Prospects for using Entomopathogenic Nematodes as a Biocontrol Agent against Western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae)

by

Thandwa Dlamini

Thesis presented in fulfilment of the requirements for the degree of Master of Science in Agriculture (Entomology), in the Faculty of AgriSciences at Stellenbosch University

UNIVERSITEIT
iYUNIVESITHI
STELLENBOSCH
UNIVERSITY

100
1918·2018

Supervisor: Prof Antoinette P Malan

Co-supervisor: Dr Elleunorah Allsopp

December 2018

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2018

i

Acknowledgements

Dedication

Dedicated to my late father

France T. Dlamini

Summary

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera), is one of the most economically important pests in greenhouses, with preference being exhibited towards feeding on flowers. WFT is a serious pest of greenhouse cultivation, because it damages plants directly by means of feeding and oviposition on foliage and flowers, and indirectly, by means of vectoring tospoviruses, such as impatiens necrotic spot virus and tomato spotted wilt virus. Approximately 7500 species of thrips have been identified to date, with 14 species being recognised as virus vectors, of which *F. occidentalis* is responsible for transmitting five species of tospoviruses. Chemical control has been the most frequently used method for the control of WFT in greenhouses. The high frequency of insecticide applications for WFT control, coupled with the short generation time of *F. occidentalis,* has led to an increasing incidence of insecticide resistance in WFT in recent years. An integrated pest management (IPM) programme offers a sustainable alternative control for WFT in undercover production. Biological control, especially the use of entomopathogenic nematodes (EPNs), has been identified as an environmentally friendly option. The use of other parasites and predators for biological control has shown only limited ability to reduce WFT populations, apparently because their movement is restricted when entering tight flower buds, meristem tissues, or narrow flower structures favoured by WFT, due to their large body size.

This study investigated the potential use of indigenous EPNs for the control of WFT under laboratory and greenhouse conditions. To achieve the above, the development and survival rate of *F. occidentalis* on two host plants, as well as its biology, were studied under laboratory conditions to identify life stages targetable with EPNs. The efficacy of the local strains of EPNs to control the different life stages of WFT, and the optimum nematode concentrations required for the suppression of WFT under laboratory conditions, were investigated. Lastly, the potential of foliar and soil applications of different concentrations of locally isolated *S. yirgalemense* for controlling *F. occidentalis* in a commercial blueberry greenhouse was investigated.

Laboratory studies were conducted to determine the life-history and host preference of adult WFT on chrysanthemum (*Dendranthema grandiflora*) leaflets and green bean pods (*Phaseolus vulgaris*). The identification of *Frankliniella occidentalis* was verified, using both morphological and molecular methods. Main morphological features included six to nine antennal segments, major setae on the head and pronotum dark, interocellar and postocular setae approximately the same length, the first vein of the anterior wing with a complete row of regularly spaced setae, and posteromarginal comb on tergite VIII of the female well-developed and complete. Molecular identification was based on amplification of the mtCOI gene

iv

sequences for the identification of four thrips species (*F. occidentalis, Thysanoptera* sp., *Gynaikothrips ficorum* and *Pseudophilothrips ichini*) collected from the study area. The *F. occidentalis* morphologically identified showed 100 % identity with sequences in the database from GenBank. One of the *Thrips* sp. could not be identified neither morphologically nor molecularly and could possibly be an unidentified species. Results from the life-history study showed that more first instar larva hatched on chrysanthemums, faster larval developmental rate and a higher survival rate on chrysanthemums indicating that chrysanthemum is a more attractive and more suitable host than green bean.

Among the 12 EPN species tested against *F. occidentalis* in laboratory bioassays, virulence ranged from 11 % to 67 %. Generally, *Heterorhabditis* spp. were more virulent than the *Steinernema* spp. *Heterorhabditis baujardi* was found to be the most potent species, with a mortality of 67 %, although it was not significantly different from *Steinernema yirgalemense* (66 %). The study showed that the commercial nematode *Steinernema feltiae* did not perform better than the local EPN species. Bioassays to determine infectivity were performed using different life stages (larva, pupa and adult) of *F. occidentalis* exposed to infective juveniles (IJs) of *S. yirgalemense*, *H. baujardi* and *Steinernema jeffreyense*. The pupae of WFT were found to be more sensitive to nematode infection than either the larvae or the adults. The highest WFT mortality was recorded for the pupae (72 %) when applying 100 IJs/insect of *H. baujardi*, with the lowest being recorded when treated with *S. jeffreyense* (17 %). *Steinernema yirgalemense* and *H. baujardi* were tested at concentrations of 0, 10, 20, 40, 80, and 160 IJs/larva. Increasing EPN concentrations gave increased thrips mortality, with a probit analysis indicating *S. yirgalemense* to be 5.49 more potent than *H. baujardi*. Results from the temporal development study showed that both *S. yirgalemense* and *H. baujardi* were able to complete their life cycles in the host within 5 days, and were able to produce a new cohort of IJs. Relatively few IJs were found to penetrate the insect, due to the small size of the insect and the IJs recovered from the host were relative in number to the IJs penetrated.

The field trial was initiated to determine the efficiency of different concentrations of *S. yirgalemense* in controlling *F. occidentalis* in a commercial blueberry greenhouse. A combination of foliar and soil applications of *S. yirgalemense* in two greenhouse trials, one at lower concentrations of 4.3, 8.6, and 17.2 IJs/cm^2, and the other at higher concentrations of 25, 50, and 100 IJs/cm^2 were applied. The results in both trials indicated thrips mortality < 50 % at the highest concentration of 100 IJs/cm^2, at mean substrate temperatures < 15 $^\circ$C, which was below optimum for *S. yirgalemense* infection. Increase in nematode concentration resulted in a decline in the number of thrips captured. The experiment with higher concentrations showed

increased thrips mortality (53 %) in relation to the experiment with lower concentration (< 40 %). *Steinernema yirgalemense* was persistent for 4 weeks, with low mortalities when mealworms were used to monitor infectivity.

The correct identification of thrips is important for further studies investigating biological control thereof. Research into the use of EPNs for the biological control of insects should not be restricted to laboratory conditions, as these conditions do not truly represent field performance. *Steinernema yirgalemense* showed potential for use as a biocontrol option for WFT, giving low to moderate results in the field trial, under suboptimal temperatures, at a concentration of 100 IJs/cm^2. The application of *S. yirgalemense* to control WFT requires further investigation under relatively warmer substrate temperatures in the Haygrove tunnels under blueberry production. Application of nematodes should target WFT populations on new growth after post-harvest pruning, when WFT causes significant economic damage. Weekly follow-up applications should be investigated as a future alternative. The feasibility of applying *S. yirgalemense* in conjunction with other biological agents and insecticide–pathogen synergistic interactions in IPM systems should also be investigated.

Opsomming

Die westelike blomblaaspootjie (WBB), *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera), is een van die belangerikste ekonomiese peste in kweekhuise en toon 'n voorliefde daarvoor om op blomme te voed. Die WBB is 'n ernstige pes van plante in kweekhuise omdat dit plante direk beskadig deur voeding en die lê van eiers op blare en blomme, asook indirek deur die dra van tospovirusse soos "impatiens necrotic spot virus" en "tomato spotted wilt virus". Ten minste 7500 spesies blaaspootjies is bekend, waarvan 14 geken is as draers. *Frankliniella occidentalis* is verantwoordelik vir die verspreiding van omtrent vyf tospovirus spesies. Chemiese beheer is die mees algemene metode wat toegepas word teen WBB in kweekhuise. Die hoë frekwensie waarteen insekdoders aangewend word vir die beheer van WBB, tesame met die kort generasietyd van die spesie, het gelei tot toenemende weerstand teen insekdoders in WBB in die afgelope paar jaar. 'n Geïntegreerde pes beheer (GPB) program, bied 'n volhoubare alternatief vir die beheer van WBB in onderdakproduksie. Biologiese beheer, veral die gebruik van entomopatogeniese nematodes (EPNs), is geïdentifiseer as 'n omgewingsvriendelike beheer metode. Die gebruik van ander parasiete en predatore vir biologiese beheer het sover slegs beperkte sukses getoon, blykbaar omdat hul beweging beperk is wanneer hul stywe blomknoppe, meristeem weefsel of nou blomstrukture binnedring, as gevolg van hul liggaamsgrootte.

Hierdie studie het die potensiaal ondersoek van die gebruik van inheemse EPNs vir die beheer van WBB in laboratorium en kweekhuis omstandighede. Om dit te bereik, was die ontwikkeling en oorlewingskoers van *F. occidentalis* op twee gasheer plante, sowel as sy biologie, bestudeer in laboratorium toestande, sodat lewensstadia wat vatbaar vir EPN infeksie is identifiseer kon word. Die vermoë van die plaaslike EPN spesies om die verskillende lewensfases van WBB te beheer, asook die optimale nematode konsentrasies vir die onderdrukking van WBB in laboratorium toestande, was ondersoek. In die laaste deel van die studie was die potensiaal van EPNs om *F. occidentalis* in 'n kommersiële bloubessie kweekhuis te beheer, ondersoek deur plaaslike *S. yirgalemense* by verskillende konsentrasies aan te wend.

Laboratorium studies om die lewensgeskiedenis en gasheer voorkeur van volwasse WBB te bepaal, was uitgevoer op krisant (*Dendranthema grandiflora*) blare en groenboontjies (*Phaseolus vulgaris*). *Frankliniella occidentalis* was geïdentifiseer en sy identiteit bevestig deur gebruik te maak van morfologiese en molekulêre metodes. Die hoof morfologiese kenmerke van *F. occidentalis* is ses tot nege antenna segmente, groot seta op die kop, donker pronotum, "interocellar" en post-okulêre seta omtrent dieselfde lengte, die eerste aar van die

voorste vlerk met eweredig gespasieërde en volledige seta, asook 'n goed ontwikkelde en volledige "posteromarginal comb" op die "tergite VIII" van die wyfie. Resultate van die lewensgeskiedenis eksperiment het vinniger ontwikkeling, meer eiers en larwes, asook 'n hoër oorlewingskoers getoon op krisante, wat beteken dat dit 'n meer gepaste gasheer is as die groenboontjie. Molekulêre identifikasie was gebaseer op die mtCOI geen vir die identifikasie van vier spesies (*F. occidentalis, Thysanoptera* sp., *Gynaikothrips ficorum* en *Pseudophilothrips ichini*) wat versamel was in die studie area. Die *F. occidentalis* was morfologies geïdentifiseer was, het 100% identiteit getoon met die inligting in die databasis van GenBank. Een van die blaaspootjie spesies kon nie morfologies of molekulêr identifiseer word nie en kan moontlik 'n onbeskryfde spesie wees.

Die virulensie van die 12 EPN spesies wat getoets was teen *F. occidentalis* in laboratorium biotoetse het gewissel van 11 % tot 67 %. Oor die algemeen het die *Heterorhabditis* spesies hoër virulensie getoon as die *Steinernema* spesies. *Heterorhabditis baujardi* was die dodelikste spesies, met 'n mortaliteit van 67 %, alhoewel dit nie 'n beduidende verskil getoon het teenoor die dodelikheid *Steinernema yirgalemense* (66 %) nie. Die studie het getoon dat die kommersiële nematode *Steinernema feltiae* nie beter gevaar het as die plaaslike EPN spesies nie. Biotoetse om infeksie te bepaal was uitgevoer op verskillende lewensstadia (larwes, papies en volwassenes) van *F. occidentalis* met die EPNs *S. yirgalemense*, *H. baujardi* en *Steinernema jeffreyense*. Die papies van WBB was meer vatbaar vir nematode infeksie as die larwes of die volwassenes. Die hoogste WBB mortaliteit was aangeteken met die aanwending van 100 Ils/insek van *H. baujardi* op WBB papies (72 %). Die laagste mortaliteit was aangeteken toe papies behandel was met *S. jeffreyense* (17 %). *Steinernema yirgalemense* en *H. baujardi* was getoets by konsentrasies van 0, 10, 20, 40, 80, en 160 ILs/larwe. 'n Toename in EPN konsentrasies het gelei tot 'n toename in die mortaliteit van blaaspootjies, met 'n pro-bit analise wat getoon het dat *S. yirgalemense* 5.49 keer meer dodelik is as *H. baujardi*. Resultate van die temporale ontwikkeling studie het getoon dat beide *S. yirgalemense* en *H. baujardi* in staat was om hul lewenssiklusse te voltooi in die gasheer binne 5 dae en ook 'n nuwe groep ILs kon produseer. Relatief min ILs het die insek gepenetreer, as gevolg van die klein liggaamsgrootte van die insek en die ILs wat gevind was in die gasheer was relatief tot die aantal ILs wat die insek gepenetreer het.

Die doel van die veldproef was om die effektiwiteit van verskillende konsentrasies van *S. yirgalemense* te toets vir die beheer van *F. occidentalis* in 'n kommersiële bloubessie kweekhuis. 'n Kombinasie van blaar- en grondaanwending van *S. yirgalemense* was toegepas in twee kweekhuis proewe, een by laer konsentrasies van 4.3, 8.6, en 17.2 ILs/cm^2, en 'n ander

by hoër konsentrasies van 25, 50, en 100 ILs/cm^2. Albei proewe het mortaliteit van < 50 % getoon in blaaspootjies, met die hoogste konsentrasie van 100 ILs/cm^2, by gemiddelde substraat temperature van < 15 $^\circ$C, wat onder die optimale temperatuur was vir *S. yirgalemense* infeksie. Die toename in nematode konsentrasie het gelei tot 'n afname in die aantal blaaspootjies wat gevang was. Die eksperiment met hoër konsentrasies het 'n verhoogde mortaliteit getoon in blaaspootjies (53 %) in vergelyking met die eksperiment by laer konsentrasies (> 40%). *Steinernema yirgalemense* het aangehou vir 4 weke, met lae mortaliteit toe meelwurms gebruik was om infektiwiteit te monitor.

Die identifikasie van blaaspootjies is belangerik vir verdere navorsing oor hul biologiese beheer. Navorsing oor die gebruik van EPSs vir die biologiese beheer van insekte moet nie beperk word tot laboratoriumtoestande nie, omdat hierdie toestande nie werklik die prestasie van EPNs in die veld verteenwoordig nie. *Steinernema yirgalemense* het potensiaal getoon as 'n biologiese beheer opsie vir WBB, met lae tot matige resultate in die veldproef, in suboptimale temperature, by 'n konsentrasie van 100 ILs/cm^2. Die aanwending van *S. yirgalemense* vir die beheer van WBB benodig verdere ondersoek, met relatief warmer substraat temperature in die Haygrove tonnels onder bloubessie produksie. Die aanwending van nematodes moet die piek in WBB populasies teiken gedurende die tydperk van nuwe groei, wanneer WBB aansienlike ekonomiese skade veroorsaak. Weeklikse opvolg aanwendings moet ondersoek word as 'n toekomstige alternatief. Die moontlikheid van die aanwending van *S. yirgalemense* in samewerking met ander biologiese beheermiddels, asook insekdoder-patogeen sinergistiese interaksies in IPM sisteme, moet ondersoek word.

Table of Contents

List of Figures

List of Tables

Chapter 1

Control of western flower thrips, *Frankliniella occidentalis*, with special reference to entomopathogenic nematodes

Abstract

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), has become a global pest of economic importance worldwide, especially for greenhouse producers. WFT is extremely polyphagous, attacking a wide range of host plants in both the field and the greenhouse. *Frankliniella occidentalis* can be of great economic importance, causing yield reductions of more than 50 %, with the greatest amount of damage caused by its ability to transmit tospoviruses, such as the tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV). Due to their minute size, thrips are often overlooked and incorrectly identified, hence the need for positive identification for effective control. The overuse of insecticides for the control of WFT has led to the development of resistance to many insecticides. This is due to characteristics of pest, such as rapid developmental time, high fecundity and polyphagous nature, and the difficulties that have been experienced with spraying and also due to the cryptic and thigmotactic behaviour of WFT. The use of natural enemies, including predatory mites and predatory bugs, has proven to be ineffective, because of the cryptic habits of the thrips. Entomopathogenic nematodes (EPNs) have become an option for control, as they have the ability to seek out hosts in enclosed spaces. *Steinernema feltiae* is commercially applied for the control of WFT and other insect pests internationally, and it is more virulent against the soil-dwelling life stages of WFT. However, *S. feltiae* has not been isolated in South Africa and its use is prohibited. Therefore, the need to test locally adapted and more virulent EPN species is necessary. This review of *F. occidentalis* as a pest of undercover production and its management, focuses on biological control by means of EPNs.

Keywords: *Frankliniella occidentalis*, entomopathogenic nematodes, greenhouse

1.1.   INTRODUCTION

The western flower thrips (WTF), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is the primary thrips species encountered in greenhouse production. The pest is extremely polyphagous, feeding on a wide variety of crops grown in both commercial and research greenhouses (Cloyd 2009). WFT causes direct plant damage and is a vector of tospoviruses, like tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV) (Bennison *et al*. 2001). Beginning in the late 1970s, WFT began to spread widely from its native range in Western North America (Kirk & Terry 2003). The exact cause for its spread is still uncertain, but increased global trade in floricultural and horticultural products has been implicated. In the 1970's and 1980's a highly resistant strain originated from California, due to intensive insecticide use in greenhouse crops (Immaraju *et al*. 1992). WFT has now established throughout North America, many European countries, as well as on the Asian, South American, African and Australian continents (Kirk & Terry 2003).

Chemical control has been the most frequently used method for the control of WFT in greenhouses. The high frequency of insecticide applications for WFT control, coupled with the short generation time of *F. occidentalis,* has led to an increasing incidence of insecticide resistance in WFT in recent years (Ebssa *et al*. 2001a). In addition, the cryptic habits of WFT (including their egg-laying in plant tissue, pupation in the soil and leaf litter, feeding on developing tissues in growth tips and inside flowers) which protect them from exposure to contact insecticides and their resistance to many insecticides have become critical limiting factors. Several pest strains of WFT have already developed resistance to most used insecticide classes (Ebssa *et al*. 2004; Gao *et al*. 2012). Biological control has become increasingly important for successful WFT management programmes. The use of other parasites and predators for biological control has shown limited ability to reduce WFT populations, related to their inability to enter tight flower buds, meristem tissues, or narrow flower structures, due to their large body size (Tourtois & Grieshop 2015).

In the process of identifying alternative and biological control measures against WFT, a comprehensive review of current control measures was conducted. Emphasis was placed on the use of entomopathogenic nematodes (EPNs) and on their potential as an environment-friendly control measure against WFT in undercover crop production.

1.2.   IDENTIFICATION

Thrips are minute insects, which are generally overlooked and incorrectly identified (Allsopp 2016). However, positive identification is necessary for effective control. The main species in the order Thysanoptera and the family Thripidae, which are known to be of economic importance on greenhouse crops include western flower thrips, *F. occidentalis*; the eastern flower thrips, *Frankliniella tritici* Fitch; the onion thrips, *Thrips tabaci* Lindeman; the greenhouse thrips *Heliothrips haemorrhoidalis* Bouché; and the banded greenhouse thrips, *Hercinothrips femoralis* Reuter (Greer & Diver 2000). The WFT is, however, the most economically damaging.

Morphological identification is limited to only adult thrips, as the immature stages have no specific characteristics for identification (Karnkowski & Trdan 2002). Just after hatching, both females and males have a pale colouration. After 48 h females develop one of three genetic colour forms, which include pale, dark or intermediate yellow (McDonald *et al.* 2002). The common one, which is the intermediate colour, is yellow with distinctive light brown markings medially, on each abdominal tergite (Cavalleri & Mound 2012). The male remains pale, has a narrow abdomen with a rounded end and is smaller than the female (Karnkowski & Trdan 2002). The head and thorax are usually orange-yellow, with the abdomen being more rounded, and ending in a point. The antennae have eight segments, with the first segment being paler than the second one. The pronotum has two large setae on each posterior and anterior angle. The ocellar setae are situated between the anterior ocellus and each of the posterior ocelli. The main post-ocular setae are much larger and darker than the others are. They have two complete rows of 20-22 setae on the main vein of the fore wing, and 15-17 on the secondary vein (Mound & Kibby 1998).

1.3.   BIOLOGY AND ECOLOGY

Life history is temperature and host-dependent, but it can be quite rapid, allowing multiple generations to occur in a single cropping season (Ishida *et al.* 2003). Development occurs when the temperature exceeds a minimum threshold of 8-10 $^{\circ}$C. At the most favourable temperatures of 25-30 $^{\circ}$C, the egg to adult development time can be as brief as 9-13 days (Reitz 2009). The life cycle of *F. occidentalis* has six developmental stages: the egg, two feeding larval stages, two non-feeding pupal stages and the adult (Lewis 1973; Kanara & Acharya 2014). The female adults have a saw-like ovipositor (Reitz 2009) that enables them to lay eggs in parenchymatous tissues of their host plant, within 72 h after emergence (Ebssa *et al.* 2004). They can deposit the eggs into leaves, petioles, flower bracts and petals, and developing fruit. (Reitz 2009).

The duration of the egg stage is relatively long, with hatching occurring after 2-4 days at optimal temperatures. Kanara & Acharya (2014) found that the duration of first, second and total larval period varied from 1-2 days, 3-6 days and 4-8 days, respectively, and that the first instar is typically about half the duration of the second (Gaum *et al*. 1994; Reitz 2008). Thrips drop to the soil to pupate most of the time, but some remain on the host plants, especially if the hosts have complex floral architecture (Broadbent *et al*. 2003). The pre-pupa and pupa are both immobile stages and total pupal period can be 3-6 days (Kanara & Acharya 2014). Winged adults emerge from the pupal stage in 1-3 days (Fig. 1.1) (Reitz 2009) and shortly after emergence from the soil, the adult WFT feeds on leaves and flowers of the host plant (Ebssa *et al*. 2004). Adults and larvae aggregate in flowers or in other concealed areas on plants, such as the developing fruits, foliage and floral buds. This preference for residing in tightly enclosed and concealed spaces of plants is termed thigmotactic behaviour (Hansen *et al.* 2003). Under controlled laboratory conditions at 28 °C, the adult longevity is relatively long, about 26 days, and can be as long as five weeks, compared with the immature development time of about 12 days (Zhi *et al*. 2005; Reitz 2008).
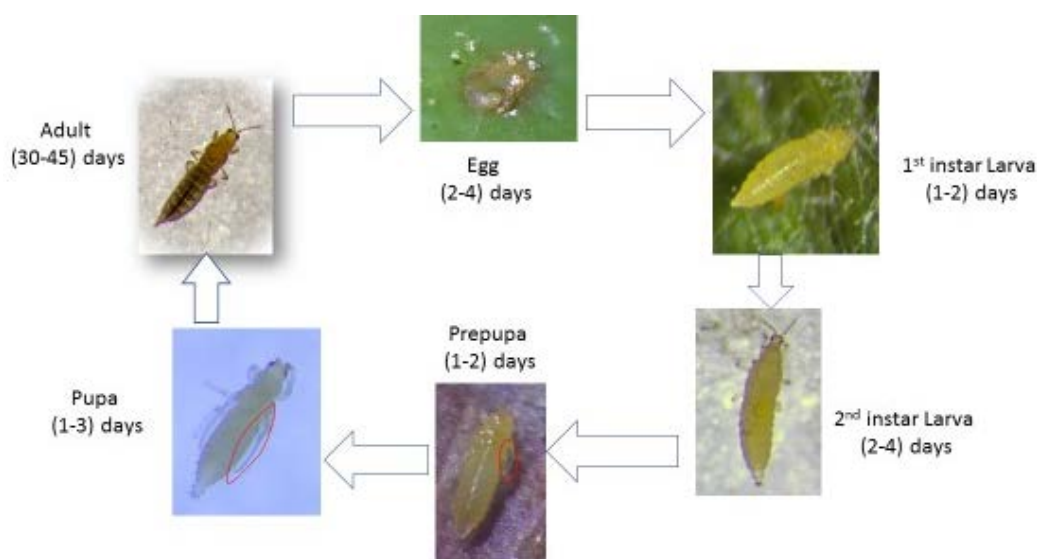
Fig. 1.1. The life cycle of *Frankliniella occidentalis*, western flower thrips. Wing buds longer in pupa than in prepupa (circled). (Photo credits for egg; Elleunorah Allsopp).

