influence the performance of these predators (Butler & O'Neil, 2007; Fathi, 2009; Bonte *et al.*, 2015; Rehman *et al.*, 2020). The efficiency of an *Orius* predator as a biological control agent of thrips is mainly supported by its high predation capacity and favourable life history characteristics, especially reproduction (van Lenteren & Manzaroli, 1999; Grenier & Clercq, 2003). In this study, we present results of several experiments that complement each other and overall support that *Orius* species show large variations in its biological parameters when they were offered different thrips species as prey.

4.1.1 Predator behaviour, predation capacity and prey preference

During the predatory behaviour experiment, observations on the search and attack behaviour of the *Orius* as well as on the behaviour of the different prey species were made. We observed that the attacking behaviour and its duration could vary from an assertive jump immediately followed by the capture of the prey, lasting less than 2 seconds; to an intense fight triggered by thrips defence, which could last up to 20 seconds. Once the prey was immobilized, the anthocorid inserted its stylets into the soft areas of the thrips body causing its death within a few seconds and further sucking out its body contents. Similar to previous studies, our observations on the body of the predated thrips suggest that these anthocorids often killed prey without fully consuming it, which increases their effectiveness as biological control agents (Isenhour & Yeargan, 1981; De Clercq & Degheele, 1994; Kohno & Kashio, 1998; Meyling *et al.*, 2003; Yano *et al.*, 2005; Fantinou *et al.*, 2008; Bonte *et al.*, 2015).

Furthermore, we show that the behaviour of thrips plays an essential role in the success of the predatory attacks of anthocorids (Reitz *et al.*, 2006). Although all adult thrips tested are winged, *F. occidentalis* was by far the most active, agile, and skilled in escaping from attacks and consequently the most challenging to capture as the low success ratios indicate (29.1% for *O. laevigatus* and 14.7% for *O. majusculus*). Nevertheless, *Orius* adults are much more successful when offered in capturing *F. occidentalis* larvae (Venzon *et al.*, 2002). Moreover, adults of *F. occidentalis* were observed a few times releasing droplets of anal fluid known as alarm pheromones that cause other thrips to walk away from the source (Teerling *et al.*, 1993). This behavioural response to the presence of predators might as well have contributed to a lower predation success on *F. occidentalis*.

Contrastingly, the leaf-dwelling *E. americanus* showed a sedentary behaviour, moving slowly and often standing motionless on the veins of the leaf while becoming an easy target of predation hence reflected by the highest success ratios (69.6% for *O. laevigatus* and 80.6% for *O. majusculus*). Slightly lower results (66.7% for *O. laevigatus* and 62.3% for *O. majusculus*), were achieved on the prey *T. setosus* which showed a more active behaviour than *E. americanus* yet not as agile and responsive to predator attacks as *F. occidentalis*.

When comparing results between predators, *O. laevigatus* preying on *F. occidentalis* was two times more successful that *O. majusculus* on the same prey. Yet, *O. majusculus* encountered more times with the prey this way counterbalancing its lower success ratio and at the end of 1 h both predators consumed approximately the same amounts of prey. On the 24 h predation capacity trial, *O. majusculus* even managed to kill more *F. occidentalis* individuals than did *O. laevigatus* (20.2 vs 17.7 thrips). We hypothesise that the lower percentage of well succeeded attacks of *O. majusculus* is due to the bigger body size and mass of this predator causing it to consume more food but also to be less agile in its predation activity. This effect was particularly marked when preying on *F. occidentalis,* the most difficult prey to capture.

Furthermore, low success ratios often imply that the energy involved in the attack exceeds the energy than can be gained from feeding on the prey which can cause the predator to cease hunt before being satiated (Meiracker & Sabelis, 1999). We found that although *O. majusculus* always consumes more prey than *O. laevigatus,* this pattern significantly increases when the prey is *E. americanus* (30.1 vs 42.2 thrips). This might be related with the fact that the associated high success ratio on this prey offers no energy constraints for *O. majusculus* and so the possibility to fulfil its bigger gut capacity.

Apart from the behaviour of the prey, predation response may also be influenced by its nutritional quality. However, higher predation capacities do not necessarily reflect a better quality of prey. In fact, Mendes *et al.* (2002) found that higher consumption rates by an *Orius* predator may occur as an attempt to fulfil the nutritional gap caused by a low quality prey like aphids. The inverse is valid for the superior nutritional quality prey *E. kuehniella* of which relatively small amounts are needed to rear anthocorids (Mendes *et al.,* 2002; Yano *et al.,* 2002).

Previous studies have found lower predation capacities in comparison to our results. At 26 °C, Tommasini *et al.* (2004) reported predation rates for 8-day-old *O. laevigatus* and *O. majusculus* adults averaging respectively 22.7 and 20.3 *F. occidentalis* adults per day per *Orius* couple. For *O. laevigatus,* Cocuzza *et al.* (1997a) found an average predation rate of 6.9 *F. occidentalis* adults per day whereas Jakobsen *et al.* (2004) reported that under similar conditions *O. majusculus* females killed 12.6 *F. occidentalis* adults in 24 h.

Several authors have examined the predation performance of other *Orius* species on *F. occidentalis,* but no records were found for the preys *E. americanus* and *T. setosus*. Under similar conditions, Bonte *et al.,* (2015) found that predation capacities of southern African anthocorid bugs preying on *F. occidentalis* adults never exceeded 8 thrips per day. Likewise, Cocuzza *et al.*, (1997b) reported a low predation capacity for *O. albidipennis* (4.59 thrips/day). However, on a diet of mixed larvae and adults Calixto *et al.* (2013) found higher predation numbers (20.4 thrips/day).

Finally, the prey preference trial showed that when given a choice between *F. occidentalis* and *E. americanus* both anthocorids consume preferably *E. americanus*. Altogether, *Orius* preference is reinforced by higher success ratios of encounters and predation capacities on the prey *E. americanus*. Nevertheless, it is impossible to make conclusions on the nutritional value of preys with only this data. Prey preference does not necessarily reflect the food quality of the prey as reported by Toft *et al.* (2020). This author found that well-nourished *O. majusculus* preferred to prey on aphids when giving a choice between this low-quality food source and *E. kuehniella* eggs.

In general, small experimental arenas used in laboratory studies may not reflect realistic scenarios. Firstly, the predator experiences much higher densities of prey than the actual densities encountered in greenhouses or fields . Secondly, the confinement of *Orius* in small arenas results in over-estimated numbers of attacking events during a period of time. While in experimental arenas the most limiting factor is the prey handling time, in the field the predation rates will be substantially more affected by the prey-searching behaviour (Isenhour & Yeargan, 1981; De Clercq & Degheele, 1994; Meiracker & Sabelis, 1999) or scramble competition (Liu *et al.*, 2018).

Additionally, the diet used for rearing *Orius* may affect its predatory behaviour. Henaut *et al.* (2000) found that *O. majusculus* reared on 4th instars of aphids during nymphal stages were more successful in attacking this prey than individuals reared on stationary eggs of *E. kuehniella*. For anthocorid generalist predators experience gained as an immature or even the conditioning to the target prey during adulthood positively affects the efficiency of the predator, suggesting a certain degree of learning. It is possible that rearing our experimental *Orius* on thrips instead of factitious preys could have resulted in experienced predators and therefore in more successful encounters, particularly with the prey *F. occidentalis.*

Besides, several other factors may play a role on the success of anthocorids in integrated pest management programmes. These include the induction of diapause under certain conditions of photoperiod and temperature (Meiracker, 1994), the ability to use alternative food resources in periods of prey scarcity (Labbé *et al.*, 2018), the influence of plant architecture and leaf characteristics of the infested crop on predation activity of the *Orius* (Eigenbrode *et al.*, 1996), or even the presence of multiple preys. For example, when spider mites are present their webs provide a refugee for thrips larvae thus reducing the risk of being predated (Venzon *et al.*, 2000). In future studies the laboratory findings reported here should be complemented with semi-field and field trials in order to fully understand the potential of these anthocorids as biological control agents of *F. occidentalis, E. americanus* and *T. setosus*.