Sex determination in WFT is through haplodiploidy. The haploid males are produced from unfertilised eggs, whereas the diploid females are produced from fertilised eggs (*i.e.* by means of arrhenotoky). Although the sex ratios of adults from field samples are often biased towards one sex, mated females do not appear to allocate the sex of their progeny (Terry & Kelly 1993). All studies of reproduction in WFT have reported high fecundity rates for the females (Reitz 2008) and the biases found in the adult sex ratios are likely because of the differences between

the sexes in their dispersal and distribution in response to host quality and longevity (Reitz 2009). After an initial pre-oviposition period, a female can oviposit throughout her lifetime (Reitz 2008). With optimal temperatures and diets, the females can produce up to seven progeny per day, as well as having average total lifetime fecundities exceeding 200 per female. Their high level of fecundity leads to high intrinsic rates of population increase, so uncontrolled populations can multiply rapidly (Gerin *et al*. 1994; Hulshof *et al*. 2003; Reitz 2009).

1.4.   DISTRIBUTION OF WESTERN FLOWER THRIPS

The increased global trade in floricultural and horticultural products contributes greatly to the spread of the *F. occidentalis* (Kirk & Terry 2003). They can also move long distances on wind currents, even though they are weak flyers, but are enabled by the fringed wings (Lewis 1997). Their spread is further enhanced by polyphagy, and by the ability of small founder populations to succeed (Reitz 2009). According to Kirk & Terry (2003), WFT was first recorded in 1969 in Pennsylvania on chrysanthemums in a glasshouse, but it only became established in 1976 and 1977. In South Africa, the WFT was first identified on chrysanthemums near Krugersdorp in 1987, and on roses and chrysanthemums in greenhouses near Cape Town in 1988 (Giliomee 1989). In 1990 it was found in apple (Rosales: Rosaceae) orchards in Grabouw in the Western Cape Province (Badenhorst 1993). An insecticide-resistant strain, which was first recorded in New Zealand in 1992, is thought to be a new arrival, rather than a change in the existing 'lupin strain' (Brødsgaard 1994). It has established itself and spread rapidly worldwide (Kirk & Terry 2003).

1.5.   ECONOMIC IMPACT

The polyphagous nature of WFT increases the number of crops on which it can be transported internationally and which enhances the chances of finding suitable hosts in new areas (Morse & Hoddle 2006). The species is known to feed on over 250 different crop plants, from more than 60 plant families in the USA (Robb 1989; Tommasini & Maini 1995; Lewis 1997). It is a significant pest of virtually all crops, including fruiting and leafy vegetables, ornamentals, trees and small fruits, and cotton (Lewis 1997). In addition, it occurs on many uncultivated plants (Chellemi *et al*. 1994; Paini *et al*. 2007). The high fecundity of females makes it possible for small founder populations to be established, and to grow rapidly. Consequently, some of these populations may readily adapt to new environments, and they may be relatively resistant to the detrimental effects of inbreeding. Also, because of their potentially long adult lifespan, rapid immature development rate, and haplodiploid sex determination, unmated founder females could produce male progeny initially, and survive long enough to

mate with the males, thus making introduced populations consisting of as few as one potentially viable (Immaraju *et al*. 1992; Reitz 2009).

Frankliniella occidentalis damages plants directly by means of feeding and oviposition. Adult and larval feeding causes considerable aesthetic damage to ornamentals and fruit crops (Reitz 2009). Both adults and larvae show preference for feeding on the flowers, but also feeds on leaves and fruits (van Dijken *et al.* 1994; Reitz 2009). The damage varies, depending on crop and growth stage at the time of attack (Greer & Diver 2000). The WFT feed by means of piercing plant cells with their mouthparts and sucking out the contents (plant fluids, pollen, and nectar). They feed on the mesophyll and epidermal cells of leaf tissues, probing and feeding removes surface waxes, epidermal cells collapse and mesophyll cells are destroyed (Hunter *et al.* 1992). Tissues develop a silvery sheen and damaged areas coalesce and wrinkle to form silvered patches and flecking on the expanded leaves and petals (Fig. 1.2), resulting in deformed plant growth and flower deformation (Chisholm & Lewis 1984; van Dijken *et al.* 1994). The WFT also feeds on pollen, which can stimulate oviposition, reduce larval development time, and increase female fecundity (Riley *et al*. 2007). They spread the pollen during feeding, resulting in pollination and premature senescence of flowers (EPPO 2002). Due to their thigmotactic behaviour, feeding damage is often inflicted on developing tissue, which in flowers or fruits goes unnoticed until fruits mature (Steiner & Goodwin 2005; Reitz 2009). The damaged patches are also contaminated with tiny greenish-black faecal specks that are left by the thrips. They also damage the appearance of some ornamentals by means of spreading pollen over the flowers, as they feed on and break open the pollen sac, causing direct yield losses (EPPO 2002; Sanderson 2003).

Fig. 1.2. A) Deformed plant growth damage caused by *Frankliniella occidentalis* (western flower thrips) on blueberries, B) Flower deformation shown by the scarring of chrysanthemums petals.

Oviposition damage occurs when the females insert eggs under the plant epidermis with their saw-like ovipositors, with about a third of the egg protruding (Allsopp 2016). This causes a physiological wound response in some plants that produces the spotting on fruits, which can lead to the downgrading of fruit quality (Reitz 2009). The resultant spots are referred to as 'pansy spots' in the case of most fruits, vegetables like tomato, beans, and peppers and as 'halo spots' in table grapes. Oviposition also causes pitting and dimpling damage in fruit, which happens when the injured tissue around the oviposition site does not develop as rapidly as does the healthy tissue, thus causing the pit, or dimple, which is sometimes surrounded by a pansy spot (Allsopp 2016).

Frankliniella occidentalis also damages plants indirectly by means of transmitting tospoviruses, causing devastating losses in terms of yield and market value (Allen & Broadbent 1986). Of the estimated 7500 thrips species known (Mound 2009), only ten species in the Thripidae family are confirmed vectors of plant viruses, with *F. occidentalis* included (Ullman *et al.* 1997). The WFT is known to vector five tospovirus species; the TSWV, tomato chlorotic spot virus (TCSV), INSV, groundnut ringspot virus (GRSV) and Chrysanthemum stem necrosis virus (CSNV) (Whitfield *et al.* 2005). The symptoms of TSWV vary according to host, cultivar, and stage of plant development (Murphy *et al.* 2014), and may include stunting of the plant, bronzing, distortion, mosaic mottling of leaves, and clearing of leaf veins and fruit (EPPO

7

2002). For INSV, symptoms and susceptibility also vary according to host, with the showing of ring spots and line patterns on the leaves, necrotic lesions, black streaking on the veins and stems, stunting, and death of growing points and crown. Eventually, the plants affected can die (Murphy *et al.* 2014). Over 1000 species of plants in 84 families are susceptible to TSWV, hence it has the broadest host range of any plant pathogen (Parrella *et al*. 2003). The WFT has an intimate, complex relationship with the viruses concerned. For a WFT to transmit TSWV, it must acquire the virus as a larva, primarily as a first instar (Ullman *et al.* 1997). The WFT may acquire TSWV as an adult, but such individuals do not become competent vectors. Second instars are physiologically capable of transmitting the virus, but, as they do not readily move from plant to plant, transmission is essentially restricted to fragile adults (Wijkamp *et al*. 1996).

Not all crops that are damaged by WFT are reproductive hosts for this species. Those that only serve as adult feeding hosts, such as tomato, can still be adversely affected (Brodbeck *et al*. 2001). In many floral and horticultural crops, WFT populations are virtually guaranteed to exceed the low to non-existent damage thresholds (Robb & Parrella 1991). According to Goldbach & Peters (1994), TSWV alone is estimated to cause over $1billion worth of damage in the form of annual losses in the United States. Further complicating the management of WFT is the fact that their feeding damage can be confused with the damage caused by other pests or diseases. Such incorrect diagnoses may result from the small size and cryptic behaviour of the WFT, and by the damage not being immediately recognisable. Unfortunately, misdiagnoses often lead to inappropriate pesticide application (Steiner & Goodwin 2005; Reitz 2009).

1.6.   MANAGEMENT OF WESTERN FLOWER THRIPS

1.6.1. Monitoring

Scouting, or monitoring, is important to determine the level of WFT present in the greenhouse. In addition, scouting can detect seasonal trends in the WFT populations, and assess the effectiveness of the management strategies implemented (Cloyd 2010). Placing either blue or yellow sticky traps above the crop canopy traps adult WFT, hence monitoring population densities. Blue traps are more attractive to WFT than yellow traps (Murphy *et al.* 2014). De Villiers & Pringle (2007) and Allsopp (2010), in their research on table grapes in South Africa, showed that the blue sticky traps hung in the full sun outside the vine canopy were more effective for monitoring WFT in vineyards than traps in the shade or yellow traps in the sun. Blue sticky traps have also shown great efficacy for monitoring *F. occidentalis* in ornamentals, when traps were placed just above the crop canopy (Brødsgaard 1993). The efficacy of traps can also be enhanced by adding semiochemicals like a synthetic aggregation pheromone or

host-plant derived attractant (Broughton & Harrison 2012). Additional methods of monitoring include visual inspection by means of looking into open flowers (Cloyd 2009) and tapping them to determine whether thrips are present, which is done by simply tapping the flowers, or foliage, over a white sheet of paper (Driesche 2013). The assessment of the economic importance of WFT has advanced recently by way of developing a few economic damage thresholds for tomato, pepper, eggplant, cucumber, and strawberry. In crops with a high threat of virus transmission, such as tomato, WFT is not tolerated at all (Mouden *et al.* 2017).

1.6.2. Cultural control

Sanitation practices such as removing weeds, old plant material and growing medium debris, help to reduce the numbers of WFT. Certain weeds, particularly those in the Compositae and Solanaceae families, and those with yellow flowers, that tend to attract WFT adults, and serve as reservoirs for the viruses transmitted by WFT adults, must be removed (Kahn *et al*. 2005; Cloyd 2009). Sanitation at the beginning and end of a cropping season is very effective, as it helps to delay infestation by thrips until another IPM initiative can be implemented (Murphy *et al*. 2014). Manipulation of cropping environment like temperature, day length, light intensity, humidity and crop maintenance could have a huge impact in optimising the effects of beneficial organisms (Jacobson 1997). Manipulation of the cropping environment by increasing relative humidity for four conservative nights on chrysanthemums resulted in good control of *F. occidentalis* with an entomopathogenic fungus (Helyer *et al*. 1992).

1.6.3. Physical control

Screening greenhouse openings such as vents and sidewalls helps to reduce the numbers of WFT entering greenhouses from outside and migrating to other greenhouses. The screen size that is appropriate for WFT is 192 µm (0.037 mm^2) (Bethke *et al*. 1994; Cloyd 2009). WFT incidence was reduced by 20 % with the use of greenhouse window screens in tomato production (Mouden *et al.* 2017). Alternative management strategies may include overhead irrigation or misting, which has been proven to decrease the abundance of WFT populations, by creating an environment that is less favourable for development than it might otherwise have been (Lindquist *et al*. 1987).

The use of ultraviolet (UV) absorbing plastic films, which influence WFT adult flight behaviour, by reducing the levels of UV light entering greenhouses, as well as the use of aluminised reflective fabrics, may inhibit or repel WFT adults from entering (McIntyre *et al*. 1996). Control can also be achieved by means of leaving greenhouses fallow for several months, and by then heating them for four to five days at 30 ºC, together with placing a weed barrier

9

underneath the benches, thus preventing the WFT from entering the soil to pupate (Cloyd 2009). The use of trap or lure crops, consisting of plants and/or flowers that attract the WFT away from the main crop, is another cultural strategy that has been successfully used in the past. Infected lure plants and/or flowers may be sprayed with an insecticide, or inoculated with biological control agents that feed on the nymphal and adult stages residing in the flowers or be totally removed from the greenhouse (Bennison *et al*. 2001).

1.6.4. Chemical control

According to Cloyd (2009), the principal management strategy that is commonly used in dealing with WFT in greenhouses is the use of insecticides. Chemicals can be integrated into an IPM system by using broad-spectrum chemicals, which have minimal effect on beneficial organisms (Jacobson 1997). Common broad-spectrum insecticides used for thrips include pyrethroids, neonicitinoids, organophosphates and carbamates, while narrow spectrum insecticides which are more selective to WFT include pyridalyl and lufenuron. (Mouden *et al.* 2017). Spinosad is a natural substance produced by a soil bacterium (*Saccharopolyspora spinose*) which is used for WFT control, and is not harmful to the natural enemies of WFT (Cloyd 2009). The effect of feeding and oviposition of *F. occidentalis* has also been reduced by use of pyrethrins targeting both the adult and immature stages (Yang *et al.* 2012). Systemic insecticides applied to the growing medium through the irrigation system are more effective, less harmful to beneficial insects (Jacobson 1997) and they penetrate and reside in the leaf tissues, forming a reservoir of active ingredient, providing residual activity, even after the spray residues have dried (Cloyd & Sadof 2003). Systemic insecticides, however, do not move into the flower parts (petals and sepals) where WFT adults normally feed (Cloyd & Sadof 1998). Short persistent insecticides such as dichlorvos are successfully used to control *F. occidentalis* in cucumber and sweet peppers after planting before releasing biological agents (Jacobson 1997).

The key to successful WFT management with insecticides is to initiate applications when the populations are low, to avoid having to deal simultaneously with the different life stages over the course of the crop production cycle. When the WFT populations are already dense, more frequent chemical applications, at three to five day intervals, usually become necessary (Cloyd & Sadof 2003). Frequent chemical applications are required to target all stages of thrips. The above is especially important where overlapping generations prevail. Three to five applications made within a seven to ten-day period might be necessary to obtain sufficient

mortality when the WFT populations are dense, and in the presence of different life stages and/or overlapping generations (Seaton *et al*. 1997; Cloyd 2009).

The heavy reliance on insecticides and frequent applications have led to WFT developing resistance to the active ingredients of most of the insecticide classes (MacDonald 1993; Jacobson 1997; Bielza *et al*. 2008; Gao *et al*. 2012; Mouden *et al.* 2017). The first instance in failing to manage WFT with insecticides was reported in 1961 when the chlorinated cyclodiene, toxaphene, was found to be ineffective in controlling WTF populations (Race 1961). The four main identified mechanisms of insect resistance include metabolic detoxification, reduced penetration of toxicants, alterations of target sites for toxicants and behavioural resistance (Gao *et al*. 2012). Metabolic detoxification is attributed to the polyphagous nature of WFT that made them inherit a great abundance and diverse genes to detoxify the great variation of plant material (Sarmiento 2014). These detoxifying genes mostly code for enzymes and work by converting hydrophobic compounds into less biologically active ones. They belong to the following families Cytochrome P450 monooxygenases (P450s), esterases and glutathione-stransferases (GSTs) (Jensen 2000). In WFT, enhanced detoxification, mediated by cytochrome P-450 mono-oxygenases, is the major mechanism imparting resistance to pyrethroids, organophosphates, and carbamates (Espinosa 2005). The penetration of the toxicant enhances other resistance mechanisms, which were observed in the resistance of *F. occidentalis* to the pyrethroid insecticide fenvalerate (Gao *et al*. 2012). Insensitivity to insecticides is another resistance mechanism, which is due to knock down or mutation of genes or due to change of the target site (Zhao *et al.* 1994). Reduced toxicant penetration of an insecticide through the insect cuticle or gut wall is not considered a powerful resistance mechanism, but can synergize the effect of other resistance mechanisms. For example, a reduced rate of entry of toxicants into the insect's body may enable metabolic detoxification to occur without the enzyme systems of the insect being affected (Jensen 2000). The behavioural resistance system relies on selection of individuals that survive insecticide sprays due to cryptic and thigmotactic behaviour. Population studies on *F. occidentalis* have indicated that this affects their life cycle and thus their strength in being invasive (Hulshof *et al.* 2003).

Another factor that plays a role in the development of resistance is the enclosed greenhouse environment, because it provides constant exposure to insecticides and limits the immigration of susceptible individuals (Reitz 2009). Because of the multiple mechanisms that confer resistance in different populations, resistance could evolve faster and persist in populations for a longer period, over many generations, which would greatly affect the development and viability of insecticide rotation schemes and resistance management programmes (Jensen

11

2000). Measures to delay resistance, such as alternating or rotating insecticides from different mode of action groups, in combination with other compatible approaches to effect WFT control have been researched (Bielza *et al.* 2008).

1.6.5. Biological control

The biological control of WFT has been tested using natural enemies and includes two groups: macrobials, which include predators and parasitoids, and microbials, which are the EPNs (macrobial, artificially classified as a microbial) and entomopathogenic fungi (Mouden *et al*. 2017). Macrobials include predatory mites of the order Arachnida, family Phytoseiidae like *Neoseiulus* (*Amblyseius*) *cucumeris* (Oudemans)*, Iphiseius (Amblyseius) degenerans* (Berlese), *Amblyseius swirskii* (Athias-Henriot), *Stratiolaelaps scimitus* (Womersley), and *Geolaelaps (Hypoaspis) aculeifer* (Canestrini), and the minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Other biological control agents include the entomopathogenic nematode, *Steinernema feltiae* Wouts, Mráček, Gerdin & Bedding, and the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) (Hypocreales: Cordycipitaceae) (Murphy *et al*. 2014).

Predatory mites regulate WFT populations by feeding on the first and/or second instar nymphs, with the exception of *Stratiolaelaps miles* (Berlese) and *Hypoaspis aculeifer* (Canestrini), which are predatory mites that reside either in the soil or in growing medium and feed on the pupal stage (Cloyd 2009). An adult female *N. cucumeris* can consume 1-10 young thrips per day, and has a 30-day lifespan (Greer & Diver 2000). Manners *et al.* (2013) states that the effectiveness of *S. scimitus*, *G. aculeifer*, and *Dalotia coriaria* (Kraatz) as biological control agents is somewhat unclear. On some occasions *S. scimitus* and *G. aculeifer* have been found not to produce noticeable control of WFT, even at high rates of release. However, on chrysanthemums, both *S. scimitus* and *G. aculeifer* reduced the number of adult WFT by about 50% (Bennison *et al*. 2002; Messelink & Van Holstein-Saj 2008).

On mini-roses, *D. coriaria* has been found to consume large numbers of thrips larvae and pupae, with the anecdotal evidence also indicating thrips reductions in commercial rose farms (Carney *et al*. 2002). The use of Black Pearl pepper (*Capsicum annuum* L.) as banker plants is being utilised in certain greenhouses where releases of the minute pirate bug are being implemented. Minute pirate bugs are predaceous anthocorid bugs that feed on the nymphal and adult stages of WFT, and consume pollen from the flowers as a supplemental food source (Cloyd 2009). *Orius insidiosus* is a very efficient predator, because it feeds on all stages of thrips, frequently inhabits the same sites as does thrips, can survive for some time in the absence

of prey, is easy to mass-produce (Silveira *et al.* 2004), and consumes 5-20 thrips per day (Greer & Diver 2000). *Iphiseius degenerans* works well in the case of crops with a pollen source (*e.g.* greenhouse peppers), with it being highly unlikely to be effective in the case of floricultural crops. *Stratiolaelaps scimitus* and *G. aculeifer* feed on thrips pupae, of which they can kill up to 30 %. They can be used in combination with other predators, as their impact alone is insignificant (Murphy *et al*. 2014).

The entomopathogenic fungus, *Beauveria bassiana*, has been preferred as another ecologically and environment-friendly management approach. This approach has been successfully used to manage WFT populations on cut flowers such as roses and carnations, attaining mortalities of 82 %, where the relative humidity was higher and more conducive for the infection of WFT rather than on foliage, where the possibility of desiccation was greater (Murphy *et al*. 1998). Adult WFT seem to be more susceptible to *B. bassiana* than are the nymphs (Cloyd 2009; Messelink & Janssen, 2014), because the adults tend to be located in the flowers, where the relative humidity is higher, and conditions are favourable for infection (Cloyd 2009). Moreover, the nymphs appear to have a thicker cuticle than do the adults (Vestergaard *et al*., 1995), which might delay the penetration of the fungus into the body cavity. The nymphs might also prevent penetration of the fungal spores through the cuticle by means of shedding their own exuvium during ecdysis (Shipp *et al*. 2003). *Beauveria bassiana* granules proved to colonise the soil and were virulent against the soil-dwelling stages of WFT in tomato and cucumber, with a reduction in population of between 75 % and 90 % (Lee *et al.* 2017).

The key to implementing a successful biological control programme is to release the selected natural enemies early enough in the cropping cycle, or as soon as the thrips are detected on the sticky traps (Greer & Diver 2000). Releases must be initiated prior to the WFT entering the terminal or flower buds. The natural enemies cannot regulate an already established or existing high WFT population, because it takes time from the release before the natural enemies are able to reduce the WTF numbers to below damaging levels. Biological control tends to work best on such long-term crops as cut flowers or perennials, more so than it does on crops like bedding plants, which, typically, have short production cycles (from four to six weeks) (Jacobson 1997). Another factor to consider is that biological control agents might not provide sufficient control (based on the percentage of mortality) of the soil-dwelling life stages to make a significant impact on the WTF populations (Ebssa *et al*. 2001a; Cloyd 2009). Combined use of biological control with different arthropods, or with arthropods and entomopathogens, can be useful as an alternative treatment. The timing and compatibility of treatments should, however, be considered carefully (Mouden *et al*. 2017).

1.7.   ENTOMOPATHOGENIC NEMATODES

EPNs are roundworms, occurring naturally in the soil environment, and are obligate parasites of insects that locate their hosts via their carbon dioxide secretions, vibration, and other chemical cues (Kaya & Gaugler 1993). Species in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological agents in pest management programmes (Grewal *et al*. 1994). These parasites of insects kill their hosts with the aid of mutualistic bacteria carried in the nematode's alimentary canal, with steinernematids carrying *Xenorhabdus* species, whereas heterorhabditids carry *Photorhabdus* species (Shapiro-Ilan *et al*. 2006). EPNs fit well into integrated pest management (IPM) programmes, because they are considered non-toxic to humans, and relatively specific to their target pest(s), while they can also be applied with standard pesticide equipment (Shapiro-Ilan *et al*. 2006).

1.7.1. Life cycle

Nematodes have a simple life cycle that includes egg stage, four juvenile stages, and adult stage (Ebssa *et al*. 2004). The only free-living stage, which is the fourth juvenile stage, is often called the dauer or the infective juvenile (IJ), living in the soil, whereas all other stages live in the body of an insect host (Stock 2015). The IJ penetrates the host insect through natural openings, like the spiracles, the mouth, or the anus, or, in some species, through the intersegmental membranes of the cuticle, whereupon it enters the haemocoel (Bedding & Molyneux 1982). The IJ releases cells of the symbiotic bacteria from its intestines into the haemocoel. The bacteria multiply in the insect haemolymph, causing the death of the infected host within 24-48 hours (Stock 2015). After the death of the host, the nematode continues to feed on the host tissue, maturing and reproducing. Depending on the available resources, one or more generations might occur within the host cadaver, with a large number of the IJs eventually being released into the environment to infect other hosts, where they continue their life cycle (Kaya & Gaugler 1993).

1.7.2. Distribution

EPNs are widespread in soils all over the world, except in the North and South Pole (Shelmith 2009). According to Abd-Elgawad (2017), studies have been published concerning EPN distribution in Africa, North and South America, Australia, Asia, and Europe. Europe is the most extensively studied continent for EPN occurrence. The first record of EPNs in South Africa was from the maize beetle, *Heteronychus arator* (Fabricius) (*Heteronychus sanctae-helenae* Blanch), in Grahamstown, Eastern Cape Province (Harington 1953).

14

Eleven EPN species (consisting of four heterorhabditids and seven steinernematids) have been found in South Africa, of which seven are endemic (Malan & Ferreira 2017). Recent surveys recovered four isolates of *Steinernema* spp. and 31 isolates of *Heterorhabditis* spp. during studies on the biological control of the false codling moth (Malan *et al*. 2011; Steyn *et al*., 2017). The *Steinernema* spp. included *Steinernema khoisanae* Nguyen, Malan and Gozel, found in the Western Cape, and *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler and Adams, found in Mpumalanga, in relation to which a first report was made in South Africa, and a third report was made for the African continent. Two new species were reported, namely *Steinernema citrae* Stokwe, Malan, Nguyen, Knoetze and Tiedt, found in the Western Cape, and *Steinernema jeffreyense* Malan, Nguyen, Knoetze and Tiedt, in the Eastern Cape. The *Heterorhabditis* species isolated were *H. bacteriophora*, found in the Eastern and Western Cape Provinces, KwaZulu-Natal, and Mpumalanga, and the most dominant species *Heterorhabditis zealandica* Poinar, in the Eastern, Northern and Western Cape, as well as in the North West and Mpumalanga provinces. *Heterorhabditis noenieputensis* Malan, Knoetze and Tiedt was found on a garden fig in the settlement Noenieput in the Northern Cape Province (Malan & Ferreira 2017). Within these EPN species, four symbiotic bacterial species have been described from South Africa (Malan & Ferreira 2017).

1.7.3. Regulation and registration

The amended Act 18 of 1989 (South African Agricultural Pests Act, No. 36 of 1947) states that the introduction of exotic animals, including non-endemic EPN species, is only allowed under permit, together with a full impact study. With only *H. bacteriophora* currently having a permit for importation for research purposes (Hatting *et al*. 2018), no other nematode is allowed to be imported into South Africa. No registration is required for EPNs in many countries, hence their introduction, release and commercialisation varies from country to country. The Organization for Economic Cooperation and Development (OECD) and the European Cooperation in Science and Technology (COST) concluded that EPNs should be treated as macro-organisms, due to them being multicellular and indigenous. Although local or indigenous EPN species should not be regulated, exotic EPNs should be (Ehlers & Hokkanen 1996; Grewal *et al*. 1994).

1.7.4. Biological control potential

The fact that the soil environment is the natural habitat for EPNs offers great potential for successful biocontrol applications using these organisms (Klein 1990; Shapiro-Ilan *et al*. 2002). EPNs are characterised by their association with symbiotic bacteria to facilitate pathogenesis,

which enables them to rapidly kill their hosts, usually within a few days after infection (Kaya & Gaugler 1993). EPNs within the genera *Heterorhabditis* and *Steinernema* are the most extensively studied, and they are most often used in biological control (Kaya & Gaugler 1993; Zhang *et al.* 2008). EPNs are highly pathogenic, and are used as biological control agents of numerous insect pests. They have been commercialised for use in a range of environments, stretching from large-scale agriculture to individual home gardens on several continents (Lu *et al.* 2016).

To achieve successful applications in the soil environment, a variety of abiotic and biotic factors must be considered (Kaya 1990). According to Lu *et al.* (2016), traits important for biological control can be grouped into three main categories: infectivity, persistence and storage stability (Burnell & Dowds 1996). Another important factor to consider is whether the nematode can be easily cultured in industrial fermenters (Bedding *et al.* 1993). Infectivity refers to the characteristics that are involved in finding, infecting, and killing a target host. For EPNs to be effective in terms of biological control, they must be able to find, and kill, the appropriate insect hosts. Thus, attempts to increase and to modify host-seeking behaviour have been popular. Host-seeking has been shown to be a highly heritable trait, which can be enhanced through selective breeding for such species as *S. feltiae* (Gaugler *et al.* 1989) and *Steinernema carpocapsae* (Weiser) Wouts, Mráček, Gerdin & Bedding (Gaugler *et al.* 1990, 1991). Research to enhance host-seeking traits has relied solely on selective breeding, with the genes that are implicated in the processes being unknown (Koppenhofer *et al.* 1997).

Persistence refers to traits that increase survival rates after application in the field, such as tolerance of varying temperature, desiccation, and UV radiation. Desiccation tolerance is important for EPN persistence and production. EPN species that forage near the soil surface tend to have improved desiccation tolerance (Koppenhofer *et al.* 1997). Desiccation can induce EPN quiescence, which leads to the lengthening of the shelf life, which might contribute to their longevity in the soil (Koppenhofer *et al.* 1997). EPNs require adequate soil moisture for survival and movement, which may vary among nematode species and isolates and among different soil types. Low soil moisture levels can be lethal to EPNs with some developing survival strategies under water stress conditions, by reducing the body surface area exposed to the air and slowing their cell metabolism (anhydrobiosis), which can be reversed when the soil becomes wet again. High moisture levels might cause oxygen deprivation and restrict mobility of EPNs. Optimum moisture levels tend to vary by nematode species and soil type. (Koppenhöfer *et al.* 1997). The soil type affects nematode movement and survival rates (Kaya 1990). Generally, compared with lighter soils, soils with higher clay content tend to restrict nematode movement, and to have

potential for reduced aeration, which can result in reduced nematode survival and efficacy rates. However, exceptions to the trend have been observed (Shapiro-Ilan *et al.* 2006). The soil pH in most agroecosystems, having a range of 4-8, is not likely to affect EPNs significantly, but a pH of 10 or higher is likely to be detrimental (Kaya 1990).

Optimum temperatures for infection and reproduction vary among nematode species and strains. (Kaya 1990; Grewal *et al.* 1994; Wright *et al.* 2005). Extreme temperatures of 0 and 40 °C are lethal to EPNs and temperatures below 10-15 °C can restrict their mobility, while temperatures higher than 30-40 °C can inactivate them (Bedding *et al.* 1993; Grewal *et al.* 1994). Some species and isolates are better adapted to heat. For example, *Heterorhabditis indica* Poinar, Karunakar & David, *S. glaseri* (Ssteiner) Wouts, Mráček, Gerdin & Bedding and *S. riobrave* Cabanillas, Poinar & Raulston are relatively heat-tolerant and they can maintain efficacy at temperatures of 29 °C and above, whereas others, like *H. megidis* Pionar, Jackson & Klein*, S. feltiae*, and *Heterorhabditis marelatus* Liu & Berry, are more cold-tolerant, maintaining efficacy at 15 °C and below (Shapiro-Ilan *et al.* 2006). As ultraviolet radiation is detrimental to nematodes, it is best to apply nematodes to the soil surface in the evening or early morning hours. Alternatively, efficacy levels can be improved, and exposure to ultraviolet radiation avoided, through subsurface application, although the advantages of such approaches have not been seen in all studies (Wilson & Gaugler 2004).

The storage stability of EPNs involves traits that increase the shelf life necessary for the distribution of EPNs. Such stability is essential for EPN longevity in the soil, and for EPN commercial production and distribution. Artificial selection and hybridisation can enhance desiccation tolerance (Salame *et al.* 2010), but the removal of selection pressure ultimately results in the loss of the desired traits (Nimkingrat *et al.* 2013a, b).

Improving the above-mentioned traits, as well as many others, has the potential, ultimately, to increase their field efficacy. Although EPNs have been used in biological control, improvement in their use is needed to realise their full potential for broader application in agriculture. No matter how well-suited an EPN is to a target pest, the application will fail if the agent is not delivered in a manner that enables access to, and infection of, the host. The effective and efficient delivery of EPNs can only be achieved with careful consideration of the available application technology, coupled with an understanding of the attributes and limitations of the biocontrol agent (Lu *et al.* 2016).

1.8. ENTOMOPATHGENIC NEMATODES TO CONTROL WESTERN FLOWER THRIPS

1.8.1. Efficacy of EPN species

EPN species vary in their virulence against different host insects (Mason & Wright 1997). Moreover, the efficacy of EPN species varies, among others, in terms of concentration, host density, and temperature (Zervos *et al.* 1991). Different strains of the same EPN species differ in their pathogenicity to different insect species (Ebssa *et al.* 2004). Ebssa *et al.* (2004) tested six strains of *Heterorhabditis* and 11 strains of *Steinernema* against different soil-dwelling stages of WFT at 200 IJs/cm^2 and found that the EPN species varied greatly in terms of efficacy against WFT, with mortality ranging between 3 % and 60 %. Mean mortality values < 50 % were recorded for 67 % and 43 % of the tested *Steinernema* and *Heterorhabditis* species, respectively. The commercial product, Nemaplus$^®$, of which the active ingredient is *S. feltiae* and the hybrid, *H. bacteriophora* strain PS8, were found to be among the least effective strains. Except for *S. feltiae* and *S. carpocapsae* strain A1 B5, WFT mortality with all tested EPN species was found to be significantly higher than control mortality, which varied from 3.3 % to 12.5 %, while twenty-five percent of the tested species resulted in ≥ 50 % mortality. A strain of *S. carpocapsae* from Egypt caused significantly higher mortality in WFT than a strain of *S. carpocapsae* from Italy (Hay & Richardson 1995). *Heterorhabditis* species are more effective against *F. occidentalis* than *Steinernema* species (Chyzik *et al.* 1996; Premachandra *et al.* 2003). Ebssa *et al.* (2004) also proved that nematodes from the genus *Heterorhabditis* were more effective (76 %), whereas the genus *Steinernema* was less effective (37 %). Other laboratory trials that were conducted with 100 strains of *S. feltiae* against WFT soil stages yielded varying results between strains, with mortality ranging between 3.7 % and 72.6 %. *Heterorhabditis* and *Steinernema* spp. applied against the mixed life stages of WFT recorded mortality between 2.6 and 60 % at a concentration of 200 IJs/cm^2, with the *Heterorhabditis* spp. being more virulent (Arthurs & Heinz 2006). Post application persistence of *H. bacteriophora* strain HK3 and *S. carpocapsae* strain DD136, applied at 200 and 400 IJs/cm^2 against late second instar larvae of *F. occidentalis*, resulted in *H. bacteriophora* giving higher thrips mortality of up to 76 % compared to 37.8 % for *S. carpocapsae,* even though both persisted for at least 6 days (Belay *et al.* 2005).

1.8.2. Susceptibility of life stages

Previous studies have shown that the soil-dwelling pupal stage of WFT is highly susceptible to several EPNs, particularly *S. feltiae* (Chyzik *et al.* 1996; Ebssa *et al.* 2001a; Premachandra

et al. 2003; Pundt 2011). Results of research over the last two decades have also shown a certain biological potential of EPNs against foliar (above-ground) insect pests, but only under specific conditions (Arthurs *et al*. 2004). The relatively lower efficiency of EPNs against foliar pests is, above all, the consequence of the exposure of the nematodes to unsuitable (low) moisture levels (Lello *et al*. 1996), extreme temperatures (Grewal *et al.* 1994), or high ultraviolet radiation (Gaugler *et al*. 1992). The above-mentioned factors are important for the survival of nematodes (Gaugler 2002). They are the main reasons that nematodes are not so efficient against foliar pests, although previously conducted laboratory tests have shown higher efficiency levels (Berry & Lewis 1993). The pathogenicity of six species of EPNs in terms of controlling *F. occidentalis* was studied under laboratory conditions. The nematodes included in the experiment were *H. bacteriophora* 'HK3', *H. bacteriophora* 'HB Brecan', *S. feltiae* 'Sylt', *S. feltiae* 'OBSIII', *S. feltiae* 'CR', and *S. carpocapsae* (Weiser) 'DD136'. All species were highly effective against soil stages of the pest, with the most effective being *S. feltiae* Sylt, *S. carpocapsae* 'DD136', and *H. bacteriophora* 'HK3'. The nematode *S. feltiae* 'OBSIII' was the most virulent against the second instar larvae and prepupae in the soil, at average moisture levels, while the effectiveness of the agents was a good deal less in dry soil (Ebssa *et al*. 2001a).

1.8.3. Effect of EPN concentration

Ebssa *et al.* (2004) also noted that the effect of increasing EPN concentrations for the control of WFT depends on the type of EPN strain used. The WFT mortality caused by *H. bacteriophora* PAL H04 and *Steinernema abbasi* Elawad, Ahamad & Reid PAL S09 at 100 IJs/cm^2 was not significantly different from that caused in the water-treated control. For all strains, a concentration of 150 IJs/cm^2 did not significantly increase WFT mortality compared to that obtained with a concentration of 100 IJs/cm^2. Similarly, even though highest mortality was recorded at 1000 IJs/cm^2, the values did not differ from the mortality obtained at 400 IJs/cm^2, except with *H. bacteriophora* PAL H04 and *Steinernema bicornutum* Tallosi, Peters & Ehlers. Conclusively, WFT mortality increased with increasing concentrations, although the degree of increment differed significantly among the species/strains involved. The three *Heterorhabditis* spp. (H. *indica* strains LN2, LN10 and *H. bacteriophora*) had significantly greater slopes than did the two *Steinernema* spp. (*S. abbasi* (PAL S09) and *S. bicornutum*), indicating that the former responded more strongly to the increase in concentration.

The activity of different species of EPNs against the juveniles of *F. occidentalis* was studied under laboratory conditions, including in Slovenia (Perme 2005). This experiment included the nematodes *H. bacteriophora, H. megidis*, *S. carpocapsae*, and *S. feltiae*. Their

activity was studied at three different suspension concentrations (500, 1000, and 5000 IJs/ml). At the highest concentration, the most effective were *H. bacteriophora* (92 % mortality) and *H. megidis* (71 % mortality), whereas, at the lower concentration, *S. carpocapsae* (90 %) and *S. feltiae* (82 %) were most effective. The author confirmed that the activity of the nematodes depends more upon temperature than it does upon concentration, since all four species of nematodes were more effective at 25 °C than at lower temperatures. The species from the genus *Steinernema* showed high enough effectiveness at lower concentrations, making them suitable biological agents for controlling the larvae of *F. occidentalis,* also due to the comparatively lower cost of their use (Laznik & Trdan 2008).

The efficiency of three EPN species (*Steinernema riobravis* Cabanillas, Poinar and Raulston, *S. feltiae* and *H. bacteriophora*) against the prepupae and pupae of WFT was studied in Israel (Chyzik *et al.* 1996). The highest mortality for thrips was shown by *H. bacteriophora* (36-49 %), whereas the two *Steinernema* species were less effective (± 10 %). At higher concentrations of the *H. bacteriophora* suspension (10 000 IJs/ml), the mortality of *F. occidentalis* was only slightly higher (42-73 %) than at a lower concentration (500 IJs/ml) where mortality was between 35 and 50 % (Chyzik *et al.* 1996).

1.8.4. Abiotic conditions

The use of EPNs (Rhabditida: Steinernematidae and Heterorhabditidae) for controlling thrips has gained importance in some European countries (Kaya *et al.* 2006). A study conducted by Kung *et al.* (1990) on the effects of soil temperature, moisture and relative humidity on EPN persistence, showed that *S. carpocapsae* was persistent at low temperatures of 5-25 °C, whereas they were only poorly persistent at a temperature of 35 °C. *Steinernema glaseri*, in contrast, as a tropical/ subtropical nematode, had high persistence at high temperatures (15-35 °C) and low persistence at the lowest temperature, 5 °C. Both *S. carpocapsae* and *S. glaseri* survived best at low soil moistures of 2 % and 4 %. The survival and pathogenicity of *S. carpocapsae* and *S. glaseri* decreased as the relative humidity decreased, with *S. glaseri* being more susceptible and persistent to low relative humidity in comparison to *S. carpocapsae*.

The parasitic nematode *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae) was investigated against *F. occidentalis* infesting greenhouse floricultural crops. At constant temperatures, *T. nicklewoodi* infected WFT over the range of 1–30 °C, and at optimum temperature of approximately 20 °C, there was 80 % infection. Conclusions were drawn that daytime temperature fluctuations in greenhouses would permit the establishment of *T. nicklewoodi* (Arthurs *et al.* 2003). Ebssa *et al.* (2004) assessed *S. bicornutum* and *H. indica*

under different moisture conditions of between 67 % and 99 % RH against the soil-dwelling stages of WFT at concentrations of 100 and 400 IJs/cm^2 and the results indicated that increasing moisture content improved efficacy of *H. indica* and *S. bicornutum* at both concentrations.

1.8.5. Above- and below-ground application

Studies from several field trials on the use of EPNs for the control of insects in above-ground habitats showed lower efficacies. The main limiting factor is attributed to the rapid desiccation of the IJs (Lacey & Georgis 2012; Shapiro-Ilan *et al*. 2012), but, when wetting agents are used, the efficacy improved (Lacey & Georgis 2012). According to Buitenhuis & Shipp (2005), the efficacy of foliar applications under greenhouse conditions was low, even when twice the recommended label rates of *S. feltiae* were used (2×10^4 to 4×10^4 IJs/µl). Mortality of < 40 % was recorded in potted chrysanthemum (*Dendranthema grandiflora*) against WFT nymphal stages and adults. Weekly applications of EPNs under greenhouse conditions in a study done by Ebssa *et al*. (2006) recorded a low WFT mortality of 53 %. However, it is inconclusive whether such mortality was caused by the EPNs, or by the water used for spraying, which washed away the WFT from the plants causing them physical injury. Foliar applications of *S. feltiae*, with the aid of a wetting agent, have also been shown to control WFT adults and larvae successfully in chrysanthemum (Buitenhuis & Shipp 2005; Arthurs & Heinz 2006). The quantitative data, on the efficacy of foliar applications under greenhouse conditions, were found to be minimal, even with using rates that were twice the recommended label rate (20 000-40 000 IJs/ ml) on potted chrysanthemum (*D. grandiflora*). A low percentage of mortality (< 40 %) against the nymphal and adult stages of WFT was recorded in terms of the above (Buitenhuis & Shipp 2005). EPN application for the control of insects in soil surface habitats has proven to be a success, and because thrips spend one-third of their life as pupae in the soil, they are relatively susceptible (Mouden *et al.* 2017).

1.8.6. Commercial application

Commercial interest has been shown in EPNs, due to the advances that have been made in mass production and formulation, and because of the efficacy that EPNs have shown in controlling pests. Currently, EPNs are produced and marketed for commercial use for greenhouse production in European countries and in the USA. The common EPN species that has been commercialised is *S. feltiae* (Cloyd 2015). *Steinernema feltiae* has been evaluated both for soil applications targeting the pupal stages that are more susceptible to attack, and for foliar applications that target the nymphs and adults that are hidden in the cryptic habitats. *Steinernema feltiae*, sold under the trade name ENTONEM® by Koppert, is recommended for

control of larvae of sciarid flies (Sciaridae), WFT and leafminers, to be applied at weekly intervals as a soil application at a concentration of 50 IJs/cm^2 or as a leaf application at 25 IJ/cm^2. Another commercial product of S. *feltiae* is NemaTrident$^{®}$F by Bionema, applied at weekly intervals as soil and foliar applications at a concentration of 125 IJs/cm^2. Other trade names for the same product used against WFT are NemaShield$^{®}$, Nemasys$^{®}$ and Scanmask$^{®}$ (Pundt 2011).

As *S. feltiae* has not yet been isolated in South Africa, its importation and use is restricted, due to government regulations that are imposed on exotic organisms. The commercialisation of EPNs in South Africa is still under investigation, in terms of the mass rearing of the local EPN species, which are adapted to our environments, especially in the case of *S. yirgalemense*, which has been effective in controlling a number of insect pests.

1.9. CONCLUSION

The WFT is an important agricultural pest in undercover production of many fresh products. WFT severely damages ornamentals and vegetables, especially in greenhouses and shade houses, with preference exhibited to feeding on flowers. They cause direct plant damage through oviposition and feeding, and indirectly by means of transmitting tospoviruses, thus causing huge losses in terms of yields and/or market value. To improve management of WFT, it is important to identify thrips correctly. WFT has previously been easily confused with other species, due to their small size, and considering the > 7500 species of thrips that have been identified to date. Biological control has become increasingly important to render WFT management programmes successful, as chemical control is difficult, and several pest strains have developed resistance to many different insecticides. The efficacy of other parasites and predators for biological control is limited, because they are hindered in entering tight flower buds, meristematic tissues, or narrow flower structures. EPNs have become an option in the natural enemy pool of WFT, because they are able to actively seek out the insect host in cryptic habitats. Previous studies on the effects of EPNs on WFT have concentrated on the use of the commercial *S. feltiae* product ENTONEM$^{®}$ devised by Koppert, which has shown tremendous efficacy in European countries. However, *S. feltiae* has not been isolated in South Africa, hence its importation is prohibited. Moreover, it is adapted to cooler climates, making it unsuitable for use in local high temperature undercover conditions. If EPNs are to be used for WFT management in undercover crop production in South Africa, locally isolated EPNs that are well adapted to our environment must be evaluated for their efficacy against various life stages of WFT.

1.10.   AIM OF THE STUDY

The main aim of the current study was to investigate the potential use of indigenous EPNs for the control of WFT under laboratory and greenhouse conditions. The objectives of the study were the following:

1. To study the development and survival rate of *Frankliniella occidentalis* on two host plants, as well as its biology under laboratory conditions, to identify life stages that could be targeted with EPNs.
2. To determine the efficacy of the local species of EPNs to control the different life stages of WFT, and the optimum nematode concentrations required for the suppression of WFT under laboratory conditions.
3. To determine the effect of different concentrations of locally isolated *Steinernema yirgalemense* on the efficacy against *F. occidentalis* in a commercial blueberry greenhouse.

The chapters of this study have been written as separate publishable papers, and, for this reason, some repetition, in the different chapters, has been unavoidable. The chapters are written according to the format of the journal *African Entomology*.

1.11   REFERENCES

ABD-ELGAWAD, M.M.M, ASKARY, T.H. & COUPLAND, J. 2017. Biocontrol agents: Entomopathogenic and slug parasitic nematodes. CABI, Wallington, UK pp. 409–428.