4.1.2 Life history traits

Nymphal developmental times of both predators were about 11 days when they were raised on either *F. occidentalis* or *E. americanus* larvae. On the first diet, Riudavets & Castañé (1998) and Venzon *et al.*, (2002) found similar developmental times (12.7 days for *O. laevigatus* and 12.2 days for *O. majusculus*; 12.4 days for *O. laevigatus*) whereas Husseini *et al.* (1993), Riudavets (1995) and Cocuzza *et al.* (1997a), reported longer times (respectively, 15.2 days for *O. majusculus*; 17.5 days for *O. laevigatus* and 16.7 days for *O. majusculus*; 14.1 days for *O. laevigatus*). At 26 °C, Tommasini *et al.* (2004) found that nymphs of both species took around 11 days to develop from 1st instars to adults when fed on *F. occidentalis* adults. The survival of nymphs developed on both thrips species (ranging from 67.2-88.9%) approached the values expected according to similar studies (Husseini *et al.*, 1993; Cocuzza *et al.*, 1997a; Riudavets & Castañé, 1998; Venzon *et al.*, 2002; Tommasini *et al.*, 2004). Overall, regarding pre-imaginal characteristics our results show fast developmental times for all predator-prey combinations and nymphal survivals approaching the expected values.

All predator-prey combinations scored similar pre-oviposition periods (ranging between 3.30 and 4.17 days) which is in accordance with an average of 2.7-3.3 for *O. laevigatus* and 2.8-3.8 for *O. majusculus* fed on *F. occidentalis* reported previously (Riudavets, 1995; Venzon *et al.*, 2002; Tommasini *et al.*, 2004). In the same way, data on the hatchability of eggs presented here does not stand out from what has been reported in the literature stated above.

Lifetime fecundity is one of the most important life history traits when assessing the potential of a population to growth. The highest fecundity was recorded for *O. laevigatus* when fed on E. *americanus* (169 eggs/female). Importantly, this value is consistent with the best values of fecundity found on a diet of *E. kuehniella* eggs, suggesting that this living-prey is of high nutritional quality (Cocuzza *et al.*, 1997b; Arijs & De Clercq, 2001, 2004; Tommasini *et al.*, 2004; Bonte & De Clercq, 2008; Aragón-Sánchez *et al.*, 2018; Mendoza *et al.*, 2020). On the other hand, the lifetime egg production of *O. majusculus* reared on *E. americanus* did not approach the numbers reported on the prey *E. kuehniella,* (Tommasini *et al.*, 2004; Pumariño & Alomar, 2012; Montoro *et al.*, 2020b) except in the study of Toft *et al.* (2020) (c. 100 eggs/female).

Our results for the fecundity of the predators on *F. occidentalis* differed from what has been reported in other studies. Whereas for *O. laevigatus* some authors reported about half of the value found here [67.8 eggs/female (Cocuzza *et al.*, 1997b); 55.6 eggs/female (Tommasini *et al.*, 2004); 109 eggs/female in the present study], others reported much higher fecundities than we did [164 eggs/female (Riudavets & Castañé, 1998); 109 eggs/female in the present study]. Likewise, for *O. majusculus* significantly higher values were found by Tommasini *et al.* (2004) and Riudavets & Castañé (1998), with the latter author reporting more than 3 times the amount of eggs than we did (87.1 and 176.6, respectively vs 49.8 eggs/female). Such big discrepancies between studies might be related with the life stage of the prey used in the experiments since consistently higher fecundities were recorded by Riudavets & Castañé (1998) for both predators reared on *F. occidentalis* larvae. Data on the longevity of *O. laevigatus* females fed on *F. occidentalis* adults (16.6 days) compared well with those obtained by Tommasini *et al.* (2004) at 26 °C (18 days) but was about 5 days shorter than those found by Cocuzza *et al.* (1997a) (22.2 days). Regarding the longevity of *O. majusculus* females, Tommasini *et al.* (2004) and Riudavets (1995) reported longer times on *F. occidentalis* adults (respectively 19.7 and 18.2 vs 10.8 days).

The body size and body mass are additional factors that can be used to assess the nutritional capacities of a diet offered to an insect predator (Grenier & Clercq, 2003). We found that independently of the predator species, adults raised on *E. americanus* were always heavier than the ones raised on *F. occidentalis*. The same pattern was reported by Toft *et al.* (2020) who compared a low-quality diet (aphids) with a rich diet (*E. kuehniella* eggs) finding the latter to result in significantly higher body weights of *O. majusculus* adults. Values for the body mass of this predator reared on *E. kuehniella* eggs [females body weights ranging from 0.65 to 0.71 mg (Montoro *et al.*, 2020a, 2020b; Toft *et al.*, 2020)] do not differ from what we found on a diet of *E. americanus* (0.72 mg for females). Also, for the body weight of *O. laevigatus* our values on a diet of *E. americanus* (0.45 mg for females and 0.39 mg for males) are very similar to what has been reported on *E. kuehniella* eggs (varying from 0.42 to 0.49 mg for females and from 0.35 to 0.39 mg for males) (Arijs & De Clercq, 2001, 2004; Bonte & Clercq, 2010). Overall, this suggests that *E. americanus* is an excellent food source for *Orius* predators.

The results of this study showed that the populations of both predators fed on *F. occidentalis* had an equally sex distribution in contrast with the populations of the predators fed on *E. americanus* that showed a female-biased sex ratio. Yet, these results have to be carefully considered. Sex determination in invertebrates includes genetic mechanisms and/or environmental mechanisms, but also cytoplasmic factors (like the endosymbionts *Wolbachia* and *Spiroplasma*) might be involved (Stouthamer *et al.*, 1999; Cook, 2002). In Heteroptera, the sex is determined genetically and therefore the sex of progeny is defined at oviposition. Previous studies have mainly documented a sex ratio of 1:1 for *Orius* species, nevertheless exceptions to this ratio have been found. Shapiro *et al.* (2009) registered a skewed field sex ratio for *O. insidiosus* towards males, probably due to sampling error or differential hatch rate or survival of one sex. Because nymphal survival was high in all our treatments, it is unlikely that the female-biased sex ratios observed here are the consequence of sexually differential survivals. Bonte *et al.* (2012a, 2012b) consistently found 2 to 11 times more females emerged in populations of *O. naivashae* (Poppius) which was later accredited to *Wolbachia* infections of the predator (Van de Walle, 2014). As in previous years, random sampling from our population has frequently yielded female skewed sex ratios, we suspect that *Wolbachia* infection can be the reason behind this skewed sex ratio. This phenomenon is not rare in arthropods, including in the predators of the genus *Orius* (Kikuchi & Fukatsu, 2003; Watanabe *et al.*, 2012, 2014).

Taken together, the fact that there is no previous evidence of similar female-biased sex-ratios in *O. laevigatus* and *O. majusculus* populations leads us to believe that at least for *O. majusculus* this was a consequence of a sampling error promoted by a small sample size. On the other hand, we hypothesize the presence of a bacterial infection as the cause of the observed female-skewed sex ratio in *O. laevigatus*. Further genetic analyses are required to confirm this hypothesis.

4.1.3 Intrinsic rate of increase

Life table analysis is the most common method used to gather life history information and study an insect population. In the present study this approach was used to study and compare the performance of *Orius* populations reared on different thrips species. The overall reproductive potential of a population is reflected by its intrinsic rate of natural increase (*Rm*), expressed as the number of females produced per female per day. In general, both predators achieved a positive and relatively high intrinsic rate of increase when reared on both studied thrips species.

Overall superior performances resulted in higher intrinsic rates of increase for both *Orius* when fed on *E. americanus* in comparison to a diet on *F. occidentalis*. Hence, *E. americanus* proved to be a more suitable prey for *Orius*. Particularly, *O. majusculus* reared on *F. occidentalis* resulted in the worst results of life history parameters and intrinsic rate of increase. This predator scored a lifetime fecundity 2 to 3 times lower than the other treatments, as well as the shortest longevity. Its overall inferior performance was reflected in the significantly lowest value of *Rm*.

The intrinsic rate of increase of both *Orius* presented by Riudavets & Castañé (1998) (0.1364 for *O. laevigatus* and 0.1409 for *O. majusculus*) approached what we found on *F. occidentalis*. However, it is important to consider that this author used a different formula to calculate the parameter (presumably the pre-imaginal mortality was not included). Also, *F. occidentalis* larvae instead of adults were used as prey which might have resulted in better performance. Under similar conditions lower values of *Rm* were given by Cocuzza *et al.* (1997a) (0.105 for *O. laevigatus*), Venzon *et al.* (2002) (0.1146 for *O. laevigatus*) and Tommasini *et al.* (2004) at 26 °C (0.094 for *O. laevigatus* and 0.097 for *O. majusculus*). A possible explanation for differences between similar studies could be related with the method used to calculate the parameters. Southwood (1978) found that estimates of *Rm* obtained with the approximate method are always lower than those calculated with the iterative method used here.