ALLEN, W.R., & BROADBENT, A.B. 1986. Transmission of *tomato spotted wilt virus* in Ontario greenhouses by *Frankliniella occidentalis. Canadian Journal of Plant Pathology* **8**: 33–38. DOI: https://doi.org/10.1080/07060668609501838

ALLSOPP, E. 2010. Seasonal occurrence of western flower thrips, *Frankliniella occidentalis* (Pergande), on table grapes in the Hex River Valley, South Africa. *South African Journal of Enology and Viticulture* **31**: 49–57. DOI: http://dx.doi.org/10.21548/31-1-1400

ALLSOPP, E. 2016. Developing an integrated management system for western flower thrips *Frankliniella occidentalis* (Pergande) on deciduous fruit, using semiochemicals in a push pull strategy. Ph.D. thesis, Stellenbosch University, Stellenbosch, South Africa. DOI: http://hdl.handle.net/10019.1/100432

ARTHURS, S., HEINZ, K.M., THOMPSON, S. & KRAUTER, P.C. 2003. Effect of temperature on infection, development and reproduction of the parasitic nematode *Thripinema nicklewoodi* in *Frankliniella occidentalis*. *BioControl* **48**: 417–429. DOI: 10.1023/A:1024798928406

ARTHURS, S., HEINZ, K.M., & PRASIFKA, J.R. 2004. An analysis of using entomopathogenic nematodes against above-ground pests. *Bulletin of Entomological Research* **94**: 297–306. DOI: 10.1079/BER2003309

ARTHURS, S. & HEINZ, K.M. 2006. Evaluation of the nematodes *Steinernema feltiae* and *Thripinema nicklewoodi* as biological control agents of western flower thrips *Frankliniella occidentalis* infesting chrysanthemum. *Biocontrol Science and Technology* **16**: 141–155. DOI: https://doi.org/10.1080/09583150500258545

BADENHORST, J. 1993. Californian thrips, *Frankliniella occidentalis*, on apples. *Deciduous Fruit Growers* **43**: 204–205.

BEDDING, R.A., & MOLYNEUX, A.S. 1982. Penetration of insect cuticle juvenile of *Heterorhabditis* spp. (Heterorhabditidae: Nematode). *Nematologica* **28**: 354–359. DOI: 10.1163/187529282X00402

BEDDING, R., AKHURST, R. & KAYA, H. 1993. Nematodes and the biological control of insect pests. CSIRO Publishers, Melbourne, Australia. DOI: https://doi.org/10.1093/ee/24.1.164

BELAY, D., EBSSA, L. & BORGEMEISTER, C. 2005. Time and frequency of applications of entomopathogenic nematodes and their persistence for control of western flower thrips *Frankliniella occidentalis*. *Nematology* **7**: 611–622. DOI: 10.1163/156854105774384822

BENNISON, J., MAULDEN, K., DEWHIRST, S., POW, E., SLATTER, P. & WADHAMS, L. 2001. Towards the development of a push-pull strategy for improving biological control of western flower thrips on chrysanthemum, In: Proceedings of the 7th International Symposium on Thysanoptera. July 2–7, 2001. Reggio Calabria, Italy. 199–206.

BENNISON, J., MAULDEN, K. & MAHER, H. 2002. Choice of predatory mites for biological control of ground-dwelling stages of western flower thrips within a 'push-pull' strategy on pot chrysanthemum. *Bulletin OILB/SROP* **25**: 9–12.

BERRY, E.C. & LEWIS, L.C. 1993. Interactions between nematodes and earthworms: enhanced dispersal of *Steinernema carpocapsae*. *Journal of Nematology* **25**: 189–192.

BETHKE, J.A., REDAK, R.A. & PAINE, T.D. 1994. Screens deny specific pests entry to greenhouses. *California Agriculture* **48**: 37–40.

BIELZA, P., QUINTO, V., GRÁVALOS, C., ABELLÁN, J. & FERNÁNDEZ, E. 2008. Lack of fitness costs of insecticide resistance in the western flower thrips (Thysanoptera: Thripidae). *Journal of Economic Entomology* **101**: 499–503. DOI: https://doi.org/10.1093/jee/101.2.499

BROADBENT, A.B., RHAINDS, M., SHIPP, L., MURPHY, G. & WAINMAN, L. 2003. Pupation behaviour of western flower thrips (Thysanoptera: Thripidae) on potted chrysanthemum. *Canadian Entomology* **135**: 741–744. DOI: https://doi-org.ez.sun.ac.za/10.4039/n03-007

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 Applicata* **99**: 165–172.

BRØDSGAARD, H.F. 1993. Coloured sticky traps for thrips (Thysanoptera: Thripidae) monitoring on glasshouse cucumbers. *International organisation of Biological Control/West Palaearctic Regional Section Bulletin* **16**:19–22.

BRØDSGAARD, H.F. 1994. Insecticide resistance in European and African strains of western flower thrips (Thysanoptera: Thripidae) tested in a new residue-on-glass test. *Journal of Economic Entomology* **87**: 1141–1146.

BROUGHTON, S. & HARRISON, J. 2012. Evaluation of monitoring methods for thrips and the effect of trap colour and semiochemicals on sticky trap capture of thrips (Thysanoptera) and beneficial insects (Syrphidae, Hemerobiidae) in deciduous fruit trees in Western Australia. *Crop Protection* **42**: 156–163. https://doi.org/10.1016/j.cropro.2012.05.004

BUITENHUIS, R. & SHIPP, J.L. 2005. Efficacy of entomopathogenic nematode *Steinernema feltiae* (*Rhabditida*: *Steinernematidae*) as influenced by *Frankliniella occidentalis* (*Thysanoptera*: *Thripidae*) developmental stage and host plant stage. *Journal of*

25

Economic Entomology **98**: 1480–1485. DOI: https://doi.org/10.1603/0022-0493-98.5.1480

BURNELL, A.M. & DOWDS, B.C. 1996. The genetic improvement of entomopathogenic nematodes and their symbiont bacteria: phenotypic targets, genetic limitations and an assessment of possible hazards. *Biocontrol Science and Technology* **6**: 435–447. DOI: https://doi.org/10.1080/09583159631406

CARNEY, V.A., DIAMOND, J.C., MURPHY, G.D. & MARSHALL, D. 2002. The potential of *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae), as a biological control agent for use in greenhouse crops. *Bulletin OILB/SROP* **25**: 37–40.

CAVALLERI, A. & MOUND, L.A. 2012. Toward the identification of *Frankliniella* species in Brazil (Thysanoptera, Thripidae). *Zootaxa* **3270**: 1–30.

CHELLEMI, D.O., FUNDERBURK, J.E. & HALL, D.W. 1994. Seasonal abundance of flower-inhabiting *Frankliniella* species (Thysanoptera, Thripidae) on wild plant species. *Environmental Entomology* **23**: 337–342. DOI: https://doi.org/10.1093/ee/23.2.337

CHISHOLM, I.F. & LEWIS, T. 1984. A new look at thrips (Thysanoptera) mouthparts, their action and effects of feeding on plant tissue. *Bulletin of entomological research* **74**: 663–675. DOI: https://doi.org/10.1017/S0007485300014048

CHYZIK, R., GLAZER, J. & KLEIN, M. 1996. Virulence and efficacy of different entomopathogenic nematode species against western flower thrips (*Frankliniella occidentalis*). *Phytoparasitica* **24**: 103–110.

CLOYD, R.A., & SADOF, C.S. 1998. Flower quality, flower number, and western flower thrips density on Transvaal daisy treated with granular insecticides. *HortTechnology* **8**: 567–570.

CLOYD, R.A. & SADOLF, C.S. 2003. Seasonal abundance and the use of an action threshold for western flower thrips, in a cut carnation greenhouse. Horticulture Technology 13: 497–500.

CLOYD, R.A. 2009. Western flower thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: have we reached an impasse? *Pest Technology* **3**: 1–9.

CLOYD, R.A. 2010. Western flower thrips: management on greenhouse-grown crops. http://www.ksre.ksu.edu. 07.05.2017.

CLOYD, R.A. 2015. Western flower thrips management in greenhouse production systems in the 21st century: alternative strategies need to be considered. *Acta Horticulture* **1104**: 381–393. DOI: 10.17660/ActaHortic.2015.1104.57

DE VILLIERS, M. & PRINGLE, K.L. 2007. Seasonal occurrence of vine pests in commercially treated vineyards in the Hex River Valley in the Western Cape Province, South Africa. *African Entomology* **15**: 241–260. DOI: https://doi.org/10.4001/1021-3589-15.2.241

DRIESCHE, R. 2013. Biological control of western flower thrips. http://biocontrol.ucr.edu/. 20.06.2017.

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & POEHLING, H.M. 2001a. Efficacy of entomopathogenic nematodes against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Invertebrate Pathology* **78**: 119–127. DOI: 10.1006/jipa.2001.5051

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & POEHLING, H.M. 2001b. Impact of entomopathogenic nematodes on different soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the laboratory and under semi-field conditions. *Biocontrol Science Technology* **11**: 515–525. DOI: https://doi.org/10.1080/09583150120067544

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & 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. DOI: https://doi.org/10.1016/S1049-9644(03)00132-4

EBSSA, L., BORGEMEISTER, C. & POEHLING, H.M. 2006. Simultaneous application of entomopathogenic nematodes and predatory mites to control western flower thrips *Frankliniella occidentalis*. *Biological Control* **39**: 66–74.

EHLERS, R.U., HOKKANEN, H.M. 1996. Insect biocontrol with non-endemic entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.): Conclusions and recommendations of a 631 combined OECD and COST workshop on scientific and

regulatory policy issues. *Biocontrol Science and Technology* **6**: 295–302. DOI: https://doi.org/10.1080/09583159631280

ESPINOSA, P. 2005. Metabolic mechanisms of insecticide resistance in the western flower thrips, *Frankliniella occidentalis* (Pergande). *Pest Management Science* **61**: 1009–1015. DOI: https://doi.org/10.1002/ps.1069

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANISATION (EPPO). 2002. Diagnostic protocols for regulated pests: *Frankliniella occidentalis. Bulletin* **32**: 241–243.

GAO, Y., LEI, Z. & REITZ, S.R., 2012. Western flower thrips resistance to insecticides: detection, mechanisms and management strategies. *Pest Management Science* **68**(8): 1111–1121. DOI: 10.1002/ps.3305

GAUGLER, R., CAMPBELL, J.F. & MCGUIRE, T.R. 1989. Selection for host-finding in *Steinernema feltiae*. *Journal of Invertebrate Pathology* **54**: 363–372.

GAUGLER, R., CAMPBELL, J.F. & MCGUIRE, T.R. 1990. Fitness of a genetically improved entomopathogenic nematode. *Journal of Invertebrate Pathology* **56**: 106–116.

GAUGLER, R., CAMPBELL, J.F. & GUPTA, P. 1991. Characterization and basis of enhanced host-finding in a genetically improved strain of *Steinernema carpocapsae*. *Journal of Invertebrate Pathology* **57**: 234–241.

GAUGLER, R., CAMPBELL, J.F., SELVAN, S. & LEWIS, E.E. 1992. Large-scale inoculative releases of the entomopathogenic nematode *Steinernema glaseri*: Assessment 50 years later. *Biological Control* **2**: 181–187.

GAUGLER, R. 2002. *Entomopathogenic Nematology*. CABI Publishing, Wallingford, U.K. 373.

GAUM, W.G., GILIOMEE, J.H. & PRINGLE, K.L. 1994. Life history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on English cucumbers. *Bulletin of Entomological Research* **84**: 219–224.

GERIN, C., HANCE, T. & VAN IMPE, G. 1994. Demographical parameters of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). *Journal of Applied Entomology* **118**: 370–377.

GILIOMEE, J.H. 1989. First record of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) from South Africa. *Journal of Entomological Society of Southern Africa* **52**: 179–180.

GOLDBACH, R. & PETERS, D. 1994. Possible causes of the emergence of tospovirus diseases. *Seminars in Virology* **5**: 113–120.

GREER, L. & DIVER, S. 2000. Greenhouse IPM: Sustainable thrips control. http://www.attra.org/attra-pub/gh-thrips.html. 09.10.2017.

GREWAL, P.S., SELVAN, S. & GAUGLER, R. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* **19**: 245–253.

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.

HARINGTON, J.S. 1953. Observation on the biology, the parasites and the taxonomic position of the maize beetle – *Heteronychus san-helenae* Blanch. *South African Journal of Science* **50**: 11–14.

HATTING, J.L., MOORE, S.D. & MALAN, A.P. 2018. Microbial control of phytophagous invertebrate pests in South Africa: current status and future. *Journal of Invertebrate Pathology* (in press). https://doi.org/10.1016/j.jip. 04.02.2018.

HAY, D.B. & RICHARDSON, P.N. 1995. Inter- and intraspecific variation in infectivity of Steinernema spp. to larvae of the mushroom fly *Lycoriella solani*. *Entomologia Experimentalis Applicata* **77**: 11–15. DOI: https://doi.org/10.1111/j.1570-7458.1995.tb01979.x

HELYER, N., GILL, G. & BYWATER, A. 1992. Control of chrysanthemum pests with *Verticillium lecanii*. *Phytoparasitica* **20**.

HULSHOF, J., KETOJA, E. & VANNINEN, I. 2003. Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. *Entomologia Experimentalis Applicata* **108**: 19–32. DOI: https://doi.org/10.1046/j.1570-7458.2003.00061.x

HUNTER, W.B., ULLMAN, D.E. & MOORE, A. 1992. Electronic monitoring: Characterizing the feeding behavior of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). In *Proceedings of an Informal Conference on Electronic Monitoring of Insect Feeding Behavior, Miscellaneous Publications of the Entomological Society of America.*

IMMARAJU, J.A., PAINE, T.D., BETHKE, J.A., ROBB, K.L., & NEWMAN, J.P. 1992. Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. *Journal of Economic Entomology* **85:** 9–14.

IRAC. 2011. Mechanisms of insecticide resistance in western flower thrips, *Frankliniella occidentalis* (Pergande). http://www.irac-online.org. 06.06.2017.

ISHIDA, H., MURAI, T., SONODA, S., YOSHIDA, H., IZUMI, Y. & TSUMUKI, H. 2003. Effects of temperature and photoperiod on development and oviposition of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Applied Entomology and Zoology* **38**: 65–68.

JACOBSON, R.J. 1997. Integrated pest management (IPM) in glasshouses. In: Lewis, T. (Ed.) *Thrips as Crop Pests*. 639–666. CAB International, Wallingford, U.K.

JENSEN, S.E. 2000. Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. *Integrated Pest Management Review* **5**: 131–146.

KAHN, N.D., WALGENBACH, J.F. & KENNEDY G.G. 2005. Summer weeds as hosts for *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). *Bulletin of IOBC/WPRS* **25**: 119–122.

KANARA, H.G. & ACHARYA, M.F. 2014. Bionomics of Rose Thrips, *Frankliniella occidentalis* Pergande. *Journal of Horticulture* **1**: 1–3. DOI: 10.4172/2376-0354.1000109

KARNKOWSKI, W. & TRDAN, S. 2002. Diagnostic protocols for regulated pests. *Bulletin OEPP/EPPO Bulletin* **32**: 241–243. DOI: https://doi-org.ez.sun.ac.za/10.1046/j.1365-2338.2002.00585.x

KAYA, H.K. 1990. Soil ecology. In: Gaugler, R. & Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control*. 93–116. CRC Press, Boca Raton, FL, U.S.A.

KAYA, H.K. & GAUGLER, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* **38**: 181–206. DOI: https://doi.org/10.1146/annurev.en.38.010193.001145

KAYA, H.K., AGUILLERA, M.M., ALUMAI, A., CHOO, H.Y., TORRE, M., DE LA FODOR, A., GANGULY, S., HAZIR, S., LAKATOS, T., PYE, A., WILSON, M., YAMANAKA, S., YANG, H. & EHLERS, R.U. 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* **38**: 134–155. DOI: 10.1016/j.biocontrol.2005.11.004

KIRK, W.D. & TERRY, L.I. 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology* **5**: 301–310. DOI: https://doi.org/10.1046/j.1461-9563.2003.00192.x

KLEIN, M.G., 1990. Efficacy against soil-inhabiting insect pests. In: Gaugler, R. & Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control*. 195–214. CRC Press, Boca Raton, FL, U.S.A.

KOPPENHOFER, A.M., BAUR, M.E., STOCK, S.P., CHOO, H.Y., CHINNASRI, B. & KAYA, H.K. 1997. Survival of entomopathogenic nematodes within host cadavers in dry soil. *Applied Soil Ecology* **6**(32): 231–240. DOI: https://doi.org/10.1016/S0929-1393(97)00018-8

KUNG, S., GAUGLER, R. & KAYA, H.K. 1990. Effects of soil temperature, moisture and relative humidity on entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* **57**: 242–249. DOI: 10.1016/0022-2011(91)90123-8

LACEY, L.A. & GEORGIS, R. 2012. Entomopathogenic nematodes for control of insect pests above and below with comments on commercial production. *Journal of Nematology* **44**(2): 218–225.

LAZNIK, Z. & TRDAN, S. 2008. Entomopathogenic and entomoparasitic nematodes as biological control agents of thrips. *Acta Phytopathologica et Entomologica Hungarica* **43**: 317–322. DOI: 10.1556/APhyt.43.2008.2.15

LEE, S.J., KIM, S., KIM, J.C., LEE, M.R., HOSSAIN, M.S., SHIN, T.S., KIM, T.H. & KIM, J.S. 2017. Entomopathogenic *Beauveria bassiana* granules to control soil-dwelling stage of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *BioControl* **62**: 639–648. DOI: 10.1007/s10526-017-9818-8

LELLO, E.R., PATEL, M.N., MATTHEWS, G.A., & WRIGHT, D.J. 1996. Application technology for entomopathogenic nematodes against foliar pests. *Crop Protection* **15**: 567–574. DOI: https://doi.org/10.1016/0261-2194(96)00026-9

LEWIS, T. 1973. Thrips. Their biology, ecology and economic importance. Academic Press, London.

LEWIS, T. 1997. Pest thrips in perspective. In: Thrips as crop pests Lewis T. (Ed.). Wallingford, U: CAB International.

LINDQUIST, R.K., CASEY, M.I., BAUERLE, W.L. & SHORT, T.L. 1987. Effects of an overhead misting system on thrips populations and spider mite-predator interactions on greenhouse cucumber. *Bulletin of the IOBC/WPRS* **10**: 97–100.

LU, D., BAIOCCHI, T. & DILLMAN, A.R. 2016. Genomics of entomopathogenic nematodes and implications for pest control. *Trends in Parasitology* **32**: 588–597. DOI: 10.1016/j.pt.2016.04.008

MACDONALD, O.C. 1993. Susceptibility of western flower thrips, *Frankliniella occidentalis* (Pergande) to fumigation with methyl bromide. *Annuals of Applied Biology* **123**: 531–537. DOI: https://doi.org/10.1111/j.1744-7348.1993.tb04925.x

MACDONALD, K.M., HAMILTON, J. G. C., JACOBSON, R. & KIRK, W. D. J. 2002. Effects of alarm pheromone on landing and take-off by adult western flower thrips. *Entomologia Experimentalis Et Applicata* **103**: 279–282.

MALAN, A.P., KNOETZE, A.P., & MOORE, S.D. 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology* **108**: 115–125. DOI: 10.1016/j.jip.2011.07.006

MALAN, A.P. & FERREIRA, T. 2017. Entomopathogenic nematodes. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S., De Waele, D. (Ed.), *Nematology in South Africa: a view from the 21st Century* 22: 459–480. Springer International Publishing, Cham, Switzerland.

MANNERS, A.G., DEMBOWSKI, R.B. & HEALEY, M.A. 2013. Biological control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae),

in gerberas, chrysanthemums and roses. *Australian Journal of Entomology* **52**: 246–258. DOI: https://doi.org/10.1111/aen.12020

MASON, J.M. & WRIGHT, D.J. 1997. Potential for the control of *Plutella xylostella* larvae with entomopathogenic nematodes. *Journal of Invertebrate Pathology* **70**: 234–242. DOI: https://doi.org/10.1006/jipa.1997.4695

MCINTYRE, J.A., HOPPER, D.A. & CRANSHAW, B. 1996. Aluminized fabric debris deters thrips from entering greenhouses. *Southwestern Entomologist* **21**: 135–140.

MESSELINK, G. & VAN HOLSTEIN-SAJ, R. 2008. Improving thrips control by the soil-dwelling predatory mite *Macrocheles robustulus* (Berlese). *Bulletin OILB/SROP* **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. DOI: 10.1016/j.biocontrol.2014.07.009

MORSE, J.G., & HODDLE, M.S. 2006. Invasion biology of thrips. *Annual Review of Entomology* **51**: 67–89. DOI: https://doi.org/10.1146/annurev.ento.51.110104.151044

MOUDEN, S., SARMIENTO. K.R., KLINKHAMER. P.G. & LEISS K.A. 2017. Integrated pest management in western flower thrips: past, present and future. *Pest Management Science* **73**: 813–822. DOI: 10.1002/ps.4531

MOUND, L.A. & KIBBY G. 1998. Thysanoptera: an identification guide. 2nd Ed. CAB International, Oxon, New York.

MOUND, L.A. 2009. *Thysanoptera* (Thrips) of the world-a-checklist. www.ento.csiro.ou/thysanoptera /worldthrips.html.

MURPHY, B.C., MORISAWA, T.A., NEWMAN, J.P., TJOSVOLD, S.A. & PARRELLA, M.P. 1998. Fungal pathogen controls thrips in greenhouse flowers. *California Agriculture* **52**: 32–36. DOI: https://doi.org/10.3733/ca.v052n03p32

MURPHY, G., FERGUSON, G. & SHIPP, L. 2014. Thrips in greenhouse crops – biology, damage and management. *Ontario Ministry of Agriculture, Food and Rural Affairs Factsheet* 03-077.

NIMKINGRAT, P., STRAUCH, O. & EHLERS, R.U. 2013a. Hybridisation and genetic selection for improving desiccation tolerance of the entomopathogenic nematode *Steinernema feltiae*. *Biocontrol Science and Technology* **23**: 348–361. DOI: https://doi-org.ez.sun.ac.za/10.1080/09583157.2012.758238

NIMKINGRAT, P., STRAUCH, O. & EHLERS, R.U. 2013b. Desiccation tolerance of dauers of entomopathogenic nematodes of the genus *Steinernema*. *Nematology* **15**: 451–458. DOI: 10.1163/15685411-00002692

PAINI, D.R., FUNDERBURK, J.E., JACKSON, C.T. & REITZ, S.R. 2007. Reproduction of four thrips species (Thysanoptera: Thripidae) on uncultivated hosts. *Journal of Entomological Sciences* **42**: 610–615.

PARRELLA, G., GOGNALONS, P., GEBRE-SELASSIE, K., VOVLAS, C. & MARCHOUX, G. 2003. An update of the host range of *Tomato spotted wilt virus*. *Journal of Plant Pathology* **85**: 227–264.

PERME, S. 2005. Testing the efficacy of entomopathogenic nematodes (Rhabditida) against foliar pests of vegetables. M.Sc. thesis, University of Ljubljana, Ljubljana, Slovenia.

PREMACHANDRA, W.T., BORGEMEISTER, C., BERNDT, O., EHLERS, R.U. & POEHLING, H.M. 2003. Laboratory bioassays of virulence of entomopathogenic nematodes against soil-inhabiting *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). *Nematology* **5**: 539–547. DOI: 10.1163/156854103322683256

PUNDT, L. 2011. Biological control: using beneficial nematodes. https://ag.umass.edu/greenhouse-floriculture/fact-sheets/. 15.03.2018.

RACE, S.R. 1961. Early-season thrips control on cotton in New Mexico. Journal of Economic Entomology **54**: 974–976. DOI: https://doi.org/10.1093/jee/54.5.974

REITZ, S.R. 2008. Comparative bionomics of *Frankliniella occidentalis* and *Frankliniella ritici*. *Florida Entomologist* **91**: 474–476.

REITZ, S.R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomologist* **92**: 6–13. DOI: https://doi.org/10.1653/024.092.0102

RILEY, D.G., CHITTURI, A. & SPARKS, J.R. 2007. Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? *Entomologia Experimentalis Applicata* **124**: 133–141. DOI: 10.1111/j.1570-7458.2007.00561.x

ROBB, K.L. & PARRELLA, M. P. 1991. Western flower thrips, a serious pest of floricultural crops. *General Technical Report NE-147*. 343-358.

ROBB, K. L. 1989. Analysis of *Frankliniella occidentalis* (Pergande) as a Pest of Floricultural Crops in California Greenhouses. Ph.D. dissertation, University of California, Riverside, CA.

SALAME, L. GLAZER, I. & CHUBINISHVILLI, M.T. 2010. Genetic improvement of the desiccation tolerance and host-seeking ability of the entomopathogenic nematode *Steinernema feltiae*. *Phytoparasitica* **38**: 359–368. DOI: 10.1007/s12600-010-0106-8

SANDERSON, J.P. 2003. Western flower thrips. http://www.greenhouse.cornell.edu/pests/pdfs/insects/WFT.pdf. 25.05.2017.

SARMIENTO, H.K. 2014. Integrated Pest Management of Western Flower Thrips (Master's thesis). University of Leiden. Netherlands.

SEATON, K.A., COOK, D.F. & HARDIE, D.C. 1997. The effectiveness of a range of insecticides against western flower thrips (*Frankliniella occidentalis*) (Thysanoptera: Thripidae) on cut flowers. *Australian Journal of Agricultural Research* **48**: 781–787. DOI: 10.1071/A96081

SHAPIRO-ILAN, D.I., GOUGE, D.H. & KOPPENHÖFER, A.M. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus. In: Gaugler, R. (Ed.) *Entomopathogenic Nematology*. 333–356. CABI Publishing, Wallingford, U.K.

SHAPIRO-ILAN, D.I., GOUGE, D.H., PIGGOT, S.J. & PATTER J. 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control* **38**: 124–133. DOI: 10.1016/j.biocontrol.2005.09.005

SHAPIRO-ILAN, D.I., BRUCK, D.J. & LACEY, L.A. 2012. Principles of epizootiology and microbial control. In: F. E. Vega and H. K. Kaya. (Ed). *Insect pathology*. 29–72.

Academic Press, San Diego. DOI: https://doi.org/10.1016/B978-0-12-384984-7.00003-8

SHELMITH, M.W. 2009. Effects of abiotic factors on entomopathogenic nematodes and their potential against the sweet potato weevil *Cylas puncticollis* Boheman. Ph.D. thesis, University of Nairobi, Nairobi, Kenya.

SHIPP, J.L., ZHANG, Y., HUNT, D.W. & FERGUSON, G. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. *Environmental Entomology* **32**: 1154–1163. DOI: https://doi.org/10.1603/0046-225X-32.5.1154

SILVEIRA, L.C., BUENO, V.H. & VAN LENTEREN J.C. 2004. *Orius insidiosus* as biological control agent of thrips in greenhouse chrysanthemums in the tropics. *Bulletin of Insectology* **57**: 103–109.

STEINER, M. Y., & GOODWIN, S. 2005. Management of thrips (Thysanoptera: Thripidae) in Australian strawberry crops: within-plant distribution characteristics and action thresholds. Australian Journal of Entomology **44**: 175-185. DOI: 10.1111/j.1440-6055.2005.00467.x

STEYN, W.P, MALAN, A.P., DANEEL, M.S. & SLABBERT, R.M. 2017. Entomopathogenic nematodes from north-eastern South Africa and their virulence against the false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Biocontrol Science and Technology* **27**: 1265–1278. http://dx.doi.org/10.1080/09583157.2017.1391174

STOCK, P.S. 2015. Diversity biology and evolutionary relationships. In: *Nematodes as pathogenesis of insects and other pest: Ecology and applied technologies for sustainable plant and crop protection,* Compos-Herrera R. (Ed.) 1–27. Springer International Publishing, Switzerland.

TERRY, L.I., & KELLY, C.K. 1993. Patterns of change in secondary and tertiary sex ratios of the Terebrantian thrips, *Frankliniella occidentalis*. *Entomologia Experimentalis et Applicata* **66**: 213–225. DOI: https://doi.org/10.1111/j.1570-7458.1993.tb00712.x

TOMMASINI, M.G. & MAINI, S. 1995. *Frankliniella occidentalis* and thrips harmful to vegetable and ornamental crops in Europe. In: Biological Control of Thrips Pests, A.J.M. Loomans, J.C. van Lenteren, S. Tommasini, S. Maini and J. Riudavets (Eds.) 1–42 Wageningen Agricultural University Papers, Wageningen, Netherlands.

TOURTOIS, J. & GRIESHOP, M.J. 2015. Susceptibility of *Dalotia coriaria* (Kraatz) (Coleoptera: Staphylinidae) to entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae). *Insects* **6**: 224–235. DOI: 10.3390/insects6010224

ULLMAN, D.E., SHERWOOD, J.L. & GERMAN, T.L. 1997. Thrips as vectors of plant pathogens. In Thrips as crop pests. Lewis T (Ed). CAB International, Oxon, UK, 539-565.

VAN DIJKEN, F.R., DIK, M.T.A., GEBALA, B., DE JONG, J. & MOLLEMA, C. 1994. Western flower thrips (Thysanoptera: Thripidae) effects on chrysanthemum cultivars: plant growth and leaf scarring in nonflowering plants. *Journal of economic entomology* **87**: 1312–1317. DOI: https://doi.org/10.1093/jee/87.5.1312

VESTERGAARD, S., GILLESPIE, A.T., BUTT, T.M., SCHREITER, G. & EILENBERG, J. 1995. Pathogenicity of the hyphomycete fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis. Biocontrol Scientific Technology* **5**: 185–192. DOI: https://doi.org/10.1080/09583159550039909

WHITFIELD, A.E., ULLMAN, D.E. & GERMAN, T.L., 2005. Tospovirus-thrips interactions. *Annual Review of Phytopathology* **43**: 459–489. DOI: https://doi.org/10.1146/annurev.phyto.43.040204.140017

WIJKAMP, I., VAN DE WETERING, F., GOLDBACH, R. & PETERS, D. 1996. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis:* median acquisition and inoculation access period. *Annuals of Applied Biology* **129**: 303–313. DOI: https://doi.org/10.1111/j.1744-7348.1996.tb05753.x

WILSON, M. & GAUGLER, R. 2004. Factors limiting short-term persistence of entomopathogenic nematodes. *Journal of Applied Entomology* **128**: 250–253. DOI: https://doi.org/10.1111/j.1439-0418.2004.00814.x

WRIGHT, D.J., PETERS, A., SCHROER, S. & FIFE, J.P. 2005. Application technology. pp. 91–106 in Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (Eds) Nematodes as biocontrol agents. Wallingford, CABI.

YANG, T., STOOPEN, G., WIEGERS, G., MAO, J., WANG, C., DICKE, M. & JONGSMA, M. A. 2012. Pyrethrins protect pyrethrum leaves against attack by western flower thrips,

Frankliniella occidentalis. Journal of Chemical Ecology **38**: 370–377. DOI: 10.1007/s10886-012-0097-7

ZERVOS, S., JOHNSON, S.C. & WEBSTER, J.M. 1991. Effect of temperature and inoculum size on reproduction and development of *Heterorhabditis heliothidis* and *Steinernema glaseri* (Nematoda: Rhabditoidea) in *Galleria mellonella. Canadian Journal of Zoology* **69**: 1261–1264. DOI: https://doi.org/10.1139/z91-177

ZHANG, C., LIU, X.H., TAN, J., WANG, Y., QIAO, L. & YEDID, G. 2008. *Heterorhabditidoides chongmingensis* gen. nov. sp. nov. (Rhabditida: Rhabditidae), a novel member of the entomopathogenic nematodes. *Journal of Invertebrate Pathology* **98**: 153–168. DOI: 10.1016/j.jip.2008.02.011

ZHAO, G.Y., LIU, W. & KNOWLES, C. O. 1994. Mechanisms associated with diazinon resistance in western flower thrips. *Pesticide Biochemistry and Physiology* **49:** 13–23. DOI: https://doi.org/10.1006/pest.1994.1030

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. *Entomology Experimental Applied* **117**: 185–192. DOI: https://doi.org/10.1111/j.1570-7458.2005.00351

Chapter 2

Identification and life history of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on two different plant hosts

Abstract

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera), is one of the most economically important pests in greenhouses. However, their identification is usually difficult, with them being easily confused with other thrips species. The identity of *F. occidentalis* was verified, using both morphological and molecular methods. Main morphological key characteristics were observed in the thrips population and they fitted well with the descriptions given for *F. occidentalis.* Molecular identification was based on amplification of the mtCOI gene sequences for the identification of five thrips species (*F. occidentalis, Thysanoptera* sp., *Gynaikothrips ficorum* and *Pseudophilothrips ichini*) collected from the study area. The *F. occidentalis*, morphologically identified, showed 100 % identity with sequences for *F. occidentalis* in the database of GenBank. One of the *Thrips* sp. which could not be identified morphologically or molecularly could possibly be an unidentified species. Accurate identification of WFT is important for further studies in biological control of the pest. The life history and success rate of WFT on chrysanthemum (*Dendranthema grandiflora*) leaflets and green bean pods (*Phaseolus vulgaris*) were studied in the laboratory. Results from the life-history characteristics showed that more first instar larva hatched on chrysanthemums and faster larval developmental rate and a higher survival rate on chrysanthemums indicated that chrysanthemum is a more attractive and more suitable host than green bean.

Key words: *Frankliniella occidentalis*, morphological, molecular, life history, survival rate, host plant, developmental stages.

2.1. INTRODUCTION

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an invasive pest that is of great economic importance worldwide (Siguna 2007). Its significance is attributed to its wide range of host plants, its high reproductive potential, and its invasiveness (Reitz 2009). WFT is known to attack more than 240 plant species that belong to 62 different plant families (Lim *et al.* 2001). WFT is also a serious pest of greenhouse cultivation, because it damages plants directly by means of feeding and oviposition on foliage and flowers, and indirectly, by means of vectoring tospoviruses (Cloyd *et al.* 2001). *Frankliniella occidentalis* transmits about five species of tospoviruses, mainly the impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV) (Riley *et al.* 2011).

The life cycle of *F. occidentalis* includes the egg , which is partly inserted in the plant tissue, two actively feeding larval instars, two pupal stages (pre-pupa and pupa), which are non-feeding, and the adult stage (Lee *et al.* 2017). Development, which is dependent on the temperature and host, can be quite rapid, resulting in multiple generations in a single cropping season (Reitz 2009). Within the most favourable temperature range of 25-30 °C, the cycle from egg to adult can be as short as 9-13 days (Gaum *et al*. 1994; Katayama 1997; Reitz 2009).

At least 7500 species of thrips exist in the world (Mound 2009). The diagnostic characters separating Thripidae species are subtle and difficult to appreciate. The small size of *F. occidentalis* contributes to it being easily confused with other species. Even when keys are available, they are difficult to use and morphological identification of species remains problematic, if not impossible, for non-specialists. Consequently, misidentification of thrips may result in serious economic losses. To manage WFT effectively, it is important to identify them to species level. Morphological characteristics such as the number, size and location of the major setae on the head and prothorax, setae on the forewing and coloration are among those used for identification (Funderburk *et al.* 2007). Their minute size, cryptic behaviour, sexual dimorphism, high degree of similarity in various developmental stages, and polymorphism (in colour, wing development, body size) make morphological identification difficult (Tyagi *et al.* 2017). Molecular identification has become an important tool to support and verify morphological identification, facilitating differentiation of morphologically similar insect species. Molecular markers are available and are able to resolve the species complex in many insects (Suganthy *et al*. 2016). In the case of insect identification, including identification of the thrips species, the nucleotide sequencing of mitochondrial *cytochrome oxidase gene subunit I* (mtCOI) is used (Glover *et al.* 2010).

An integrated pest management (IPM) programme offers a sustainable alternative for the control of WFT in greenhouses, and the use of entomopathogenic nematodes (EPNs) for biological control has been identified as an environmentally friendly option. Laboratory bioassay tests need to be done to test the best performing EPN species. The laboratory bioassays require a constant, reliable source of thrips, making laboratory rearing of thrips essential. A major factor, other than environmental conditions and contamination, that has been reported to limit the success in rearing thrips, like *F. occidentalis*, is that they tend to cannibalise one another (Loomans & Murai 1997). Previously used methods of rearing, which required the employment of specialised equipment, were prone to mite infestations (De Graaf & Wood 2009). The use of whole plants is usually used for maintaining stock cultures of thrips, but monitoring life history components in such a rearing system proves to be difficult (Brodbeck *et al*. 2002). Numerous studies have investigated the life-history components of *F. occidentalis* on different host plants, including chrysanthemums (Robb & Parrella 1991) and French beans *Phaseolus vulgaris* L. (Gerin *et al*. 1994). Zhang *et al.* (2007) investigated the preference of *F. occidentalis* for five vegetables, including cabbage (*Brassica oleracea* L.), cucumber (*Cucumis sativus* L.), capsicum (*Capsicum annuum* L.), kidney bean (*P. vulgaris*), and tomato (*Lycopersicon esculentum* M.). Chaisuekul and Riley (2005) also reported that host plants significantly affect *F. occidentalis* oviposition preference.

Identification and culturing of WFT is difficult and often inaccurate. To evaluate the efficacy of EPNs against WFT, both in laboratory bioasssays and in the field, accurate identification of the thrips is necessary. Before recommendations regarding thrips control are given to producers, it is also important to first identify the thrips species present on the crop. The aim of the current study was, therefore, to use both morphological and molecular techniques to ensure that *F. occidentalis* can be identified accurately. In addition, the development, number of eggs hatched and survival rate of *F. occidentalis* on leaflets of *Dendranthema grandiflora* Ramat (Asteraceae) and pods of *P. vulgaris* L. (Fabaceae) were compared under laboratory conditions.

2.2.   MATERIALS AND METHODS

2.2.1. Morphological identification

Proper morphological identification requires the use of such laboratory facilities as good microscopes and good quality specimens (Vierbergen *et al.* 2012). In the current study, good-quality slides were prepared for the observation of the morphological features of thrips. The thrips specimens were mounted on microscope slides using the modified method used by Moritz

(2001). Adults were soaked in a NaOH (10 %) solution overnight, at room temperature to remove pesticide residues from plant parts. Specimens were transferred to acetic acid to neutralise the alkali, and then transferred to oil of clove to complete clearing for about 60 min. The specimens were then dehydrated by transferring to xylol. To make microscope mounts, specimens were mounted in Canada balsam on microscope slides, turning insects onto their ventral side, with their appendages arranged in an extended position, using fine forceps, before sealing with a coverslip. The slides were placed on a slide holder that was positioned horizontally in an oven at 45 °C until the Canada balsam had dried out. The adult thrips specimens were observed with a compound microscope (ZEISS Axio Scope.A1). Images were captured under a light microscope equipped with a camera and a differential interference contrast (DIC), and connected to a computer with ZEN 2.3 Lite software, to ascertain the key characteristics. Morphological identification was based on external anatomy, using the identification keys provided by Moritz (1994), Karnkowski & Trdan (2002), Reed *et al*. (2006), Wang *et al*. (2010), Cavalleri & Mound (2012), and Tyagi & Kumar (2015).

2.2.2. Molecular identification

Specimens from different locations (Table 2.1), which had first been morphologically identified, were used for molecular identification. DNA was extracted from the adult thrips, using a column-based QIAamp® DNA Micro extraction kit method. Whole thrips specimens were ground in a lysis buffer in a micro tube, using a micro pestle, with the homogenate lysing overnight at 56 °C in ATL lysis buffer (QIAGEN), with proteinase K. A Nanodrop ND-1000 Spectrophotometer was used to estimate the DNA concentration.

Table 2. 1. Morphological identification of thrips species collected at two locations in the Western Cape Province, South Africa.

Sample #	Location	GIS coordinates	Crop	Sample ID	Species
1	Simonsvlei Estate	33°49'41"S 18°33'6.48"E	Blueberries	WFTS1	*F. occidentalis*
2	Simonsvlei Estate	33°49'41"S 18°33'6.48"E	Blueberries	TRS1	*Thysanoptera* sp.
3	Oak Valley	34°9'22.68"S 19°3'21.15"E	Chrysanthemums	WFTS2	*F. occidentalis*
4	Oak Valley	34°9'22.68"S 19°3'21.15"E	Chrysanthemums	T1	*Gynaikothrips ficorum*
5	Oak Valley	34°9'22.68"S 19°3'21.15"E	Chrysanthemums	T2	*Pseudophilothrips* sp.

The PCR was done with the primers mtD7. 2F, 5' ATTAGGAGCHCCHGAYATAGCATT 3' and mtD 9.2 R, 5' CAGGCAAGATTAAAATATAAACTTCTG 3' that target the 5' region of mtCOI gene (Brunner *et al*. 2002; Suganthy *et al.* 2016). The 25 µl PCR reaction mixture contained 12 µl KAPA master mix, 2.0 µl of each primer (forward and reverse), 4.0 µl DNA water, and 5.0 µl DNA. The PCR cycle on the Eppendorf was 94 °C for 30 sec for denaturing, 1 cycle; 53 °C for 45 sec, and 72 °C for 1 min for annealing, amplification was at 35 cycles, and at 72 °C for 20 min for extension. The PCR products were visualised by agarose gel electrophoresis containing 1.2 % agar, with ethidium bromide (10 µg/ml), with 5 µl of the PCR product, positive and negative control, and ladder at 1000 bp for 30 min. The PCR product was sequenced either at the DNA Sequencing Facility at Stellenbosch University or at Inqaba Biotech.

The forward and reversed generated sequences were aligned and edited using CLC Main Workbench (ver. 8.0.1). Edited sequences were submitted for homology using BLAST (US National Library of Medicine, National Center for Biotechnology Information [s.d.]), so as to verify the morphological identification of the thrips species. Sequences of closely related species were retrieved from GenBank, and aligned using CLC. The phylogenetic relationship of South African species was analysed with the type specimens obtained from GenBank (Table 2.2).

43

Table 2.2. Thrips species downloaded in GenBank for nucleotide analysis.

Sample #	Thrips species	GenBank Acc. No.
1	*Frankliniella occidentalis*	EU004554
2	*Frankliniella occidentalis*	EU004556
3	*Frankliniella occidentalis*	KJ576881
4	*Frankliniella occidentalis*	KY688343
5	*Frankliniella occidentalis*	KY775404
6	*Frankliniella occidentalis*	MF993429
7	*Pseudophilothrips ichini*	GU942815
8	*Pseudophilothrips ichini*	GU942818
9	*Gynaikothrips ficorum*	JN181197
10	*Gynaikothrips ficorum*	JN181198
11	*Thrips sp.*	KM537823
12	*Thysanoptera sp.*	KM536079
13	*Aeolothrips sp.**	KP845633
14	*Scirtothrips dorsalis*	KF778773

*outgroup

2.3.   Laboratory bioassay

2.3.1. Source of thrips

The laboratory population of *F. occidentalis* was initiated from adults obtained from chrysanthemums grown undercover on Oak Valley farm in Elgin (34°9'22.68"S 19°3'21.15"E) and from blueberries on Simonsvlei Estate near Paarl (33°49'41"S 18°33'6.48"E), in the Western Cape Province, South Africa (Fig. 2.1). The collecting of adult thrips was done by means of taking flower and leaf samples, which were then shaken into a clean white container to dislodge the thrips. The culture was maintained in a plastic container ($20 \times 20 \times 30$ cm^3), with a screened hole in the lid for ventilation, and lined with moist tissue paper at the bottom to prevent plant desiccation. The plastic container was kept under growth chamber conditions (25 ± 2 °C, 60-70 % (RH). The thrips were fed on chrysanthemum flowers, which were changed weekly so as to maintain high fecundity. The rearing was continued until the emergence of the adults, which were used for further investigations.
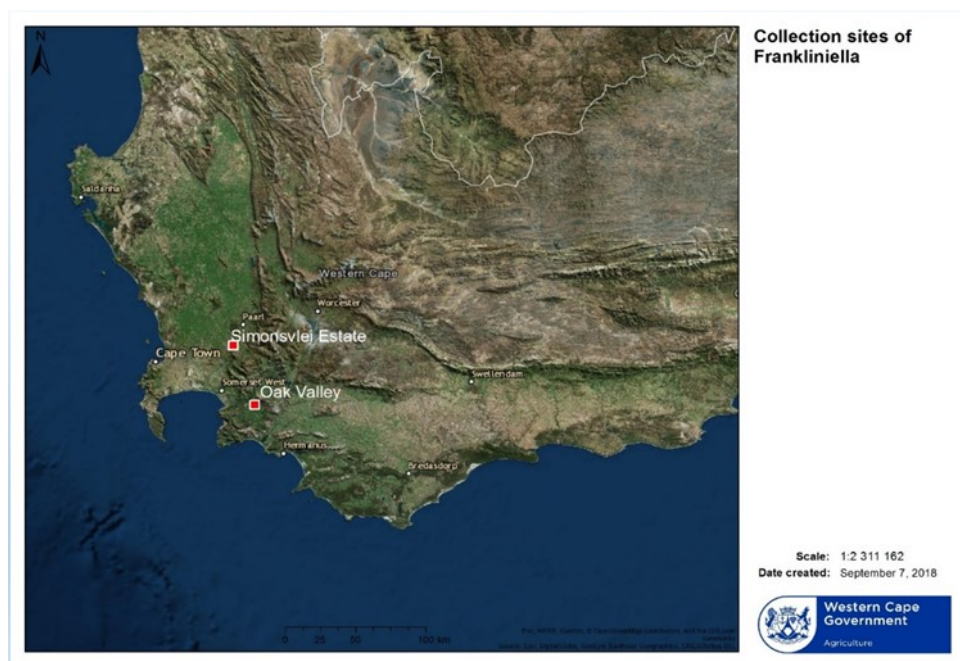
Fig. 2.1. Localities where thrips species were collected in the Western Cape Province, South Africa.

2.3.2. Source of plants

Two host plants, *D. grandiflora* (chrysanthemum) leaflets and *P. vulgaris* (green bean) pods, were used for comparing life history stages in this study, using modified protocols employed by Kanara & Acharya (2014) and Reiter *et al.* (2015), respectively. Chrysanthemums were obtained from a commercial farm, whereas the green beans were bought in supermarkets. To remove the insecticide residues, all the bean pods and chrysanthemum leaves were soaked in an abluent solution of 0.5 % sodium allylsulfonate for 1 to 2 h, thoroughly washed with water, and air-dried (Shan *et al*. 2012).

2.4.   Development and survival on two host plants

Five adult female and two male WFT were enclosed on a chrysanthemum leaf or bean pod in a plastic vial (5×4 cm), screened on top and maintained in an incubator (25 ± 2 °C, 60-70 % RH) for 4-5 days oviposition period. After this time, the adults were removed and the new cohorts of larvae, emerging from host plant material, were transferred to new green bean pods or chrysanthemum leaves in new vials with the above-mentioned specifications. After 2-3 weeks in an incubator, emerged new adults were counted as a new generation.

2.4.1. Egg stage

Five adult female and two male WFT from the new generation were enclosed in a plastic vial (5×4 cm), screened on top, containing a chrysanthemum leaflet or green bean pod for

oviposition. The ten vials that were used for each treatment were kept in a plastic container (20 × 20 × 30 cm³), maintained under climate chamber conditions (25 ± 2 °C, 60-70 % RH). After 24 h each leaflet or bean pod was transferred individually into another vial above a piece of folded tissue paper for the eggs to hatch. The duration of the egg stage was recorded from the time when the eggs were laid to when the larvae emerged and the number of larvae that emerged, were recorded.