Other studies have reported the life table parameters of *Orius* species reared on various foods. These include *O. thripoborus* (Hesse) and *O. naivashae* on *F. occidentalis* (*Rm* = 0.1232 and *Rm* = 0.1311, respectively) and on *Myzus persicae nicotianae* Blackman (*Rm* = 0.0966 and *Rm* = 0.0017, respectively) (Bonte *et al.*, 2015); *O. laevigatus* on eggs of *Spodoptera exigua* (Hübner) (*Rm* = 0.1132) (Aragón-Sánchez *et al.*, 2018); and several commercial populations of *O. laevigatus* reared on *E. kuehniella* eggs (values of *Rm* ranging between 0.103 and 0.155) (Mendoza *et al.*, 2020). Importantly, the latter *Rm*-values on a nutritional superior food are lower than what we reported in the present study for *O. laevigatus* reared on *E. americanus* (*Rm* = 0.1662).

Additionally, the results of intrinsic rates of increase are consistent with the results of the predation capacity experiment. *O. laevigatus* and *O. majusculus* killed higher numbers of *E. americanus* than did the other prey and when reared on this diet both species obtained the best *Rm*-values. The contrary was observed when the prey was *F. occidentalis*. This trend is in accordance with previous finding for species like *O. sauteri* (Poppius) (Kohno & Kashio, 1998), *O. laevigatus* (Bonte & de Clercq, 2010), *O. thripoborus* or *O. naivashae* (Bonte *et al.*, 2015).

4.2 Biology of *Thrips setosus*

4.2.1 Effects of supplemental food on *T. setosus*

The performance of thrips is strongly influenced by the quality of their food (Brodbeck *et al.*, 2002). Host plant species and dietary supplements vary in their suitability to support different species of thrips, causing changes in nymphal development, adult longevity, and fecundity, amongst others. Faster developmental rates, longer longevity and higher lifetime fecundity indicate a better suitability of the host plant or supplemental food (Brodbeck *et al.*, 2001). In the present study, we found that none of the tested supplemental foods promoted the performance of *T. setosus*.

The evaluated *T. setosus* females performed well on bean leaves without any food supplementation. The oviposition of 8-9 days-old females was measured daily during the following four days. This lifetime period corresponded with the peak oviposition of *T. setosus* reported by Murai (2001) on bean pods at 25 °C. The same author reported a daily peak fecundity ranging from 4.5 to 6.5 eggs/female and an adult longevity of 24.6 days on bean pods, consistent with our results (Figure 3.2 and Figure 3.3). Furthermore, in comparison with other thrips species, life history traits reported here for *T. setosus* on bean were similar or even higher than that of *F. occidentalis* on the same host plant (Zhi *et al.*, 2005; Zhang *et al.*, 2007; Vangansbeke *et al.*, 2016).

Some kinds of honey and pollen provided by flowers can benefit the individual or population development of several insects, including flower-dwelling thrips (Hulshof *et al.*, 2003; Zhi *et al.*, 2005; Riley *et al.*, 2011; Fu *et al.*, 2019). Pollen is an accessible source of amino acids, proteins, lipids and vitamins (Millar *et al.*, 2003) whereas honey provides valuable carbohydrates (Jervis *et al.*, 2008). Several types of pollen (cattail, corn, apple) have been found to substantially increase the fecundity and/or the longevity of *F. occidentalis* (Zhi *et al.*, 2005; Leman & Messelink, 2015; Vangansbeke *et al.*, 2016). The same positive effects were found for *Thrips hawaiiensis* (Morgan) when supplied with tea pollen or a water solution of honey (Fu *et al.*, 2019). On the other hand, cattail pollen did not support the population growth of *E. americanus* as expected for a leaf-feeding thrips species (Ghasemzadeh *et al.*, 2017). Likewise, in this study the performance of *T. setosus* females was not enhanced by the addition of pollen confirming that this species does not feed on pollen in accordance with the findings of Murai (1991).

Although several studies have described the benefits of factitious foods for biological control agents, little information is available on the potential positive effects on pest densities, particularly thrips. *E. kuehniella* eggs increase the oviposition rate of *F. occidentalis* at a similar extent to pollen (Leman & Messelink, 2015). This effect was not observed for *T. setosus* in our study. In line with what we found, *A. franciscana* cysts were reported to have no impact or a very limited impact (depending on the nature of the cysts) on the population growth of *F. occidentalis* (Leman & Messelink, 2015; Vangansbeke *et al.*, 2016).

Evaluating if supplemental foods have a positive effect on the performance of a particular insect may contribute to a better understanding of its population dynamics, which is particularly important in the case of the understudied species *T. setosus*. These supplemental foods are known to improve the establishment and population growth of important biological control agents against thrips, such as the predatory mites *Neoseiulus cucumeris* (van Rijn & Tanigoshi, 1999), *Iphiseius degenerans* (Vantornhout *et al.*, 2004), *Amblyseius swirskii* (Nguyen *et al.*, 2014; Vangansbeke *et al.*, 2016), mirids like *Macrolophus pygmaeus* (Oveja *et al.*, 2012) and many species of *Orius* predators (Calixto *et al.*, 2013; Labbé *et al.*, 2018; Sade *et al.*, 2019). However, the addition of supplemental foods has to be carefully considered because it can promote the population growth of both predator and pest. Moreover, pollen was found to reduce the predation capacity of the predator *N. cucumeris* (van Rijn & Tanigoshi, 1999) even though the opposite effect is more common, as it happens for example with *O. laevigatus* (Hulshof *et al.*, 2003).

In order to improve biological control results, it is essential to know if and to what extent thrips and predators benefit from supplemental foods. Summarizing, our findings support that the food sources assessed (pollen, honey, *E. kuehniella* eggs and *A. franciscana* cysts) can be included in biological control programmes with the aim of supporting the establishment and performance of predators without the risk of increasing the density of *T. setosus*. Nevertheless, studies that can contribute to a better understanding of predator-prey dynamics at a population level should be performed.

4.2.2 Determination of the reproductive mode in *T. setosus*

Additionally, we also aimed to determine the reproductive mode of *T. setosus*. Thysanoptera are haplodiploid, which means usually males develop from haploid eggs and females from diploid eggs, and can exhibit different reproductive modes: thelytoky, arrhenotoky and deuterotoky (Krüger, 2016). Frequent observations on our rearing populations allowed us to preliminarily exclude the hypothesis of thelytoky form of reproduction, once both females and males could be easily found in the rearing plants. Sexes were easily distinguished as they are morphologically distinct. Males are yellow coloured in contrast with the black colouration of the females (Vierbergen & Loomans, 2016) (Figure 4.1).

Figure 4.1 Adult male (on the left) and female (on the right) of *T. setosus*. Photo by M. Ulitzka, 2020, http://www.thrips-id.com/en/photo-video/macros/

The exclusively male progeny obtained from unmated females of *T. setosus* showed that the studied population does not reproduce through thelytoky (unfertilized females produce only females) or deuterotoky (unfertilized females produce females and males). We provide evidence that *T. setosus* reproduces through arrhenotoky in consistency with what Vierbergen & Loomans (2016) proposes based on the field population sex-ratio found. Nevertheless, the parthenogenetic production of males is by itself no proof of haplodiploidy. The demonstration of haploidy requires cytological and genetic evidence including the investigation of the chromosome number of the somatic cells of different males stages (Kumm, 2002). Using molecular tools, Toda and Murai (2007) reported a time saving technique for differentiation of the mode of reproduction using nucleotide sequences encoding the mitochondrial cytochrome oxidase I (COI). However, this technique should be used with caution as it was detected that some arrhenotoky insects were phylogenetically clustered incorrectly into the clade of thelytoky according to COI-based technique (Aizawa *et al.*, 2016). To find more about the sex determination in *T. setosus*, further experiments including genetic and cytological analysis as well as the investigation of different geographical populations should be undertaken.

5 Overall findings and conclusions

We present evidence based on several experiments that complement each other that *Orius* species show large variations in some of its biological parameters when they were offered different thrips species (*F. occidentalis*, *E. americanus* and *T. setosus*). Both *Orius* consumed significantly less individuals of *F. occidentalis* on which they also recorded the lowest success ratio of encounters. Moreover, *F. occidentalis* showed a very agile behaviour, contrasting with the lower mobility levels observed for *T. setosus* and *E. americanus*, which we suggest as one of the factors influencing the lower predation capacity of *Orius* on *F. occidentalis*.