2.4.2. Larval stage

To study larval instars and their duration, newly emerged larvae were transferred to a vial containing a chrysanthemum leaflet or bean pod, using a camel hair brush. The leaflets and pods were changed daily to keep the diet fresh. Individual larvae were examined under a ZEISS stereo Discovery V8 microscope, fitted with Axiocam ERc 5s, every day until they died or matured. The morphology of the first instar larva was differentiated from that of the second instar larva by means of the individual's size, and the exuviae of the first instar. The number of instars, instar duration, and total larval duration were recorded.

2.4.3. Pupal stage

To facilitate pupation, a piece of tissue paper was kept at the bottom of the vial. The pupae, when formed, were collected and kept individually in vials until adult emergence. The prepupal and pupal periods and pupal numbers were recorded. The prepupa was distinguished by its short wing sheaths and erect antennae, whereas the pupa had long wing sheaths reaching almost the end of the abdomen, with its antennae bent backwards along the head.

2.4.4. Adult stage

After counting the winged adults that emerged from the pupae, both the females and males were transferred into separate vials on the same day to study their longevity. The sexes of the adults were identified on the basis of their body colour, size and abdominal tip. The males were smaller in size, and pale in colour, with rounded abdominal tip, whereas the females were darker in colour, with pointed abdominal tip and the ovipositor visible. Fresh leaflets or pods were placed in each vial for food every 24 h, until the adults died.

2.4.5. Statistical analysis

Analysis of variance (ANOVA) was used to test for significant differences ($p < 0.05$) in the development periods, in the longevity of the females and males, and in the fecundity of *F. occidentalis* on different host plants. Statistical analyses were conducted using STATISTICA

46

13.2 software (StatSoft Inc. 2016). Homogeneity of variance tests were performed using Levene's test (Levene 1960) and the Games-Howell test (Games & Howell 1976), used for unequal variances caused by unequal group sizes.

2.5. RESULTS

2.5.1. Morphological identification

Five specimens exhibiting characteristic features of *F. occidentalis,* were observed from each location. The adults are yellow, with distinctive light brown markings medially on each abdominal tergite, with the head greater in width than length (Fig. 2.2). The *F. occidentalis* male has a narrow, rounded-end abdomen, while the female has a pointed abdomen (Fig. 2.3) with the ovipositor clearly visible. Antennae have eight segments; I yellow; II yellowish-brown; III-V yellow with brown distal end; VI-VIII brown (Fig 2.4), with a smooth antennal pedicel (Fig 2.6), and spines arising from the second antennal segment that are relatively light (Fig 2.5). Antennal segment VIII is twice the length of VII (Fig. 2.3). The pronotum has five pairs of major setae (Fig. 2.7); anteromarginal setae slightly shorter than anteroangulars, one pair of minor setae present medially between posteromarginal submedian setae. The metanotum has two pairs of setae at the anterior margin, campaniform sensilla present (Fig. 2.7). Three pairs of ocellar setae are present on the head, pair III longer than the distance between the external margins of hind ocelli, arising on anterior margins of the ocellar triangle; postocular setae pair I present, pair IV longer than the distance between hind ocelli. A pair of ocular setae separated by at least one-and-a-half times the diameter of a single ocellus, are present (Fig. 2.8). Four small setae arise on the anterior margin of the prothorax, between the major anteromarginal setae. The first vein of the anterior wing has a complete row of 14-21 (most often 16-17) regularly spaced setae (Fig. 2.9). Setae A on Tergite IX slightly shorter than B and C (Fig. 2.10). Posteromarginal comb on tergite VIII of female well-developed, complete, with about 10-14 long teeth on broad base (Fig. 2.11).

Fig. 2.2. Female *Frankliniella occidentalis* with distinctive light brown markings medially on each abdominal tergite and head greater in width than length (100 × magnification).
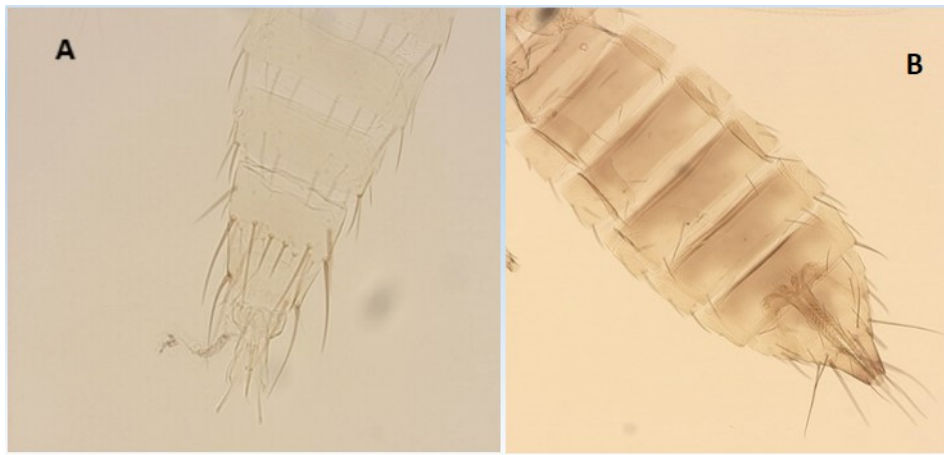
Fig. 2.3. A: Male *Frankliniella occidentalis* with narrow, rounded-end abdomen, and B: female *Frankliniella occidentalis* with pointed abdomen (200 × magnification).
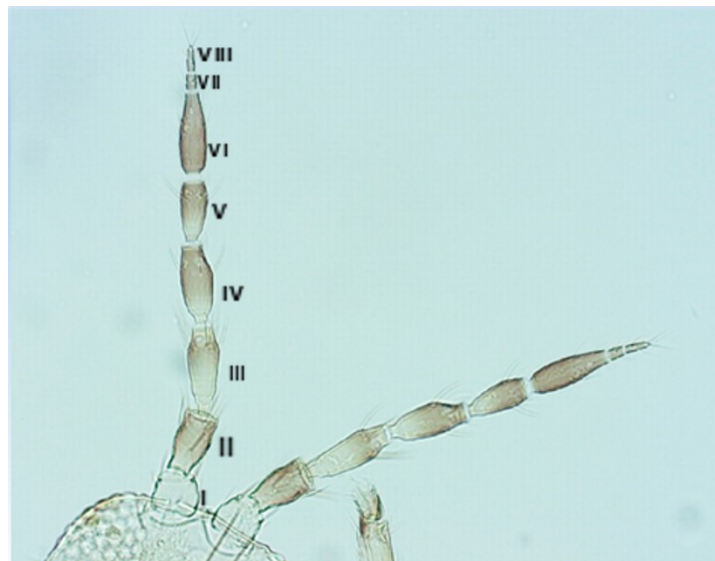
Fig. 2.4. Antennae of *Frankliniella occidentalis* with eight segments (200 × magnification).

Fig. 2.5. Antennal segments III and IV of *Frankliniella occidentalis*, with sense cones (400 × magnification).

Fig. 2.6. Pedicel of antennal segment III of *Frankliniella occidentalis* simple (400 × magnification).
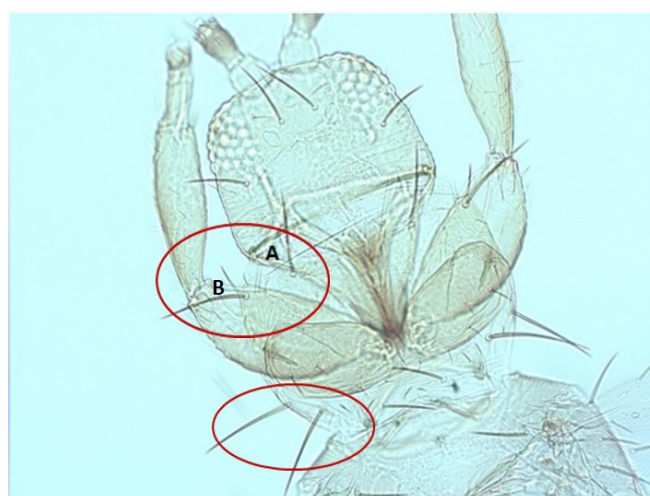
Fig. 2.7. Adult *Frankliniella occidentalis* head and pronotum, showing two pairs of large setae on the front and back of pronotum (circled). A: pronotal anteroangular setae, B: pronotal anteromarginal setae (200 × magnification).

49
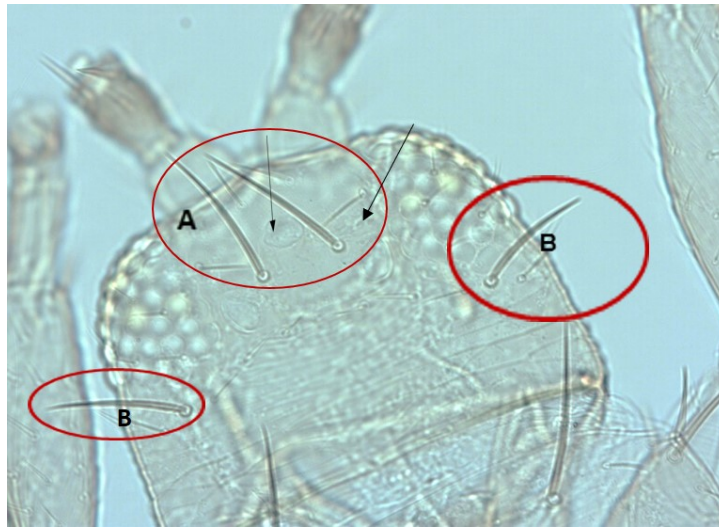
Fig. 2.8. Adult *Frankliniella occidentalis*, showing a large pair of setae (circled) between ocelli (indicated by arrows), relatively broad. A: interocellar setae/ocella setae, B: major postocular setae (400 × magnification).
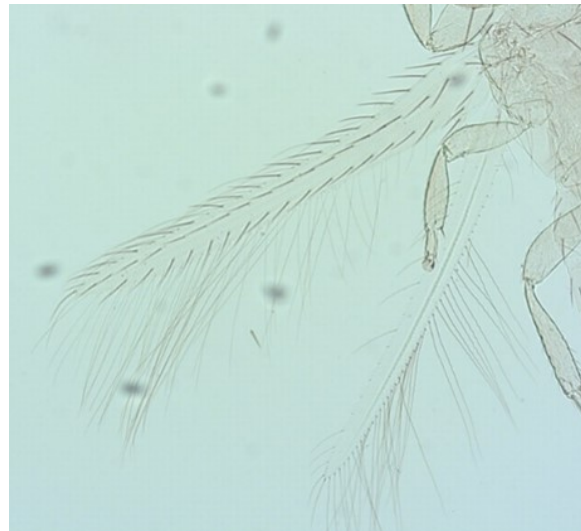
Fig. 2.9. Adult *Frankliniella occidentalis*, showing forewing's first vein, with complete row of setae (200 × magnification).
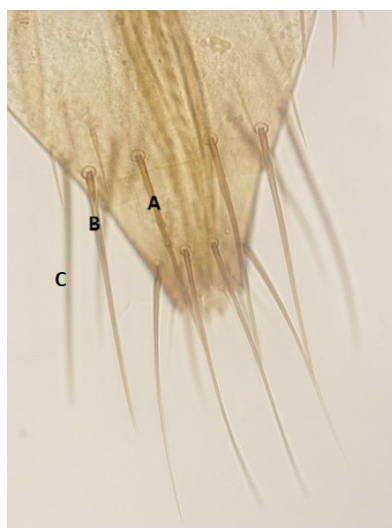
Fig. 2.10. Tergite IX, setae A of *Frankliniella occidentalis,* slightly shorter than B and C (400 × magnification).
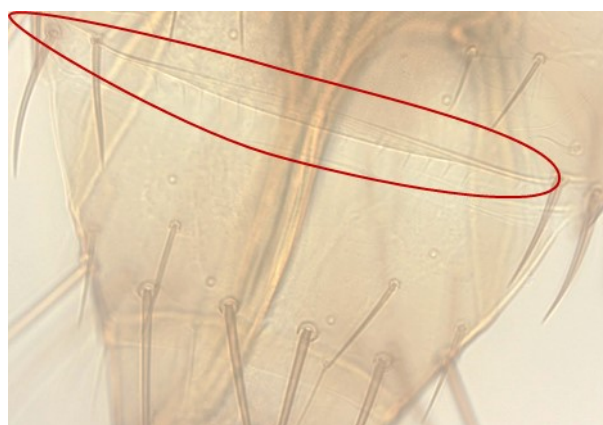
Fig. 2.11. Complete comb on abdominal tergite VIII (circled) of *Frankliniella occidentalis* (400 × magnification).

2.5.2. Molecular identification

The homology search was performed in BLAST (*www.ncbi.nlm.nih.gov*). The length of the sequence of the amplicons varied from 379-610 bp. The WFTS1 *F. occidentalis* population (Table 2.1) showed maximum identity of 100 % with GenBank accession numbers KJ576881, HQ214660, and GU148036 (Table 2.2), while the WFTS2 *F. occidentalis* population (Table 2.1) showed 100 % identify with sequences with accession numbers MF993429, KY775404, and KC008075 (Table 2.2). The sequence of the T1 *Gynaikothrips ficorum* population showed identity of 100 % with KC513156 and JN181198. T2 *Pseudophilothrips* sp. showed 90 % identity with GU942818 and GU942817. TRS1 *Thysanoptera* sp. showed 84 % identity with KM536079, and 82 % with KP845633.

51

Individuals of the same order, family and species formed distinct clusters (Fig 2.12). The cluster distinctively showed that the sequences are from the Thysanoptera, with 99 % bootstrap value. The main cluster branched into a cluster showing three families: Thripidae (EU004554, EU004556, KJ576881, KY688343, KY775404, MF993429, KM536079, KF778773, WFTS1, and WFTS2) with an 85 % bootstrap value; Phlaeothripidae (GU942815, GU942818, JN181197, JN181198, T1, and T2) with a bootstrap value of 100 % and Aeolothripidae with bootstrap of 79 %. The latter two families, which showed a very close relationship, clustered together with KP845633 in Aeolothripidae. All species of Aeolothripidae formed a monophyletic group. The further branching showed that the WFTS2 *F. occidentalis* from the study formed one cluster with the reference sequences EU004556, KY688343, KY775404, and MF993429, strongly supported by the bootstrap value of 100 %. The other *F. occidentalis* (WFTS1) from Simonsvlei formed a cluster with EU004554 and KJ576881, with a bootstrap value of 98 %. The *Gynaikothrips ficorum* sequence generated in the study showed a bootstrap value of 97 % with the reference sequence JN181198. *Pseudophilothrips ichini* showed a bootstrap value of 98 % with the reference sequences GU942815 and GU942818. The clade with *Thrips* sp. TRS1 indicated a bootstrap value of 79 % with reference sequence KM536079.
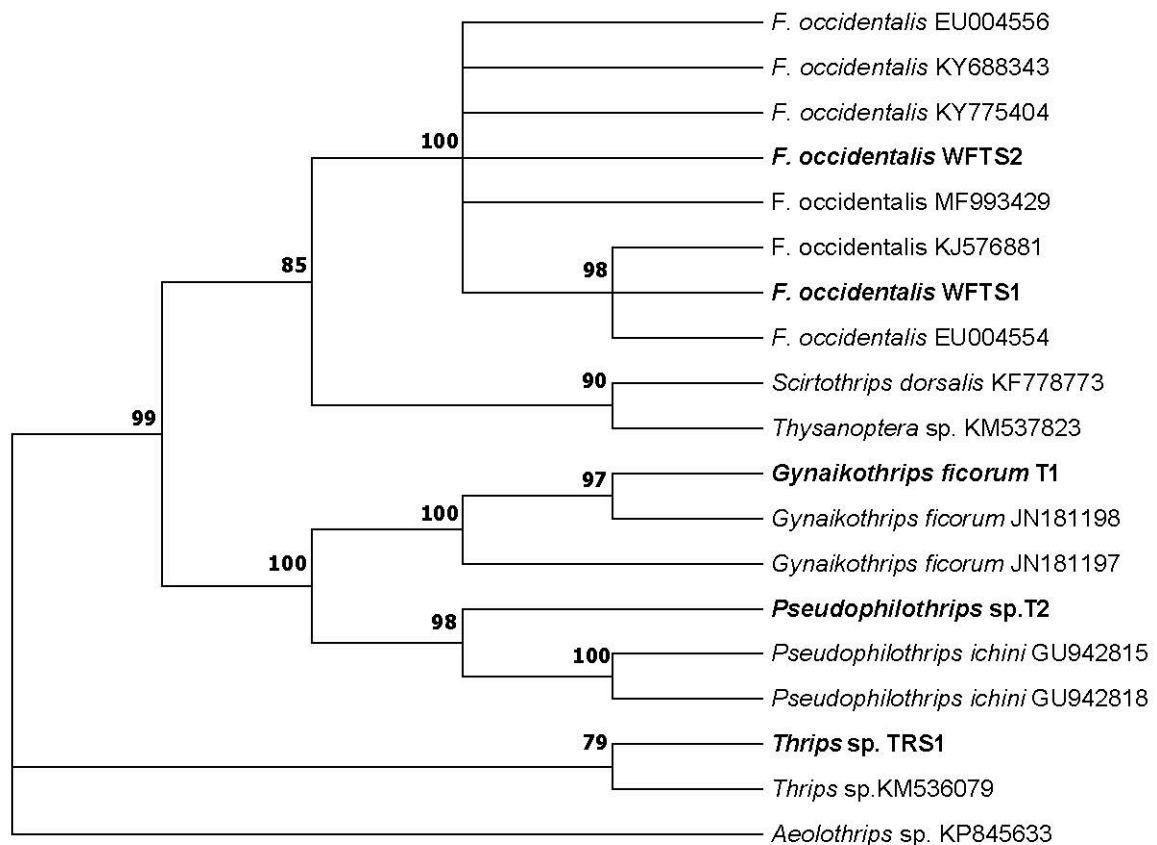
Fig. 2.12. Maximum parsimony analysis of *Frankliniella occidentalis, Gynaikothrips ficorum, Pseudophilothrips ichini,* and a *Thrips* sp. collected from blueberries and chrysanthemums, with 13 reference sequences obtained from GenBank. *Aeolothrips* sp. was used as the outgroup. Numbers at nodes represent the percentage bootstrap values and the samples of this study are shown in bold font.

The evolutionary history was inferred according to the maximum parsimony method, using the sequences generated from the study, and the reference sequences. The most parsimonious tree length was 404. The consistency index is (0.701087), with the retention index being (0.858974), and the composite index 0.625095 (0.602216) for all sites, including parsimony-informative ones (in parentheses). The percentage of replicate trees in which the associated taxa cluster together in the bootstrap test (1000 replicates) are shown next to the relevant branches (Felsenstein 1985). The MP tree was obtained using the subtree-pruning-regrafting (SPR) algorithm (Nei & Kumar 2000). The analysis involved 19 nucleotide sequences, with there being 402 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016). *Aeolothrips* sp. downloaded from GenBank was used as an outgroup.

2.5.3. Development and survival on two host plants

Analysis of the data showed a significant difference (p = 0.84288) between the number of individuals of each developmental stage obtained on the chrysanthemum leaflets and on the

green bean pods. The number of first instar larvae hatching on the chrysanthemum leaves was 31.2 ± 3.59, whereas it was 21.0 ± 3.59 on the green bean pods. The number of second instar larvae was 28.3 ± 3.44 and 18.5 ± 3.44 for chrysanthemum leaves and green bean pods, respectively. The chrysanthemums produced a higher number of pupae compared to the green bean pods, with the number of pre-pupae being 24.2 ± 3.03 and the number of pupae being 22.4 ± 3.09 for the chrysanthemum leaves. In contrast, the number of pre-pupae was 13.8 ± 3.03, with the number of pupae being 11.5 ± 3.09 for the green bean pods. The number of adults also differed significantly on the two host plants, with a total of 19.8 ± 3.00 adults emerging from chrysanthemums and 8.7 ± 3.00 emerging from green bean pods (Fig. 2.13).
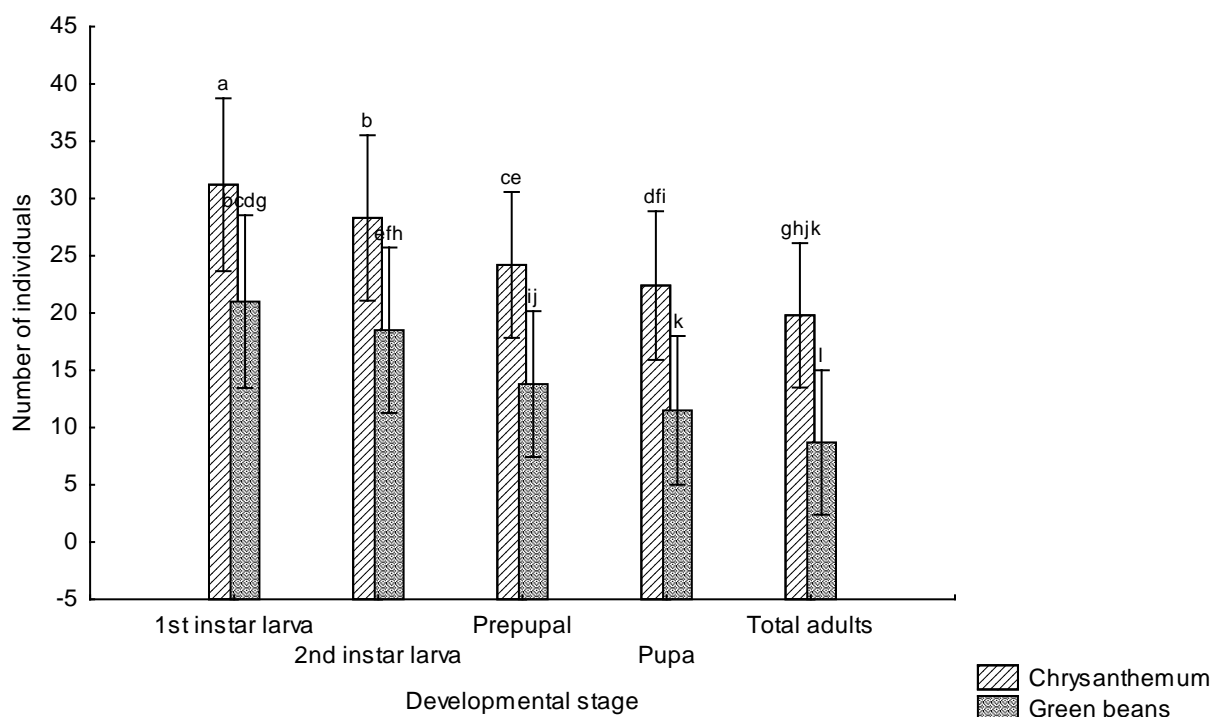
Fig. 2.13. Number of individuals of different developmental stages of *Frankliniella occidentalis* when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: F $_{(4, 72)}$ = 0.35049; p = 0.84288). The different letters above the error bars denote significant differences between treatments (p < 0.05).

The percentage survival from egg to adult was also significantly different (p = 0.00319) between the two host plants. The percentage survival on chrysanthemums was 60.7 ± 4.49 and 39.3 ± 4.49 on the green bean pods (Fig. 2.14).

54
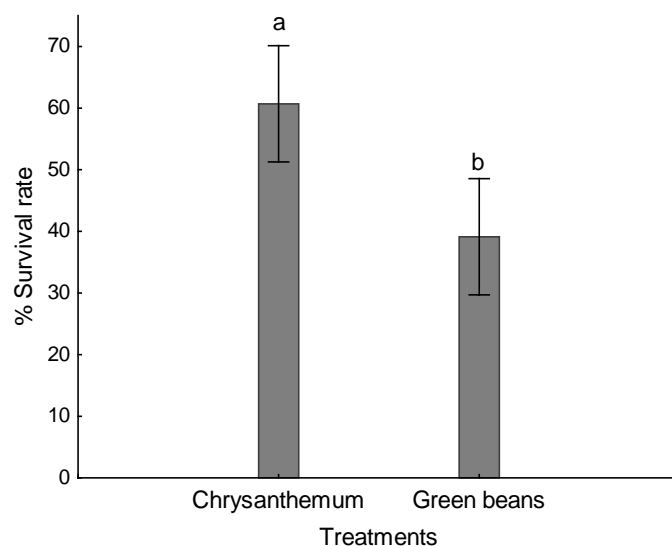
Fig. 2.14. Percentage survival rate (95 % confidence interval) from egg to adult of *Frankliniella occidentalis* when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: $F_{(1, 18)}$ = 11.562, p = 0.00319). The different letters above the error bars denote significant differences between treatments (p < 0.05).

Female *F. occidentalis* was more abundant on both hosts, compared to the number of males. A significant difference in the number (p = 0.01243) of females on the two host plants was detected, but there was no significant difference in the number (p = 0.70242) of males between the hosts. More adult females were present on the chrysanthemum leaves (15.9 ± 2.70) than on the green bean pods (5.3 ± 2.70). The number of adult males was also higher on the chrysanthemum leaves (3.9 ± 0.911) than on the green bean pods (3.4 ± 0.911) (Fig. 2.15).

Treatments
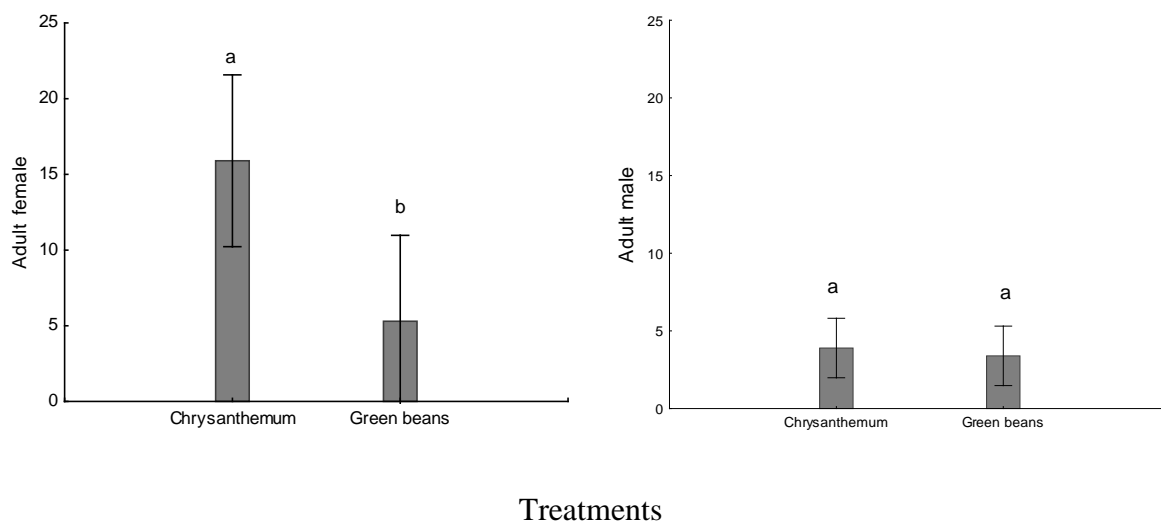
Fig. 2.15. Total number of adult females of *Frankliniella occidentalis* produced when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: $F_{(1, 18)} = 7.7135$, $p = 0.01243$; male: $F_{(1, 18)} = 0.1570$, $p = 0.70242$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

The duration of incubation and larval stages did not differ significantly ($p = 0.81936$) between the two host plants (Fig. 2.16). The incubation period of *F. occidentalis* eggs varied between the chrysanthemum leaves (5.2 ± 0.35 days) and the green bean pods (5.5 ± 0.35 days). On chrysanthemum leaves the duration of the first instar was shorter (1.7 ± 0.28 days) than on the green bean pods (1.9 ± 0.28 days). The second instar lasted 5.0 ± 0.23 days on the chrysanthemum leaves, and 4.8 ± 0.23 days on the green bean pods. However, the total duration of the larval period (6.7 ± 0.40 days) was similar on the chrysanthemum leaves and on the green bean pods (Fig. 2.16).
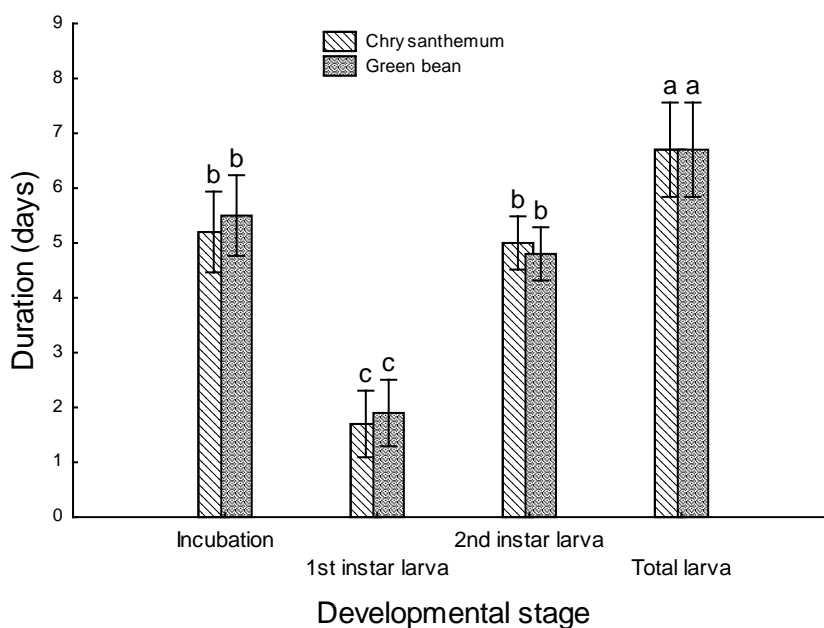
Fig. 2.16. Duration of larval period of *Frankliniella occidentalis*, when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: $F_{(3, 54)} = 0.30818$, $p = 0.81936$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

The incubation period of *F. occidentalis* in the current study, which was derived from the number of larvae to emerge from the eggs, varied from 5.2 to 5.5 days at 25 °C. The larval stages in WFT consist of the first instar, which lasts between 1.7 and 1.9 days, and of the second instar, which lasts 4.8 to 5 days, with the total larval stage lasting for 6.7 days. Duration of the pre-pupal stage varied from 2 to 2.3 days, that of the pupa from 2.2 to 2.7 days, and that of the total pupal stage from 4.2 to 5 days. No significant difference ($p = 0.37366$) was found in the pupal development time on the two host plants, for both the pre-pupa and the pupa, but significant differences in the total pupal stage. (Fig. 2.17).
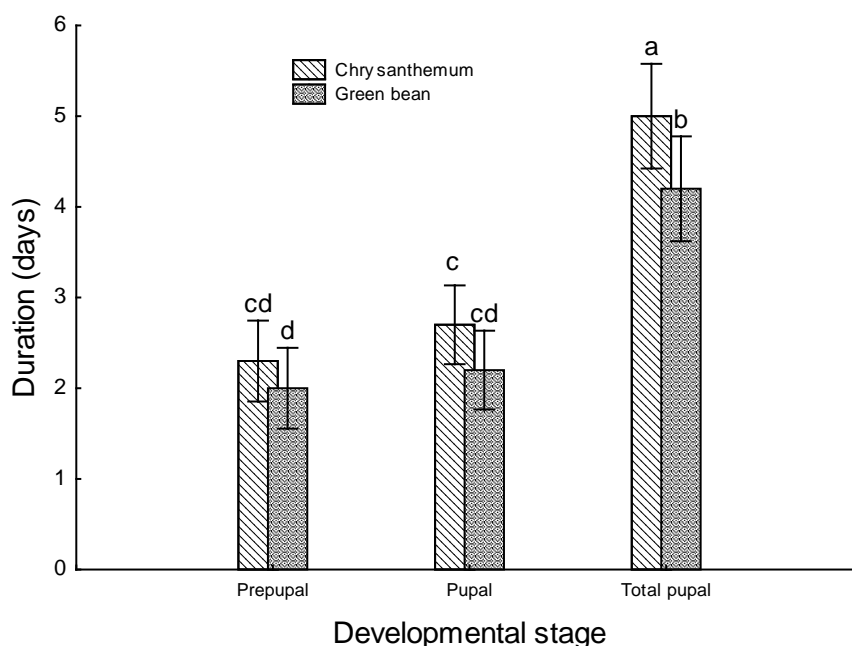
Fig. 2.17. Duration of pupal period of *Frankliniella occidentalis*, when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: $F_{(2, 36)} = 1.0118$, $p = 0.37366$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

The longevity of the female and male on chrysanthemum leaflets and green bean pods varied from 27.2 to 29.1 days and from 18.6 to 18.8 days, respectively. Longevity of both the male and the female did not differ significantly between the different host plants. In the case of the green bean pods, the longevity for the female was 29.1 ± 0.725 days, which is 1.9 days longer than it was on chrysanthemum (27.2 ± 0.725 days). Male longevity on green bean pods was 18.6 ± 0.587 days and 18.8 ± 0.587 days on chrysanthemums (Fig. 2.18).

Treatment
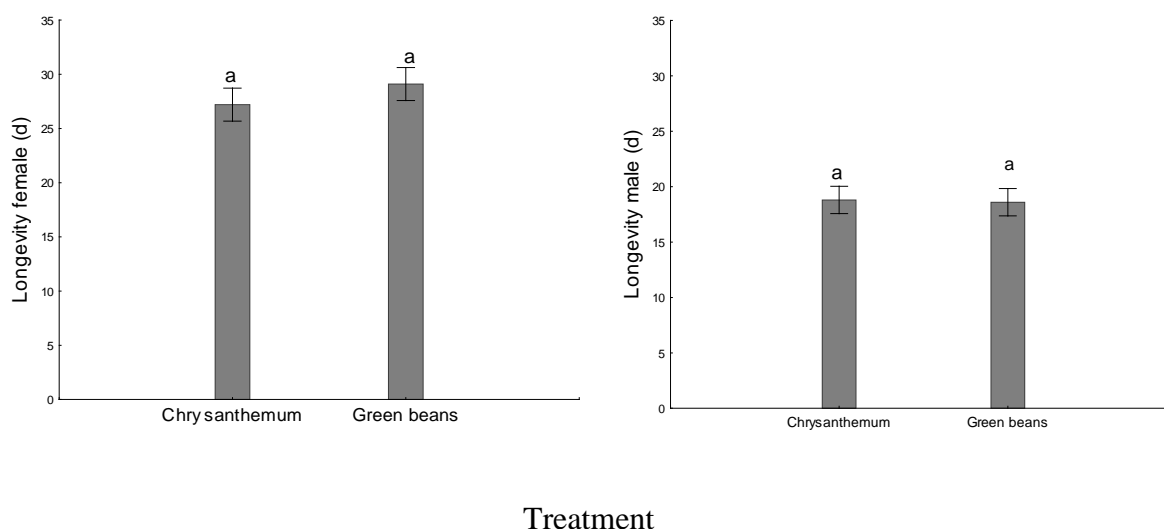
Fig. 2.18. The longevity of the adult female and male of *Frankliniella occidentalis*, when reared on chrysanthemum leaves and green bean pods (one-way ANOVA: female: $F_{(1, 18)} = 3.4381$, p

< 0.08017; male: F $(1, 18) = 0.05806$, $p = 0.81231$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

2.6. DISCUSSION

The identification of WFT is a challenge, and because of its small size and morphological similarity to several other thrips species, it is often incorrectly identified. As WFT is the most economically damaging thrips species in the Thripidae family, proper identification and differentiation from other species is important for effective control. The adult female has three colour 'morphs': dark-brown, light brown and intermediate (Cluever *et al.* 2015), with the colour of the thrips depending on the temperature at which it pupates (Cavalleri & Mound 2012). The most common WFT colour morph is the intermediate, which is yellow, with distinctive light-brown markings arranged medially on each abdominal tergite (Cavalleri & Mound 2012). Based on their morphology, using the relevant keys, adult thrips were collected from chrysanthemums and blueberries grown under cover on commercial farms in the Western Cape. They were identified as *F. occidentalis*, with the intermediate colour (light brown) being the dominant one. Although some of the morphology is generally uniform for the order Thysanoptera, the major morphometric key characters for *F. occidentalis* were confirmed.

Brunner *et al.* (2002), Brunner & Frey (2010) and Suganthy *et al.* (2016) demonstrated the application of the mtCOI gene for the differentiation of thrips species to be a success. The amplicon of *F. occidentalis* in the current study was less than 400 bp, with the reduced amplicon size being due to differences in the primer binding region, which agrees with the results that were obtained by Suganthy *et al.* (2016), in terms of which they discerned under 500 bp. The diverse nature of the population is relatively evident in phylogenetic analysis, in terms of which the grouping of thrips into one main cluster was observed. Morphological identification was verified, using molecular identification, with the following species being identified during the study: *F. occidentalis*, *G. ficorum*, *P. inchini*, and an unknown thrips species. The clustering distinctively showed that the sequences were from the Order Thysanoptera. The main cluster branched into clades showing three families: Thripidae, Phlaethripidae, and Aeolothripidae. All species of the Aeolothripidae family formed a monophyletic group. The *Thrips* sp. clustered with another *Thrips* sp. from GenBank with an identity of 84 %, suggesting that the former might be an unknown species. The species *F. occidentalis* were all in one cluster, with 100 % identity. Their reference sequences from GenBank were originally from China, Australia, Mexico, and Kenya, with one having previously being found in South Africa. The above indicates that the species present in South Africa might have been introduced from the above-mentioned countries by way of global trade. *Frankliniella occidentalis* WFTS1 branched

separately, together with closely related species from China and Kenya. The two species of *F. occidentalis* collected from the two localities, from a distance of about 150 km apart, showed no variation, which meant that they were from the same population. Brunner & Frey (2010) found that *F. occidentalis* populations showed no isolation by distance pattern, with their variation being due to their habitat. From the limited number of *F. occidentalis* that were analysed from the two localities, no clear indication could be given of the different populations of *F. occidentalis* from the two localities.

The development of *F. occidentalis* is host dependent (Reitz 2009). Different plant species vary in terms of their suitability as hosts for *F. occidentalis* (Brown *et al.* 2002; Zhang *et al.*, 2007). *Frankliniella occidentalis* was able to complete its life cycle on both the chrysanthemums and the green beans, but the two host plants significantly affected the oviposition of WFT. More first instar larva hatched on the chrysanthemum leaves than on bean pods, possibly because the chrysanthemum leaves were more attractive to ovipositing females, and the survival rate to the adult stage was also higher on chrysanthemum leaves. All developmental stages periods were not significantly different from each other except for the total pupal stage. The incubation period and larval period developed most rapidly on chrysanthemum leaves than on green beans, but the pupal stages period was shorter in green beans than in chrysanthemums. The differences in the prepupal and pupal periods were not significant but significant in the total pupal period. Because the pupal stage is a non-feeding stage, it could not have had preference in feeding on the different hosts. The faster larval developmental rates, larger number of eggs and larvae and higher survival rate indicate that chrysanthemum leaves was a more suitable host than bean pods. The faster the developmental rates and the higher the fecundity of insects on a host plant, the more suited the host plant is to the insects (Van Lenteren & Noldus 1990). Egg hatching is affected by the quality of the food and by the varying morphology of the plant (Zhang *et al.* 2007). Numerous studies suggest that plant defences, both morphological and chemical, are responsible for host selection by herbivorous insects, like thrips (Brown & Simmonds 2006). A study of the comparison between *P. vulgaris* pods and leaves showed the superiority of the pods to the leaves for WFT (Zhi *et al.* 2010). Shan *et al.* (2012) compared the development of three bean host species, *Canavalia gladiata (Jacq.)* (sword bean), *Lablab purpureus (L.)* (lablab), and *P. vulgaris* (French bean), with the number of eggs hatched being 543, 296 and 85, respectively, when forty female WFT were introduced. WFT prefers feeding on flowers of plants, as it prefers enclosed spaces. However, flowers cannot be kept fresh for long periods of time, which limits the rearing of this thrips on flowers. Microclimatic conditions are also important for successful rearing of thrips,

especially temperature and relative humidity and these vary according to species and growth stage. Having adequate light and ventilation is key to egg hatching (Loomans & Murai 1997). When rearing WFT in culture for experimental purposes, it is important to ensure sufficient light and ventilation. Diapause is linked with photoperiod in thrips, but research on *F. occidentalis* shows that this species undergoes no reproductive diapause (Brødsgaard 1994; Ishida *et al*. 2003). In *Frankliniella intosa* (Trybom)*,* reproductive diapause of adult females reared under long photoperiod began to oviposit within three days after emergence, but they did not oviposit for more than 20 days under short photoperiod (Murai 1988), and this shows how short photoperiod can induce diapause.

Significant differences were found in the survival rates from egg to adult between the two host plants in the study, with a higher survival rate on chrysanthemum than on green beans. Shan *et al*. (2012), in their study, found no significant difference in the survival rates of the first to second instar, the egg period, and the second instar period between the three hosts and the survival rates of WFT on *L. purpureus* (60.6 %), and on *P. vulgaris* (44.2 %). MacDonald (2003) found that, of the 40 larvae isolated, only 17 reached adulthood in a bean pod culture. The rates of survival, reproduction and development of herbivorous insects like thrips are linked to the nitrogen levels in plants (Strong *et al.* 1984) and, within a plant, the levels of proteins and carbohydrates may vary, which could influence the preferred feeding sites of thrips (Ullman *et al.* 1992). The results from the current study showed that the female *F. occidentalis* were more abundant on both hosts, compared to the males. Baez *et al*. (2011) also found a high female-biased ratio of *F. occidentalis* in peppers. On chrysanthemums, adult male longevity was longer than it was on the green bean pods, with female longevity being shorter on chrysanthemums, compared to on green beans and attributed to the food quality and morphology of the two hosts. Longevity is usually longer in natural environments compared to the artificial environments, because of factors like temperature, space stress, and the lack of suitable humidity (Grundy *et al*. 2000).

In the current study, *F. occidentalis* was successfully identified using both morphological and molecular methods. Since morphological identification is time-consuming and difficult for some species, and given that taxonomic expertise is getting scarce and not always readily available, it is a valuable alternative to use molecular identification, which, in the current study, was used to verify all other species found in the study locations. PCR was successfully used to identify WFT accurately and verify the morphological identification. Although quicker and accurate, the expense is significant and sometimes a unique mtCOI will not supply ample resolution. The use of a combination of PCR and morphological characteristics seems to be a

better option, especially using morphological keys for day-to-day identification, but also regular use of molecular identification as a kind of quality control. *Frankliniella occidentalis* showed preference for chrysanthemum leaves compared to green bean pods, although the precise morphological and chemical characteristics of the hosts that influence the host selection behaviour of the thrips were not determined. When rearing WFT for laboratory bio-assays it is important to use the most suitable host plant that is practical to obtain as many insects as possible and to ensure sufficient light and ventilation, which is key for egg hatching. From these results and literature it is evident that WFT can be expected to increase more rapidly on more suitable host plants and that WFT can develop to epidemic levels faster on more suitable hosts than on less suitable ones.

2.7.   REFERENCES

BAEZ, I., REITZ, S.R., FUNDERBURK, J.E. & OLSON, S.M. 2011. Variation within and between *Frankliniella* thrips species in host plant utilization. *Journal of Insect Science* **11**: 1–18. 1–18. DOI: 10.1673/031.011.0141

BRODBECK, B.V. FUNDERBURK, J. STAVISKY, J. ANDERSEN, P.C. & 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, Australian National Insect Collection (ANIC), Can-berra, 145–153

BRØDSGAARD, H. F. 1994. Effect of photoperiod on the bionomics of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). Journal of Applied Entomology **117**: 498–507. https://doi.org/10.1111/j.1439-0418.1994.tb00767.x

BROWN, A.S. SIMMONDS, M.S. & BLANEY, W.M., 2002. Relationship between nutritional composition of plant species and infestation levels of thrips. *Journal of Chemical Ecology* **28**: 2399–2409. DOI https://doi.org/10.1023/A:1021471732625

BROWN, A.S. & SIMMONDS, M.S. 2006. Leaf morphology of hosts and non-hosts of the thrips *Heliothrips haemorrhoidalis* (Bouché). *Botanical Journal of the Linnean Society* **152**: 109–130. DOI: https://doi.org/10.1111/j.1095-8339.2006.00560.x

BRUNNER, P.C., FLEMING, C. & FREY, J.E. 2002. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing

and a PCR-RFLP-based approach. *Agricultural and Forest Entomology* **4**: 127–136. DOI: https://doi.org/10.1046/j.1461-9563.2002.00132.x

BRUNNER, P.C. & FREY, J.E. 2010. Habitat-specific population structure in native western flower thrips, *Frankliniella occidentalis* (Insecta, *Thysanoptera*). *Journal of Evolutionary Biology* **23**: 797–804. doi:10.1111/j.1420-9101.2010.01946.x

CAVALLERI, A. & MOUND, L.A. 2012. Toward the identification of *Frankliniella* species in Brazil (Thysanoptera, Thripidae). *Zootaxa* **3270**: 1–30.

CHAISUEKUL, C. & RILEY, D.G. 2005. Host plant, temperature, and photoperiod effects on ovipositional preference of *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae). *Journal of Economic Entomology* **98**: 2107–2113. DOI: https://doi.org/10.1603/0022-0493-98.6.2107

CLOYD, R.A., WARNOCK, D.F. & HOLMES, K. 2001. Technique for collecting thrips for use in insecticide efficacy trials. *HortScience* **36**: 925–926.

CLUEVER, J.D., SMITH, H.A., FUNDERBURK, J.E. & FRANTZ G. 2015. Western flower thrips (*Frankliniella occidentalis* [Pergande]). http://edis.ifas.ufl.edu. 11.09.2017.

DE GRAAF, H.E. & WOOD, G.M. 2009. An improved method for rearing western flower thrips *Frankliniella occidentalis*. *Florida Entomologist* **92**: 664–666. DOI: https://doi.org/10.1653/024.092.0424

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**: 783–791. DOI: 10.1111/j.1558-5646.1985.tb00420.x

FUNDERBURK, J., DIFFIE, S., SHARMA, J., HODGES, A. & OSBORNE, L. 2007. *Thrips of Ornamentals in the Southeastern US*. University of Florida, IFAS, ENY–845 (IN754).