Our results indicate that amongst the thrips species tested, *E. americanus* is the most suitable food for both *O. laevigatus* and *O. majusculus*. This is clearly demonstrated by high predation capacities, high survival rates, long longevities, favourable reproductive parameters and intrinsic rates of increase. In fact, some of these results approached what has been found on an optimal diet of *E. kuehniella* eggs. If indeed these *Orius* species prove to successfully suppress *E. americanus* in field conditions, feeding on this prey can positively affect the biological control effectiveness of anthocorids. While reducing the number of *E. americanus*, feeding on this prey will bring extra benefits for the predator by increasing its performance. As a result, fast population growth rates will be achieved, possibly cascading into better control of less preferred preys as well.

Several authors have previously examined the predation capacity and life table parameters of *Orius* species on *F. occidentalis*. However, to the best of our knowledge, this is the first study evaluating in depth several developmental parameters of *Orius* predators on the leaf-inhabiting thrips *E. americanus* and *T. setosus.* Although thrips are generally known to be a good food source for *Orius* predators, thrips identity itself further affects the predators' fitness. Based on our findings, we hypothesize that less mobile thrips are preferably predated and lead to better reproductive characteristics of the predator population.

Parallelly, we present information on the effects of supplemental food for *T. setosus* and its mode of reproduction, thus contributing to a broader knowledge of the biology characteristics of this recent invader. Unlike what is known for thrips like *F. occidentalis,* so far there is no evidence of omnivorous habits of *T. setosus*. Most importantly, these results encourage the introduction of common food supplements (like pollen, *E. kuehniella* eggs and *A. franciscana* eggs) in biological control programmes without risking an increase in the densities of *T. setosus*. Regarding the mode of reproduction, we present strong evidence that *T. setosus* reproduces though haplodiploid arrhenotoky. Yet confirmation with genetic and cytological analysis is recommended.

6 References

Aizawa, M., Watanabe, T., Kumano, A., Miyatake, T. & Sonoda, S. (2016) Cypermethrin resistance and reproductive types in onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae). *Journal of Pesticide Science*, **41**, 167–170.

Aragón-Sánchez, M., Román-Fernández, L.R., Martínez-García, H., Aragón-García, A., Pérez-Moreno, I. & Marco-Mancebón, V.S. (2018) Rate of consumption, biological parameters, and population growth capacity of *Orius laevigatus* fed on *Spodoptera exigua*. *BioControl*, **63**, 785–794.

Arijs, Y. & Clercq, P. De. (2001) Rearing *Orius laevigatus* on cysts of the brine shrimp *Artemia franciscana*. *Biological Control*, **21**, 79–83.

Arijs, Y. & Clercq, P. De. (2004) Liver-based artificial diets for the production of *Orius laevigatus*. *BioControl*, **49**, 505–516.

Bakker, J.C., Bot, G.P.A., Challa, H. & Braak, N.J. van de. (1995) *Greenhouse Climate Control*: *An integrated approach*. Wageningen Academic Publishers, Wageningen, The Netherlands.

Bielza, P. (2008) Insecticide resistance management strategies against the western flower thrips, *Frankliniella occidentalis*. *Pest Management Science*, **64**, 1131–1138.

Blaeser, P., Sengonca, C. & Zegula, T. (2004) The potential use of different predatory bug species in the biological control of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Journal of Pest Science*, **77**, 211–219.

Bonte, J., Hauwere, L. De, Conlong, D. & Clercq, P. De. (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.

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

Bonte, J., Vangansbeke, D., Maes, S., Bonte, M., Conlong, D. & Clercq, P. De. (2012b) 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.

Bonte, M. & clercq, P. De. (2008) Developmental and reproductive fitness of *Orius laevigatus* (Hemiptera: Anthocoridae) reared on factitious and artificial diets. *Journal of Economic Entomology*, **101**, 1127–1133.

Bonte, M. & Clercq, P. de. (2010) Influence of diet on the predation rate of *Orius laevigatus* on *Frankliniella occidentalis*. *BioControl*, **55**, 625–629.

Bonte, M. & Clercq, P. De. (2010) Impact of artificial rearing systems on the developmental and reproductive fitness of the predatory bug, *Orius laevigatus*. *Journal of Insect Science*, **10**, 1–11.

Bouagga, S., Urbaneja, A. & Pérez-Hedo, M. (2018) Comparative biocontrol potential of three predatory mirids when preying on sweet pepper key pests. *Biological Control*, **121**, 168–174.

Brent, R. (1973) *Algorithms for minimization without derivatives*. Prentice-Hall, Englewood Cliffs, NJ.

Brodbeck, B., Funderburk, J., Stavisky, J., Andersen, P. & Hulshof, J. (2002) Recent advances in the nutritional ecology of Thysanoptera, or the lack thereof. In *Thrips and tospoviruses: Proceedings of the 7th international symposium on Thysanoptera* (ed. by Marullo, R. & Mound, L.), pp. 145-153. Australian National Insect Collection (ANIC), Canberra, Australia.

Brodbeck, B. V., Stavisky, J., Funderburk, J.E., Andersen, P.C. & Olson, S.M. (2001) Flower nitrogen status and populations of *Frankliniella occidentalis* feeding on *Lycopersicon esculentum*. *Entomologia Experimentalis et Applicata*, **99**, 165–172.

Buitenhuis, R., Murphy, G., Shipp, L. & Scott-Dupree, C. (2015) *Amblyseius swirskii* in greenhouse production systems: a floricultural perspective. *Experimental and Applied Acarology*, **65**, 451–464.

Butler, C.D. & O'Neil, R.J. (2007) Life history characteristics of *Orius insidiosus* (Say) fed diets of soybean aphid, *Aphis glycines* Matsumura and soybean thrips, *Neohydatothrips variabilis* (Beach). *Biological Control*, **40**, 339–346.

Calixto, A.M., Bueno, V.H.P., Montes, F.C., Silva, A.C. & Lenteren, J.C. van. (2013) Effect of different diets on reproduction, longevity and predation capacity of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). *Biocontrol Science and Technology*, **23**, 1245–1255.

Calvo, F.J., Knapp, M., Houten, Y.M. van, Hoogerbrugge, H. & Belda, J.E. (2015) *Amblyseius swirskii*: what made this predatory mite such a successful biocontrol agent? *Experimental and Applied Acarology*, **65**, 419–433.

Castañé, C., Arnó, J., Gabarra, R. & Alomar, O. (2011) Plant damage to vegetable crops by zoophytophagous mirid predators. *Biological Control*, **59**, 22–29.

Chambers, R.J., Long, S. & Helyer, N.L. (1993) Effectiveness of *Orius laevigatus* (Hem.: Anthocoridae) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. *Biocontrol Science and Technology*, **3**, 295–307.

Childers, C.C. (1997) Feeding and oviposition injuries to plants. In *Thrips as crop pests* (ed. by Lewis, T.), pp. 505-537. CABI Press, Wallingford, UK.

Childers, C.C. & Achor, D.S. (1995) Thrips feeding and oviposition injuries to economic plants, subsequent damage and host responses to infestation. In *Thrips biology and management* (ed. by Parker, B.L., Skinner, M. & Lewis, T.), pp. 31-51. Springer, Boston, MA.

Chitturi, A., Riley, D.G. & Joost, P.H. (2006) Effect of pine pollen on settling behavior of *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) on tomato and peanut. *Environmental Entomology*, **35**, 1396–1403.

Clercq, P. De, Arijs, Y., Meir, T. Van, Stappen, G. Van, Sorgeloos, P., Dewettinck, K., *et al.* (2005) Nutritional value of brine shrimp cysts as a factitious food for *Orius laevigatus* (Heteroptera: Anthocoridae). *Biocontrol Science and Technology*, **15**, 467–479.

Clercq, P. De & Degheele, D. (1994) Laboratory measurement of predation by *Podisus maculiventris* and *P. sagitta* (Hemiptera: Pentatomidae) on beet armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **87**, 76–83.

Cock, M.J.W., Lenteren, J.C. van, Brodeur, J., Barratt, B.I.P., Bigler, F., Bolckmans, K., *et al.* (2010) Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? *BioControl*, **55**, 199–218.

Cocuzza, G.E., Clercq, P. De, Lizzio, S., Veire, M. Van De, Tirry, L., Degheele, D., *et al.* (1997a) Life tables and predation activity of *Orius laevigatus* and *O. albidipennis* at three constant temperatures. *Entomologia Experimentalis et Applicata*, **85**, 189–198.

Cocuzza, G.E., Clercq, P. De, Veire, M. Van de, Cock, A. De, Degheele, D. & Vacante, V. (1997b) Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs. *Entomologia Experimentalis et Applicata*, **82**, 101–104.

Coll, M. & Guershon, M. (2002) Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annual Review of Entomology*, **47**, 267–297.

Cook, J. (2002) Genetics of sex ratio and sex determination: Sex determination in invertebrates. In *Sex Ratios: Concepts and research methods* (ed. by Hardy, I.), pp. 178-194. Cambridge University Press, Cambridge, UK.

DeBach, P. (1964) *Biological control of insect pests and weeds*. Chapman and Hall, London, UK.

Doğramaci, M., Arthurs, S.P., Chen, J., McKenzie, C., Irrizary, F. & Osborne, L. (2011) Management of chilli thrips *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on peppers by *Amblyseius swirskii* (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae). *Biological Control*, **59**, 340–347.

Dowds, B. & Peters, A. (2002) Virulence mechanisms. In *Entomopathogenic nematology* (ed. by Gaugler, R.), pp. 79-98. CABI Press, Wallingford, UK.

Ebssa, L., Borgemeister, C. & Poehling, H.-M. (2004) Effectiveness of different species/strains of entomopathogenic nematodes for control of western flower thrips (*Frankliniella occidentalis*) at various concentrations, host densities, and temperatures. *Biological Control*, **29**, 145–154.

Eigenbrode, S.D., Castagnola, T., Roux, M.-B. & Steljes, L. (1996) Mobility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. *Entomologia Experimentalis et Applicata*, **81**, 335–343.

El-Wakeil, N., Saleh, M., Gaafar, N. & Elbehery, H. (2017) Conservation biological control practices. In *Biological control of pest and vector insects* (ed. by Shields, D.C.V.). Intech Open Access, London, UK.

Fantinou, A.A., Perdikis, D.C., Maselou, D.A. & Lambropoulos, P.D. (2008) Prey killing without consumption: does *Macrolophus pygmaeus* show adaptive foraging behaviour? *Biological Control*, **47**, 187–193.

FAO. (2017) *The future of food and agriculture – Trends and challenges*. *Food and Agriculture Organization of the United Nations*, Rome, Italy.

Faraji, F., Janssen, A. & Sabelis, M.W. (2001) Predatory mites avoid ovipositing near counterattacking prey. *Experimental and Applied Acarology*, **25**, 613–623.

Fathi, S.A.A. (2009) The abundance of *Orius niger* (Wolf.) and *O. minutus* (L.) in potato fields and their life table parameters when fed on two prey species. *Journal of Pest Science*, **82**, 267–272.

Fu, B., Li, Q., Qiu, H., Tang, L., Zeng, D., Liu, K., *et al.* (2019) Oviposition, feeding preference, and biological performance of *Thrips hawaiiensis* on four host plants with and without supplemental foods. *Arthropod-Plant Interactions*, **13**, 441–452.

Funderburk, J., Stavisky, J. & Olson, S. (2000) Predation of *Frankliniella occidentalis* (Thysanoptera:

Thripidae) in field peppers by *Orius insidiosus* (Hemiptera: Anthocoridae). *Environmental Entomology*, **29**, 376–382.

Gao, Y., Lei, Z. & Reitz, S.R. (2012) Western flower thrips resistance to insecticides: detection, mechanisms and management strategies. *Pest Management Science*, **68**, 1111–1121.

Ghasemzadeh, S., Leman, A. & Messelink, G.J. (2017) Biological control of *Echinothrips americanus* by phytoseiid predatory mites and the effect of pollen as supplemental food. *Experimental and Applied Acarology*, **73**, 209–221.

Gonzalez, F., Tkaczuk, C., Dinu, M.M., Fiedler, Ż., Vidal, S., Zchori-Fein, E., *et al.* (2016) New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. *Journal of Pest Science*, **89**, 295–311.

Grenier, S. & Clercq, P. de. (2003) Comparison of artificially vs. naturally reared natural enemies and their potential for use in biological control. In *Quality control and production of biological control agents: Theory and testing procedures* (ed. by Lenteren, J.C. van), pp. 115-131. CABI Press, Wallingford, UK.

Gurr, G. & Wratten, S. (Eds.). (2000) *Biological control: Measures of success*. Springer, Dordrecht, The Netherlands.

Hanafi, A. (2003) Integrated production and protection today and in the future in greenhouse crops in the Mediterranean region. *Acta Horticulturae*, **614**, 755–765.

Hansen, E.A., Funderburk, J.E., Reitz, S.R., Ramachandran, S., Eger, J.E. & Mcauslane, H. (2003) Within-plant distribution of *Frankliniella* species (Thysanoptera: Thripidae) and *Orius insidiosus* (Heteroptera: Anthocoridae) in field pepper. *Environmental Entomology*, **32**, 1035–1044.

Henaut, Y., Alauzet, C., Ferran, A. & Williams, T. (2000) Effect of nymphal diet on adult predation behavior in *Orius majusculus* (Heteroptera: Anthocoridae). *Journal of Economic Entomology*, **93**, 252–255.

Hoogerbrugge, H., Oude Lenferink, K., Houten van, Y. & Bolckmans, K. (2014) Screening of three phytoseiid mite species as biocontrol agents of *Echinothrips americanus*. *IOBC/WPRS Bulletin*, **102**, 97–101.

Hulshof, J., Ketoja, E. & Vänninen, I. (2003) Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. *Entomologia Experimentalis et Applicata*, **108**, 19–32.

Hunter, W.B. & Ullman, D.E. (1992) Anatomy and ultrastructure of the piercing-sucking mouthparts and paraglossal sensilla of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *International Journal of Insect Morphology and Embryology*, **21**, 17–35.

Husseini, M., Schumann, K. & Sermann, H. (1993) Rearing immature feeding stage of *Orius majesculus* Reut. (Het., Anthocoridae) on the acarid mite *Tyrophagus putriscentiae* Schr. as new alternative prey. *Journal of Applied Entomology*, **116**, 113–117.

Ingegno, B.L., Bodino, N., Leman, A., Messelink, G.J. & Tavella, L. (2017) Predatory efficacy of *Dicyphus errans* on different prey. *Acta Horticulturae*, **1164**, 425–430.

Isenhour, D.J. & Yeargan, K. V. (1981) Predation by *Orius insidiosus* on the soybean thrips, *Sericothrips variabilis*: effect of prey stage and density. *Environmental Entomology*, **10**, 496–500.

Ivanova, G.P., Sukhoruchenko, G.I. & Kudryashova, L.Y. (2017) Tactics to control a new invasive pest - *Echinothrips americanus*. *Zashchita i Karantin Rasteniĭ*, **10**, 38–41 (in Russian with English summary).

Jacobson, R.J., Chandler, D., Fenlon, J. & Russell, K.M. (2001) Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. *Biocontrol Science and Technology*, **11**, 391–400.

Jakobsen, L., Enkegaard, A. & Brødsgaard, H.F. (2004) Interactions between two polyphagous predators, *Orius majusculus* (Hemiptera: Anthocoridae) and *Macrolophus caliginosus* (Heteroptera: Miridae). *Biocontrol Science and Technology*, **14**, 17–24.

Jervis, M.A., Ellers, J. & Harvey, J.A. (2008) Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annual Review of Entomology*, **53**, 361–385.

Kikuchi, Y. & Fukatsu, T. (2003) Diversity of *Wolbachia* endosymbionts in heteropteran bugs. *Applied and Environmental Microbiology*, **69**, 6082–6090.

Kirk, W.D.J. (1996) *Thrips. Naturalists' handbooks 25*. Richmond Publishing Co. Ltd., Slough, UK.

Kirk, W.D.J. & Terry, L.I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*, **5**, 301–310.

Knapp, M., Houten, Y. van, Baal, E. van & Groot, T. (2018) Use of predatory mites in commercial biocontrol: current status and future prospects. *Acarologia*, **58**, 72–82.

Knapp, M., Palevsky, E. & Rapisarda, C. (2020) Insect and mite pests. In *Integrated pest and disease management in greenhouse crops. Plant Pathology in 21st century*, Vol. **9** (ed. by Gullino, M., Albajes, R. & Nicot, P.), pp. 101-146. Springer, Cham, Switzerland.

Kobatake, H. (1984) Ecology and control of spotted wilt disease of tomato in Nara Prefecture. *Proceedings of the Kansai Plant Protection Society.*, **26**, 23–28 (In Japanese with English summary).

Kobatake, H. & Nakazawa, K. (1988) *Thrips setosus*. In *Pest thrips in Japan* (ed. by Umeya, K., Kudo, I. & Miyazaki, M.). Zenkoku Noson Kyoiku Kyokai, Tokyo, pp. 256–260 (in Japanese).

Kohno, K. & Kashio, T. (1998) Development and prey consumption of *Orius sauteri* (Poppius) and *O. minutus* (L.) (Heteroptera: Anthocoridae) fed on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Applied Entomology and Zoology*, **33**, 227–230.

Koizumi, S. (1985) Studies on Thysanoptera found on tobacco plants in the western Japan I. Species of Thysanoptera infesting tobacco plants. *Bulletin of the Kagoshima Tobacco Experiment Station*, **26**, 55–74 (in Japanese with English summary).

Krüger, S. (2016) *Reproductive strategies of plant-sap sucking insects with special focus on Thysanoptera*. PhD dissertation, Martin Luther Universität Halle-Wittenberg. https://opendata.uni-halle.de//handle/1981185920/8501

Kumm, S. (2002) *Reproduction, progenesis, and embryogenesis of thrips (Thysanoptera, Insecta).* PhD Dissertation, Martin Luther Universität Halle-Wittenberg. https://opendata.uni-halle.de/bitstream/1981185920/9904/1/prom.pdf

Kumm, S. & Moritz, G. (2008) First detection of *Wolbachia* in arrhenotokous populations of thrips species (Thysanoptera: Thripidae and Phlaeothripidae) and its role in reproduction. *Environmental*

Entomology, **37**, 1422–1428.

Labbé, R.M., Gagnier, D., Kostic, A. & Shipp, L. (2018) The function of supplemental foods for improved crop establishment of generalist predators *Orius insidiosus* and *Dicyphus hesperus*. *Scientific Reports*, **8**, 1–12.

Landis, D.A., Wratten, S.D. & Gurr, G.M. (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology*, **45**, 175–201.

Leman, A., Ingegno, B.L., Tavella, L., Janssen, A. & Messelink, G.J. (2020) The omnivorous predator *Macrolophus pygmaeus,* a good candidate for the control of both greenhouse whitefly and poinsettia thrips on gerbera plants. *Insect Science*, **27**, 510–518.

Leman, A. & Messelink, G.J. (2015) Supplemental food that supports both predator and pest: A risk for biological control? *Experimental and Applied Acarology*, **65**, 511–524.

Lenteren, J. van. (1988) Biological and integrated pest control in greenhouses. *Annual Review of Entomology*, **33**, 239–269.

Lenteren, J.C. van. (2000) A greenhouse without pesticides: fact or fantasy? *Crop Protection*, **19**, 375–384.

Lenteren, J.C. van (Ed.). (2012a) IOBC *Internet book of biological control*. https://www.iobc-wprs.org/pub/IOBC_InternetBookBiCoVersion6Spring2012.pdf

Lenteren, J.C. van. (2012b) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, **57**, 1–20.

Lenteren, J.C. van, Bolckmans, K., Köhl, J., Ravensberg, W.J. & Urbaneja, A. (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. *BioControl*, **63**, 39–59.

Lenteren, J.C. van & Manzaroli, G. (1999) Evaluation and use of predators and parasitoids for biological control of pests in greenhouses. In *Integrated pest and disease management in greenhouse crops* (ed. by Albajes, R., Gullino, M.L., Lenteren, J.C. van & Elad, Y.), pp. 183-201. Springer, Dordrecht, The Netherlands.

Lenteren, J.C. van & Tommasini, M.G. (2003) Mass production, storage, shipment and release of natural enemies. In *Quality control and production of biological control agents: Theory and testing procedures* (ed. by Lenteren, J.C. van), pp. 181-189. CABI Press, Wallingford, UK.

Leon-Beck, M. & Coll, M. (2009) The mating system of the flower bug *Orius laevigatus*. *Biological Control*, **50**, 199–203.

Leppla, N.C. & Clercq, P. De. (2019) History of the international organization for biological control global working group on mass rearing and quality assurance. *Journal of Insect Science*, **19**.

Lewis, T. (1973) *Thrips: Their biology, ecology and economic importance*, 1st edn. Academic Press, London, UK.

Lewis, T. (1997) *Thrips as crop pests*. CABI Press, Wallingford, UK.

Liu, P., Jia, W., Zheng, X., Zhang, L., Sangbaramou, R., Tan, S., *et al.* (2018) Predation functional response and life table parameters of *Orius sauteri* (Hemiptera: Anthocoridae) feeding on *Megalurothrips usitatus* (Thysanoptera: Thripidae). *Florida Entomologist*, **101**, 254–259.

Lundgren, J.G. (2011) Reproductive ecology of predaceous Heteroptera. *Biological Control*, **59**, 37–52.

Lundgren, J.G., Fergen, J.K. & Riedell, W.E. (2008) The influence of plant anatomy on oviposition and reproductive success of the omnivorous bug *Orius insidiosus*. *Animal Behaviour*, **75**, 1495–1502.

Maanen, R. van, Broufas, G., Oveja, M.F., Sabelis, M.W. & Janssen, A. (2012) Intraguild predation among plant pests: western flower thrips larvae feed on whitefly crawlers. *BioControl*, **57**, 533–539.

Maia, A. de H.N., Luiz, A.J.B. & Campanhola, C. (2000) Statistical inference on associated fertility life table parameters using jackknife technique: computational aspects. *Journal of Economic Entomology*, **93**, 511–518.

Maia, A.D.H.N., Pazianotto, R.A.D.A., Luiz, A.J.B., Marinho-Prado, J.S. & Pervez, A. (2014) Inference on arthropod demographic parameters: computational advances using R. *Journal of Economic Entomology*, **107**, 432–439.

McCullagh, P. & Nelder, J.A. (1989) *Generalized linear models*, 2nd edn. Chapman & Hall/CRC, London, UK.

Meiracker, R.A.F. (1994) Induction and termination of diapause in *Orius* predatory bugs. *Entomologia Experimentalis et Applicata*, **73**, 127–137.

Meiracker, R.A.F. & Sabelis, M.W. (1999) Do functional responses of predatory arthropods reach a plateau? A case study of *Orius insidiosus* with western flower thrips as prey. *Entomologia Experimentalis et Applicata*, **90**, 323–329.

Meiracker, R.A.F. van den & Ramakers, P.M.J. (1991) Biological control of the western flower thrips *Frankliniella occidentalis*, in sweet pepper, with the anthocorid predator *Orius insidiosus*. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **56**, 241–249.

Mendes, S.M., Bueno, V.H.P., Argolo, V.M. & Silveira, L.C.P. (2002) Type of prey influences biology and consumption rate of *Orius insidiosus* (Say) (Hemiptera, Anthocoridae). *Revista Brasileira de Entomologia*, **46**, 99–103.

Mendoza, J.E., Balanza, V., Cifuentes, D. & Bielza, P. (2020) Selection for larger body size in *Orius laevigatus*: Intraspecific variability and effects on reproductive parameters. *Biological Control*, **148**.

Messelink, G.J. (2017) Pest management in organic greenhouse horticulture. *Acta Horticulturae*, **1164**, 361–370.

Messelink, G.J., Bennison, J., Alomar, O., Ingegno, B.L., Tavella, L., Shipp, L., *et al.* (2014) Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. *BioControl*, **59**, 377–393.

Messelink, G.J. & Holstein-Saj, R. van. (2008) Improving thrips control by the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese). *IOBC/WPRS Bulletin*, **32**, 135–138.

Messelink, G.J. & Janssen, A. (2014) Increased control of thrips and aphids in greenhouses with two species of generalist predatory bugs involved in intraguild predation. *Biological Control*, **79**, 1–7.

Messelink, G.J., Maanen, R. van, Steenpaal, S.E.F. van & Janssen, A. (2008) Biological control of thrips and whiteflies by a shared predator: two pests are better than one. *Biological Control*, **44**, 372–379.

Messelink, G.J., Steenpaal, S.E.F. Van & Ramakers, P.M.J. (2006) Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. *BioControl*, **51**, 753–768.

Meyer, J.S., Ingersoll, C.G., McDonald, L.L. & Boyce, M.S. (1986) Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecology,* **67**, 1156–1166.

Meyling, N.V., Enkegaard, A. & Brødsgaard, H. (2003) Two anthocoris bugs as predators of glasshouse aphids - voracity and prey preference. *Entomologia Experimentalis et Applicata*, **108**, 59–70.

Millar, J.G., Paine, T.D., Joyce, A.L. & Hanks, L.M. (2003) The effects of eucalyptus pollen on longevity and fecundity of eucalyptus longhorned borers (Coleoptera: Cerambycidae). *Journal of Economic Entomology*, **96**, 370–376.

Montoro, M., Clercq, P. De, Overgaard, J. & Sigsgaard, L. (2020a) Fitness consequences of artificial diets with different macronutrient composition for the predatory bug *Orius majusculus*. *Entomologia Experimentalis et Applicata*, **168**, 492–501.

Montoro, M., Fine Licht, H.H. De & Sigsgaard, L. (2020b) Nutritional quality of *Drosophila melanogaster* as factitious prey for rearing the predatory bug *Orius majusculus*. *Insect Science*.

Morales-Ramos, J., Guadalupe Rojas, M. & Shapiro-Ilan, D. (Eds.). (2014) *Mass production of beneficial organisms: Invertebrates and entomopathogens*. Elsevier, Amsterdam.

Morse, J.G. & Hoddle, M.S. (2006) Invasion biology of thrips. *Annual Review of Entomology,* **51**, 67–89.

Mouden, S., Sarmiento, K.F., Klinkhamer, P.G.L. & Leiss, K.A. (2017) Integrated pest management in western flower thrips: past, present and future. *Pest Management Science*, **73**, 813–822.

Murai, T. (1991) Thysanoptera: *Frankliniella intonsa*, *Thrips tabaci* and *Thrips setosus*. In *Rearing method of insects* (ed. by K. Yushima, S. Kamano & Y. Tamaki). Japan Plant Protection Association, Tokyo, pp. 21–24 (in Japanese).

Murai, T. (2011) Life history study of *Thrips setosus*. *Entomologia Experimentalis et Applicata,* **100**, 245–251.

Nagai, K. (1990) Suppressive effect of *Orius* sp. (Hemiptera: Anthocoridae) on the population density of *Thrips palmi* Karny (Thysanoptera: Thripidae) in eggplant in an open field. *Japanese Journal of Applied Entomology and Zoology,* **34**, 109–114 (In Japanese with English summary).

Nakao, S. (1998) Effects of photoperiod and temperature on induction and termination of reproductive diapause of *Thrips setosus* Moulton (Thysanoptera: Thripidae). *Japanese Journal of Applied Entomology and Zoology,* **42**, 172–173 (in Japanese with English summary).

Nault, B.A., Shelton, A.M., Gangloff-kaufmann, J.L., Clark, M.E., Werren, J.L., Cabrera-la Rosa, J.C., *et al.* (2006) Reproductive modes in onion thrips (Thysanoptera: Thripidae) populations from New York onion fields. *Environmental Entomology*, **35**, 1264–1271.

Nguyen, D.T., Vangansbeke, D. & Clercq, P. De. (2014) Artificial and factitious foods support the development and reproduction of the predatory mite *Amblyseius swirskii*. *Experimental and Applied Acarology*, **62**, 181–194.

Northfield, T.D., Barton, B.T. & Schmitz, O.J. (2017) A spatial theory for emergent multiple predator-prey interactions in food webs. *Ecology and Evolution*, **7**, 6935–6948.

Opit, G.P., Peterson, B., Gillespie, D.R. & Costello, R.A. (1997) The life cycle and management of *Echinothrips americanus* (Thysanoptera: Thripidae). *Journal of the Entomological Society of British Columbia*, **94**, 3–6.

Oveja, M.F., Arnó, J. & Gabarra, R. (2012) Effect of supplemental food on the fitness of four omnivorous predator species. *IOBC/WPRS Bulletin*, **80**, 97–101.

Oveja, M.F., Riudavets, J., Arnó, J. & Gabarra, R. (2016) Does a supplemental food improve the effectiveness of predatory bugs on cucumber? *BioControl*, **61**, 47–56.

Pijnakker, J., Leman, A., Vangansbeke, D. & Wackers, F. (2017a) *Echinothrips americanus*: a bottleneck for integrated pest management in ornamentals? *Communications in Applied Biological Sciences, Ghent University*, **82**, 105–111.

Pijnakker, J., Overgaag, D., Guilbaud, M., Vangansbeke, D., Duarte, M. & Wackers, F. (2019) Biological control of the Japanese flower thrips *Thrips setosus* Moulton (Thysanoptera: Thripidae) in greenhouse ornamentals. *IOBC/WPRS Bulletin*, **147**, 107–112.

Pijnakker, J., Vangansbeke, D., Wäckers, F. & Arijs, Y. (2017b) Building a 'standing army' of beneficials: a reality in greenhouse crops. In *AFPP – 6e conférence sur les moyens alternatifs de production pour une production intégrée*. Lille, France, 21-23 mars 2017, pp. 464–473.

Pimentel, D. (2005) Environmental and economic costs of the application of pesticides primarily in the United States. *Environment, Development and Sustainability*, **1**, 229–252.

Pizzol, J., Nammour, D., Ziegler, J.P., Voisin, S., Maignet, P., Olivier, N., *et al.* (2008) Efficiency of *Neoseiulus cucumeris* and *Franklinothrips vespiformis* for controlling thrips in rose greenhouses. *Acta Horticulturae*, **801**, 1493–1498.

Pozzebon, A., Boaria, A. & Duso, C. (2015) Single and combined releases of biological control agents against canopy- and soil-dwelling stages of *Frankliniella occidentalis* in cyclamen. *BioControl*, **60**, 341–350.

Pumariño, L. & Alomar, O. (2012) The role of omnivory in the conservation of predators: *Orius majusculus* (Heteroptera: Anthocoridae) on sweet alyssum. *Biological Control*, **62**, 24–28.

Rajabpour, A., Seraj, A.A., Allahyari, H. & Shishehbor, P. (2011) Evaluation of *Orius laevigatus* Fiber (Heteroptera: Anthocoridae) for biological control of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) on greenhouse cucumber in south of Iran. *Asian Journal of Biological Sciences*, **4**, 457–467.

Ramakers, P., Meiracker, R. van den & Mulder, S. (2000) Predatory thrips as thrips predators. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent*, **65**, 343–350.

Ramakers, P. & O'Neill, T. (1999) Cucurbits. In *Integrated pest and disease management in greenhouse crops* (ed. by Albajes, R., Gullino, M.L., Lenteren, J.C. van & Elad, Y.), pp. 435-453. Springer, Dordrecht, The Netherlands.

Rehman, S.U., Zhou, X., Ali, S., Asim Rasheed, M., Islam, Y., Hafeez, M., *et al.* (2020) Predatory functional response and fitness parameters of *Orius strigicollis* Poppius when fed *Bemisia tabaci* and *Trialeurodes vaporariorum* as determined by age-stage, two-sex life table. *PeerJ*, **8**, e9540.

Reitz, S.R. (2009) Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomologist*, **92**, 7–13.

Reitz, S.R., Funderburk, J.E. & Waring, S.M. (2006) Differential predation by the generalist predator *Orius insidiosus* on congeneric species of thrips that vary in size and behavior. *Entomologia Experimentalis et Applicata*, **119**, 179–188.

Reitz, S.R., Gao, Y., Kirk, W.D.J., Hoddle, M.S., Leiss, K.A. & Funderburk, J.E. (2020) Invasion biology, ecology, and management of western flower thrips. *Annual Review of Entomology*, **65**, 17–37.

Rijn, P.C.J. van, Houten, Y.M. Van & Sabelis, M.W. (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. *Ecology,* **83**, 2664–2679.

Rijn, P.C.J. van & Tanigoshi, L.K. (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. *Experimental and Applied Acarology,* **23**, 785–802.

Riley, D.G., Angelella, G.M. & McPherson, R.M. (2011) Pine pollen dehiscence relative to thrips population dynamics. *Entomologia Experimentalis et Applicata,* **138**, 223–233.

Riudavets, J. (1995) Predators of *Frankliniella occidentalis* (Perg.) and *Thrips tabaci* Lind.: a review. *Wageningen Agricultural University Papers,* **95–1**, 43–87.

Riudavets, J. & Castañé, C. (1998) Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. *Environmental Entomology,* **27**, 86–93.

Roermund, H.J.W. van, Lenteren, J.C. van & Rabbinge, R. (1997) Biological control of greenhouse whitefly with the parasitoid *Encarsia formosa* on tomato: an individual-based simulation approach. *Biological Control*, **9**, 25–47.

Rotenberg, D. & Whitfield, A.E. (2018) Molecular interactions between tospoviruses and thrips vectors. *Current Opinion in Virology,* **33**, 191–197.

Sade, A., Grosman, A. & Steinberg, S. (2019) Improved western flower thrips control through *Artemia*-based early introduction of *Orius laevigatus* in commercial pepper greenhouses. *IOBC/WPRS Bulletin,* **147**, 39–46.

Schelt, G. van, Hoorgerbrugge, H., Houten, Y. van & Bolckmans, K. (2002) Biological control and survival of *Echinothrips americanus* in pepper. *IOBC/WPRS Bulletin,* **25**, 285–288.

Shapiro, J.P., Shirk, P.D., Reitz, S.R. & Koenig, R. (2009) Sympathy of *Orius insidiosus* and *O. pumilio* (Hemiptera: Anthocoridae) in north central Florida. *Florida Entomologist*, **92**, 362–366.

Shipp, J.L., Gillespie, D.R., Fry, K.M. & Ferguson, G.M. (2001) *Echinothrips americanus* (Morgan), *Frankliniella occidentalis* (Pergande), western flower thrips, and *Thrips tabaci* Lindeman, onion thrips (Thysanoptera: Thripidae). In *Biological control programmes in Canada*, *1981-2000,* pp. 115-119. CABI Press, Wallingford, UK.

Southwood, T.R.E. (1978) The construction, description and analysis of age-specific life-tables. In *Ecological methods* (ed. by Henderson, P.A. & Southwood, T.R.E.), pp. 429-464. Springer, Dordrecht, The Netherlands.

Steiner, M.Y., Spohr, L.J. & Goodwin, S. (2011) Relative humidity controls pupation success and dropping behaviour of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Australian Journal of Entomology*, **50**, 179–186.

Stouthamer, R., Breeuwer, J.A.J. & Hurst, G.D.D. (1999) *Wolbachia Pipientis*: microbial manipulator of arthropod reproduction. *Annual Review of Microbiology,* **53**, 71–102.

Teerling, C.R., Pierce, H.D., Borden, J.H. & Gillespie, D.R. (1993) Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. *Journal of Chemical Ecology*, **19**, 681–697.

Toda, S. & Murai, T. (2007) Phylogenetic analysis based on mitochondrial COI gene sequences in *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographic distribution. *Applied Entomology and Zoology*, **42**, 309–316.

Toft, S., Jensen, K., Sørensen, J.G., Sigsgaard, L. & Holmstrup, M. (2020) Food quality of *Ephestia* eggs, the aphid *Rhopalosiphum padi* and mixed diet for *Orius majusculus*. *Journal of Applied Entomology*, **144**, 251–262.

Tommasini, M.G. (2003) *Evaluation of Orius species for biological control of Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae)*. PhD Dissertation, Wageningen Univeristy. https://edepot.wur.nl/121434

Tommasini, M.G. & Lenteren, J.C. van. (2003) Occurrence of diapause in *Orius laevigatus*. *Bulletin of Insectology*, **56**, 225–251.

Tommasini, M.G., Lenteren, J.C. Van & Burgio, G. (2004) Biological traits and predation capacity of four *Orius* species on two prey species. *Bulletin of Insectology*, **57**, 79–93.

Trdan, S., Milevoj, L., Raspudic, E. & Zezlina, I. (2003) The first record of *Echinothrips americanus* Morgan in Slovenia. *Acta Phytopathologica et Entomologica Hungarica*, **38**, 157–166.

Trdan, S., Žnidaračič, D. & Vidrih, M. (2007) Control of *Frankliniella occidentalis* on glasshouse-grown cucumbers: an efficacy comparison of foliar application of *Steinernema feltiae* and spraying with abamectin. *Russian Journal of Nematology*, **15**, 25–34.

Trichilo, P.J. & Leigh, T.F. (1986) Predation on spider mite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), an opportunist in a cotton agroecosystem. *Environmental Entomology*, **15**, 821–825.

Tsuda, S., Fujisawa, I., Ohnishi, J., Hosokawa, D. & Tomaru, K. (1996) Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus*. *Phytopathology*, **86**, 1199.

Ugine, T.A., Wraight, S.P. & Sanderson, J.P. (2007) Effects of manipulating spray-application parameters on efficacy of the entomopathogenic fungus *Beauveria bassiana* against western flower thrips, *Frankliniella occidentalis*, infesting greenhouse impatiens crops. *Biocontrol Science and Technology*, **17**, 193–219.

Ulitzka, M. (2020) *Male and female of Thrips setosus* [Photograph]. Thrips-iD. http://www.thrips-id.com/en/photo-video/macros/ [accessed on 18th November 2020].

Umeya, K., Kudo, I. & Miyazaki, M. (1988) *Pest thrips in Japan*. Zenkoku Noson Kyoiku Kyokai Publishing Co.

Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L., *et al.* (2014) Performance of the predatory mite *Amblydromalus limonicus* on factitious foods. *BioControl*, **59**, 67–77.

Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L., *et al.* (2016) Supplemental food for *Amblyseius swirskii* in the control of thrips: feeding friend or foe? *Pest Management Science*, **72**, 466–473.

Vantornhout, I., Minnaert, H., Tirry, L. & clercq, P. de. (2004) Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*. *BioControl*, **49**, 627–644.

Venzon, M., Janssen, A., Pallini, A. & Sabelis, M.W. (2000) Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. *Animal Behaviour*, **60**, 369–375.

Venzon, M., Janssen, A. & Sabelis, M.W. (2002) Prey preference and reproductive success of the generalist predator *Orius laevigatus*. *Oikos,* **97**, 116–124.

Vierbergen, G., Cean, M., Hataláné Szellér, I., Jenser, G., Masten, T. & Simala, M. (2006) Spread of two thrips pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). *Acta Phytopathologica et Entomologica Hungarica,* **41**, 287–296.

Vierbergen, G. & Loomans, A.J.M. (2016) *Thrips setosus* (Thysanoptera: Thripidae), the Japanese flower thrips, in cultivation of *Hydrangea* in the Netherlands. *Entomologische Berichten*, **76**, 103–108.

Vierbergen, G.B. (1998) *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society,* **9**, 155–160.

Vox, G., Teitel, M., Pardossi, A., Minuto, A., Tinivella, F. & Schettini, E. (2010) Sustainable greenhouse systems. In *Sustainable agriculture: Technology, planning & management* (ed. by Salazar, A. & Rios, I.), pp 1-79. Nova Science Publishers Inc., New York, NY.

Walle, A. Van de (2014) *Alternatieve voedingsbronnen voor de kweek van Orius thripoborus en Orius naivashae*. Master's thesis, Universiteit Gent. https://lib.ugent.be/catalog/rug01:002166620

Watanabe, M., Tagami, Y., Miura, K., Kageyama, D. & Stouthamer, R. (2012) Distribution patterns of *Wolbachia* endosymbionts in the closely related flower bugs of the genus *Orius*: implications for coevolution and horizontal transfer. *Microbial Ecology,* **64**, 537–545.

Watanabe, M., Yukuhiro, F., Maeda, T., Miura, K. & Kageyama, D. (2014) Novel strain of *Spiroplasma* found in flower bugs of the genus *Orius* (Hemiptera: Anthocoridae): transovarial transmission, coexistence with *Wolbachia* and varied population density. *Microbial Ecology*, **67**, 219–228.

Weintraub, P.G., Pivonia, S. & Steinberg, S. (2011) How many *Orius laevigatus* are needed for effective western flower thrips, *Frankliniella occidentalis*, management in sweet pepper? *Crop Protection*, **30**, 1443–1448.

Yano, E., Jiang, N., Hemerik, L., Mochizuki, M., Mitsunaga, T. & Shimoda, T. (2005) Time allocation of *Orius sauteri* in attacking *Thrips palmi* on an eggplant leaf. *Entomologia Experimentalis et Applicata*, **117**, 177–184.

Yano, E., Watanabe, K. & Yara, K. (2002) Life history parameters of *Orius sauteri* (Poppius) (Het., Anthocoridae) reared on *Ephestia kuehniella* eggs and the minimum amount of the diet for rearing individuals. *Journal of Applied Entomology,* **126**, 389–394.

Zhang, Z.-J., Wu, Q.-J., Li, X.-F., Zhang, Y.-J., Xu, B.-Y. & Zhu, G.-R. (2007) Life history of western flower thrips, *Frankliniella occidentalis* (Thysan., Thripae), on five different vegetable leaves. *Journal of Applied Entomology*, **131**, 347–354.

Zhi, J., Fitch, G.K., Margolies, D.C. & Nechols, J.R. (2005) Apple pollen as a supplemental food for the western flower thrips, *Frankliniella occidentalis*: response of individuals and populations. *Entomologia Experimentalis et Applicata,* **117**, 185–192.