GAMES, P.A. & HOWELL, J. F. 1976. Pair wise multiple comparison procedures with unequal numbers and/or variances. *Journal of Educational Statistics* **1**:13–125. DOI: 10.2307/1164979

GAUM, W.G., GILIOMEE, J.H. & PRINGLE, K.L. 1994. Life history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on English cucumbers. *Bulletin of Entomological Research* **84**: 219–224. DOI: 10.1017/S0007485300039729

GERIN, C., HANCE, T. & IMPE, G.V. 1994. Demographical parameters of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Journal of Applied Entomology* **118**: 370–377. DOI: https://doi.org/10.1111/j.1439-0418.1994.tb00814.x

GLOVER, R.H., COLLINS, D.W., WALSH, K. & BOONHAM, N. 2010. Assessment of loci for DNA barcoding in the genus Thrips (*Thysanoptera*: *Thripidae*). *Molecular Ecology Resources* **10**: 51–59. DOI: https://doi.org/10.1111/j.1755-0998.2009.02723.x

GRUNDY, P., MAELZER, A., BRUCE, A. & HASSAN, E. 2000. A mass-rearing method for the assassin bug *Pristhesancus plagipennis* (Hemiptera: Reduviidae). *Biological Control* **18**: 243–250. DOI: https://doi.org/10.1006/bcon.2000.0832

ISHIDA, H., MURAI, T., SONODA, S., YOSHIDA, H., IZUMI, Y. & TSUMUKI, H. 2003. Effects of temperature and photoperiod on development and oviposition of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Applied entomology and zoology* **38***: 65–68. https://doi.org/10.1303/aez.2003.65

KANARA, H.G. & ACHARYA, M.F. 2014. Bionomics of rose thrips, *Frankiliniella occidentalis* Pergande. *Journal of Horticulture* **1**: 1–3. DOI: 10.4172/2376-0354.1000109

KARNKOWSKI, W. & TRDAN, S. 2002. Diagnostic protocols for regulated pests. *Bulletin OEPP/EPPO Bulletin* **32**: 241–243.

KATAYAMA, H. 1997. Effect of temperature on development and oviposition of western flower thrips *Frankliniella occidentalis* (Pergande). *Japanese Journal of Applied Entomology and Zoology* **41**: 225–231. DOI: https://doi.org/10.1303/jjaez.41.225

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870–1874. 1870–1874. DOI: 10.1093/molbev/msw054

LEE, S.J., KIM, S., KIM, J.C., LEE, M.R., HOSSAIN, M.S., SHIN, T.S., KIM, T.H. & KIM, J.S. 2017. Entomopathogenic *Beauveria bassiana* granules to control soil-dwelling stage of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *BioControl* **62**: 639–648. DOI: 10.1007/s10526-017-9818-8

LEVENE, H. 1960. Robust testes for equality of variances. In: Olkin, I. (Ed.) *Contributions to Probability and Statistics.* 278–292. Stanford University Press, Palo Alto, CA.

LIM, U.T., VAN DRIESCHE, R.G. & HEINZ, K.M. 2001. Biological attributes of the nematode, *Thripinema nicklewoodi*, a potential biological control agent of western flower thrips. *Biological Control* **22**: 300–306. DOI: https://doi.org/10.1006/bcon.2001.0979

LOOMANS, A.J. & MURAI, T. 1997. Culturing thrips and parasitoids. In: Lewis, T. (Ed.) *Thrips as Crop Pests*. 639–666. CAB International, Wallingford, UK.

MACDONALD, K.M. 2003. *The Alarm Pheromone of the Western Flower Thrips*. PhD thesis, Keele University, Keele, Staffordshire, UK.

MORITZ, G. 1994. Pictorial key to the economically important species of Thysanoptera in Central Europe. *Bulletin OEPP/EPPO* **24**: 181–208.

MORITZ, G. 2001. The biology of thrips is not the biology of their adults: a developmental view. *Proceedings of the 7th International Symposium on Thysanoptera, Reggio Calabria, Italy*, 2–7 July 2001. 259–267.

MORITZ, G., MORRIS, D. & MOUND, L. 2001. *Thrips ID: Pest Thrips of the World*. CSIRO Publishing, Collingwood, Australia.

MOUND, L.A. 2009. *Thysanoptera* (Thrips) of the world-a-checklist. www.ento.csiro.ou/thysanoptera /worldthrips.html.

MURAI, T. 1988. Studies on the ecology and control of flower thrips, *Frankliniella intonsa* (Trybom). Bulletin of Shimane Agricultural Experimental Station 23: 1–73

NEI, M. & KUMAR, S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

REED, T., ALLEN, C., BAGWELL, R., COOK, D., BURRIS, E., FREEMAN, B., LEONARD, R. & LENTZ, G. 2006. *A Key to the Thrips (Thysanoptera: Thripidae) on Seedling Cotton in the Mid-Southern United States*. https://www.cottoninc.com/wp-content/uploads/2015/12/REED-J-et-al-A-Key-to-the-Thrips-Thysanoptera-Thripidae-on-Seedling-Cotton-in-the-Mid-Southern-United-Staates-2006.pdf.

REITER, D., FARKAS, P., SOJNOCZKI, K. & FAIL, J. 2015. Laboratory rearing of *Thrips tabaci* Lindeman: a review. *Die Bodenkultur* **66**: 33–40.

REITZ, S.R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): the making of a pest. *Florida Entomologist* **92**: 6–13. DOI: https://doi.org/10.1653/024.092.0102

RILEY, D.G., JOSEPH, S.V., SRINIVASAN, R. & DIFFIE, S. 2011. Thrips vectors of tospoviruses. *Journal of Integrated Pest Management* **2**: I1–I10. https://doi.org/10.1603/IPM10020

ROBB, K.L. & PARRELLA, M.P. 1991. Western flower thrips, a serious pest of floricultural crops. In: Parker, B.L., Skinner, M. & Lewis, T. (Eds) *Towards Understanding the Thysanoptera*. General Technical Report NE-147. 343–358. US Department of Agriculture, Forest Service, Radnor, PA.

SHAN, C., MA, S., WANG, M. & GAO, G. 2012. Evaluation of insecticides against the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), in the laboratory. *Florida Entomologist* **95**: 454–460. DOI: https://doi.org/10.1653/024.095.0229

SIGUNA, A.D. 2007. Cultural and botanical methods for the management of thrips on French beans *Phaseolus vulgaris*. Thesis M.Sc. (Agricultural Entomology), School of Pure and Applied Sciences, Kenyatta University, Nairobi. URI: http://hdl.handle.net/123456789/137

STATSOFT INC. 2016. *STATISTICA (Data Analysis Software System) Version 13.0*. StatSoft, Tulsa, OK.

STRONG, D.R., LAWTON, J.H. & SOUTHWOOD, T.R. 1984. *Insects on Plants: Community Patterns and Mechanisms*. Blackwell Scientific Publications, Oxford, United Kingdom.

SUGANTHY, M., RAGESHWARI, S., SENTHILRAJA, C., NAKKEERAN, S., MALATHI, V.G., RAMARAJU, K. & RENUKADEVI, P. 2016. New record of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in South India. *International Journal of Environment, Agriculture and Biotechnology*, **1**(4): 857–867. DOI: http://dx.doi.org/10.22161/ijeab/1.4.33

TYAGI, K. & KUMAR, V. 2015. First report of western flower thrips, *Frankliniella occidentalis* (Pergande) (*Thripidae*: *Thysanoptera*) from India – a potential havoc to Indian agriculture. *Halteres* **6**: 1–3.

TYAGI, K., KUMAR, V., SINGHA, D., CHANDRA, K., LASKA, B.A., KUNDU, S., CHAKRABORTY, R. & CHATTERJEE, S. 2017. DNA barcoding studies on thrips in

India: cryptic species and species complexes. *Scientific Reports* **7**: 48–98. DOI: 10.1038/s41598-017-05112-7

ULLMAN, D.E., CHO, J.J., MAU, R.F., HUNTER, W.B., WESTCOT, D.M. & CUSTER, D.M. 1992. Thrips-tomato spotted wilt virus interactions: morphological, behavioural and cellular components influencing thrips transmission. *Advances in Disease Vector Research* **9**: 195–240. DOI: 10.1007/978-1-4612-2910-0_6

US NATIONAL LIBRARY OF MEDICINE. NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. [s.d.]. *Basic Local Alignment Search Tool*. https://blast.ncbi.nlm.nih.gov/Blast.cgi.

VAN LENTEREN, J.C. & NOLDUS, L.P. 1990. Whitefly-plant relationships: behavioural and ecological aspects. In: Gerling, D (Ed.) *Whiteflies: Their Bionomics, Pest Status and Management*. 47–89. Intercept, Andover, UK,

VIERBERGEN, G., KOCH, J. & LOOMANS, A. 2012. Morphological identification of *Thrips palmi* in the frame of ISO/IEC 17025. *Acta Phytopathologica et Entomologica Hungarica* **47**: 167–178. DOI: 10.1556/APhyt.47.2012.1.21

WANG, C., LIN, F., CHIU, Y. & SHIH, H. 2010. Species of *Frankliniella Trybom* (Thysanoptera: Thripidae) from the Asian-Pacific Area. *Zoological Studies* **49**(6): 824–838.

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* (Thysanoptera: Thripidae), on five different vegetable leaves. *Journal of Applied Entomology* **131**: 347–354. DOI: https://doi.org/10.1111/j.1439-0418.2007.01186.x

ZHI, J.R., LI, J.Z. & GAI, H.T. 2010. Life table for experimental population of *Frankliniella occidentalis* feeding on leguminous vegetables. *China Bulletin of Entomology* **47**: 313–317.

Chapter 3

Efficacy of entomopathogenic nematodes for control of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) under laboratory conditions

Abstract

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an important field and greenhouse pest of many crops worldwide. To control *F. occidentalis* is extremely challenging, because of its cryptic behaviour, short life cycle and resistance to many insecticides. The use of entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabdidae) for controlling thrips has gained importance in some European countries, and, hence, has attracted interest in South Africa. The objective of the study was to screen different South African isolates of EPNs against different life stages of WFT. A total of 11 EPN species reported from South Africa, and the exotic *Steinernema feltiae,* were tested for pathogenicity against WFT under laboratory conditions. Virulence against *F. occidentalis* in laboratory bioassays ranged from 11 % to 67 %. Generally, however, *Heterorhabditis* spp. were more virulent than the *Steinernema* spp. *Heterorhabditis baujardi* was found to be the most potent species, resulting in mortality of 67 %, although it was not significantly different from *Steinernema yirgalemense* (66 % mortality). Bioassays for determining infection were performed using different life stages (larva, pupa and adult) of *F. occidentalis* with *S. yirgalemense*, *H. baujardi* and *Steinernema jeffreyense*. The pupae of western flower thrips (WFT) were found to be more sensitive to nematode infection than were either the larvae or the adults. The highest mortality against WFT was recorded for the pupae (72 %) when applying 100 IJs/insect of *H. baujardi*, and the lowest was recorded for treatment with *S. jeffreyense* (17 %). *Steinernema yirgalemense* and *H. baujardi* were tested at concentrations of 0, 10, 20, 40, 80, and 160 IJs/larva. Increasing EPN concentrations gave increased thrips mortality, with a probit analysis indicating *S. yirgalemense* to be 5.49 more potent than *H. baujardi*. Results from the temporal development study showed that both *S. yirgalemense* and *H. baujardi* were able to complete their life cycles in the host within five days, and to produce a new cohort of IJ. The study showed that *S. feltiae* did not perform well compared to the local EPNs under optimum laboratory conditions, and that locally isolated *S. yirgalemense, H. baujardi* and *H. bacteriophora* have outstanding potential for the control of

F. occidentalis, in terms of targeting the soil-dwelling stages, as they gave the best control, and could be tested further under field conditions.

Keywords: Entomopathogenic nematodes (EPNs), virulence, life stages, temporal development, bioassays.

3.1.   INTRODUCTION

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is native to the west coast of California, United States of America and was first recorded in South Africa on chrysanthemums (Asterales: Asteracece) near Krugersdorp in 1987 (Giliomee 1989). According to Kirk & Terry (2003), since the late 1970s, the WFT has spread from its original distribution range to become a major worldwide crop pest, with its spread being attributable to the movement of horticultural material like cuttings, seedlings and potted plants.

Frankliniella occidentalis severely damages ornamentals and vegetables directly, through feeding and ovipositioning, and indirectly by transmitting virus diseases (e.g. tomato spotted wilt virus), thus causing extensive losses in the form of reduced yield and/or market value (Duan *et al.* 2013). The high impact of WFT is due to its extreme polyphagy, rapid developmental cycle (which ranges from 14-21 days, depending on temperature), high reproductive rate, small size, and cryptic habits, occurring especially in growing tips and flower buds (Manners *et al.* 2013).

The WFT is an extremely challenging pest to control, because it develops resistance to insecticides rapidly (Lewis 1997), hence biological control has become increasingly important for successful WFT management programmes. Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabdidae) have become an option for biocontrol. EPNs from the Heterorhabdidae and Steinernematidae families, which are widely distributed in soils throughout the world, are one of the best non-chemical alternatives for insect pest control (Campos-Herrera & Guierrez 2008; Hominick 2002; Kaya *et al.* 2006).

The use of EPNs for controlling thrips has gained importance in some European countries (Kaya *et al.* 2006). Research into EPNs to control pests in South Africa has been done on other insects, but not yet on WFT. The efficacy of EPN species varies according to different host insects, abiotic and biotic factors. Previous studies have shown a high susceptibility of the soil-dwelling stages of WFT to EPNs (Ebssa *et al.* 2001), and, recently, the efficacy of the nematode *Steinernema feltiae* Wouts, Mráček, Gerdin & Bedding, has been intensively studied against the above-ground stages of thrips (Laznik & Trdan 2008). Other EPN species that have been

69

tested include *Steinernema carpocapsae* (Weiser) Wouts, Mráček, Gerdin & Bedding, *Steinernema glaseri* Wouts, Mráček, Gerdin & Bedding, and *Heterorhabditis bacteriophora* Poinar. *Steinernema feltiae* has shown outstanding efficacy in the control of WFT in most European countries, hence its commercialisation for WFT control. Limitations do, however, exist in the use of the nematode, as, of almost all African countries, it has only been isolated in Algeria (Tarasco *et al.* 2009), leading to it being proscribed from importation into South Africa. *Steinernema feltiae* also has other biological limitations in terms of higher temperatures (Jagdale *et al*. 2004) experienced in undercover production in warmer climates, since it is a cool-temperature-active nematode. This limitation may also apply to other EPNs considered as potential biocontrol agents.

The use of South African isolated EPN species adapted to the local environment was hypothesised as a potential option for enhanced control of WFT. The objective of the current study was to screen available isolates of EPNs for efficacy against the different life stages of WFT, to determine the optimum concentration for the best performing EPNs in the laboratory and to determine whether they can complete their life cycles in WFT.

3.2. MATERIALS AND METHODS

3.2.1 Source of thrips

Frankliniella occidentalis was obtained from blueberries and chrysanthemums cultivated on commercial farms in the Western Cape Province, South Africa. Collecting adult thrips from the localities was done by means of sampling flowers and leaves, which were shaken into a clean, white container, to dislodge the thrips. The thrips were then taken to the laboratory for morphological identification to confirm species identity, and for a laboratory culture.

3.2.2 Thrips culture

To ensure a constant, reliable supply of thrips for experiments, a laboratory culture was established on a diet of insecticide-free chrysanthemum (*Dendranthema grandiflora*) flowers obtained from a commercial farm. The thrips were kept in a plastic container ($20 \times 20 \times 30$ cm^3), with a screened hole on top, and maintained in a controlled environmental chamber (25 ± 2 °C, 60-70 % RH). To prevent desiccation, the flowers were kept in a petri dish, together with a layer of moist tissue paper. The thrips were provided with fresh flowers weekly, and the old flowers removed to maintain conditions conducive for high fecundity. To obtain the desired life stages of *F. occidentalis* for the different experiments, petri dishes (11 cm in diameter, and 3 cm in height) were used for breeding the thrips. Fresh green leaves of chrysanthemum plants

70

were placed in the petri dish and 20 to 30 adult females of *F. occidentalis* were extracted from the stock culture, and transferred to each of the petri dishes for egg laying. The petri dishes were lined with moistened filter paper to keep the leaves fresh. After 24 h, the adult females were removed to a new petri dish to obtain more eggs. The eggs from the petri dishes were kept in an incubator at the above-mentioned climatic conditions, for development until the thrips reached the desired life stage for experiments.

3.2.3 Nematode cultures

All EPN species were sourced from the Department of Conservation Ecology and Entomology, Nematology Laboratory collection held at Stellenbosch University (Malan *et al*. 2006, 2011; Abate *et al*. 2018), comprising nematodes collected during previous surveys, except *S. feltiae* which had originally been obtained from e-nema, Schwentinental, Germany. (Table 3.1). Infective juveniles (IJs) of the selected species were cultured *in vivo* at room temperature*,* using the last instar of the greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (Griffin *et al*. 2005). The rearing and harvesting procedures for the infective juveniles (IJs) were conducted according to the methods employed by Kaya & Stock (1997). The IJs from white traps, which were harvested within the first week of emergence, were stored horizontally in 500-ml vented culture flasks, containing approximately 150 ml distilled water at 14 °C. The nematodes were used within a month after harvesting. To aid in aeration and nematode survival during storage, the culture flasks were shaken weekly. The nematode concentrations used for different experiments were calculated using the equation developed by Navon & Ascher (2000).

Table 3.1. Locally isolated *Steinernema* and *Heterorhabditis* species (except for *S. feltiae*), isolate number, habitat, origin, Genbank accession number and size.

Species	Isolate	Morphological/ molecular group	Habitat	Origin (town/ province)	Genbank accession number	Length of IJs: mean and range µm
*S. jeffreyense**	J194	bicornutum	Guava tree	Jeffrey's Bay, Eastern Cape	KC897093	924 (784-1043)
S. feltiae	e-nema	feltiae	n/a	Germany	-	876 (766-928)
*S. khoisanae**	SF87	glaseri	Apple orchard	Villiersdorp, Western Cape	DQ314287	1062 (994-1159)
S. yirgalemense	157-C	bicornutum	Citrus orchard	Friedenheim, Mpumalanga	EU625295	635 (548-693)
*S. litchii**	WS9	glaseri	Litchi orchard	Mbombela, Mpumalanga	KP325086	1054 (953-1146)
*S. sacchari**	SB10	cameroonense	Sugarcane	KwaZulu-Natal	KC633095	680 (630-722)
*S. innovationi**	SGI-60	glaseri	Grain field	Free State	KJ578793	1053 (1000-1103)
H. zealandica	SF41	megidis	Natural	Brenton-on-Sea, Western Cape	EU699436	685 (570-740)
H. bacteriophora	SF351	bacteriophora	Grapevine	Wellington, Western Cape	-	588 (512-671)
*H. noenieputensis**	SF699	indica	Fig tree	Noenieput, Northern Cape	JN620538	528 (484-563)
H. baujardi	MT19	indica	Natural vegetation	KwaZulu-Natal	MF535520	551 (497-595)
H. indica	SGS	indica	Grapevine	Bonnievale, Western Cape	GQ377411	528 (497-573)

*Type specimen from South Africa

3.2.4   Screening EPN species for pathogenicity against WFT

A total of 11 EPN species, all reported from South Africa, of which five were indigenous and one, *S. feltiae,* was imported, were tested for pathogenicity against second instar larvae of *F. occidentalis* (Table 3.1). Ten second instar thrips larvae were released onto cell culture dishes with a diameter of 35 × 10 mm, lined with filter paper. All the EPN strains were applied at a concentration of 1000 IJs / 100 µl, (100 IJs per insect) following the introduction of the WFT. The dishes were sealed with PARAFILM® and placed in a plastic container lined with wet paper towels to create 100 % humidity. They were kept in a controlled environment chamber at 25 ± 2 °C. After 24 h and 48 h the thrips were checked for mortality, which was determined by cadaver colour change, and by means of dissecting the dead larvae to determine whether death had occurred due to nematode infection. The control treatment was treated with distilled water only. Each experiment was replicated five times (*n* = 50 insects) and repeated on a different test date, using a fresh batch of nematodes.

3.2.5   EPN efficacy against different life stages

Ten thrips each of three developmental stage (larvae, pupae, adult females or males) were released into separate cell culture dishes with a diameter of 35 × 10 mm, and lined with filter paper. *H. baujardi*, *S. yirgalemense* or *S. jeffreyense* were then applied at a concentration of 1000 IJs / 100 µl into each dish. Thereafter, the dish was sealed with Parafilm®, and placed in a plastic container lined with wet paper towels to maintain 100 % humidity. The container was kept in a controlled environment chamber at 25 ± 2 °C. After 24 h and 48 h the thrips were checked for mortality and dissected to detect the presence of nematodes. Each experiment was replicated five times (*n* = 50 insects), and repeated on a different test date, using a fresh batch of nematodes.

3.2.6   Optimal nematode concentration

Ten second instar WFT larvae were released onto cell culture dishes with a diameter of 35 × 10 mm, and which were lined with filter paper. The two most effective EPN species, *S. yirgalemense* and *H. baujardi*, were inoculated in concentrations of 0, 10, 20, 40, 80, and 160 IJs/larva. Each dish was then sealed with Parafilm® and placed in a plastic container lined with wet paper towels to maintain 100 % humidity. The container was kept in a controlled environment chamber at 25 ± 2 °C. After 48 h, the thrips were checked for mortality and dissected to detect the presence of nematodes. The experiment was repeated for each nematode

species on a different test date, using a fresh batch of nematodes replicated five times ($n = 50$ insects).

3.2.7   Temporal development

Second instar larvae of *F. occidentalis* were inoculated with *S. yirgalemense,* and *H. baujardi* to determine the temporal growth of the EPNs inside the larvae. Ten larvae were transferred to small culture dishes with a diameter of 35×10 mm, which were lined with filter paper, with five culture dishes being used per treatment. A total of 4000 IJs of EPN species / 100 µl of distilled water were inoculated in each culture dish, which was sealed with Parafilm®. The culture dishes were then placed in plastic containers lined with moist tissue paper to create 100 % humidity. They were then transferred to the growth chamber at 25 ± 2 °C. After 24 h, the dead larvae were removed and rinsed with distilled water to remove the surface nematodes. The procedure was repeated for each EPN species. After each 24 h period, a random petri dish was removed, with the contents being observed under a light stereo microscope. On dissection, each nematode's development was noted. Individual thrips were assessed for colour change, infection and stage of nematode development. After observation, when IJs had started emerging from the larvae, the experiment was terminated.

3.2.8   Statistical analysis

Statistical analyses were conducted using STATISTICA 13.2 software (StatSoft Inc. 2016) for EPN screening and efficacy against different life stages. In the absence of significant differences between the test dates and treatments, the data were pooled and analysed using ANOVA, and a post-hoc comparison of means was undertaken, using Bonferroni's method. A bootstrap multi-comparison was performed (Efron & Tibshirani 1993), in case the residuals were not normally distributed. The data were expressed as weighted means ± standard error for EPN screening and efficacy against different life stage trials, and as least square means ± standard error for the optimum concentration trials. A probit analysis was performed using Polo PC (LeOra Software 1987) to determine the lethal dosages (LD_{50} and LD_{90}) (Finney 1971).

3.3 RESULTS

3.3.1   Screening EPN species for pathogenicity against WFT

Great variation was found in the thrips mortality between the different EPN species, ranging from 11 to 67 % (Fig. 3.1). A significant difference ($F_{(12, 104)} = 99.03$, $p < 0.001$) was found among the treatments with respect to mortality levels after 48 h. *Heterorhabditis baujardi*

caused the highest level of mortality against second instar WFT larvae (67.0 % ± 3.55 %), followed by *S. yirgalemense* (66.0 % ± 3.55 %), and then by *H. bacteriophora* (60.0 % ± 3.55 %). However, mortality did not differ significantly between these three species (p < 0.05). In addition, no significant difference in mortality (p < 0.05) was found between *S. feltiae*, *S. sacchari*, *S. litchii*, *S. khoisanae*, *S. innovationi*, and *S. jeffreyense*. The lowest mortality was obtained with *S. jeffreyense* (11.0 % ± 3.55 %).
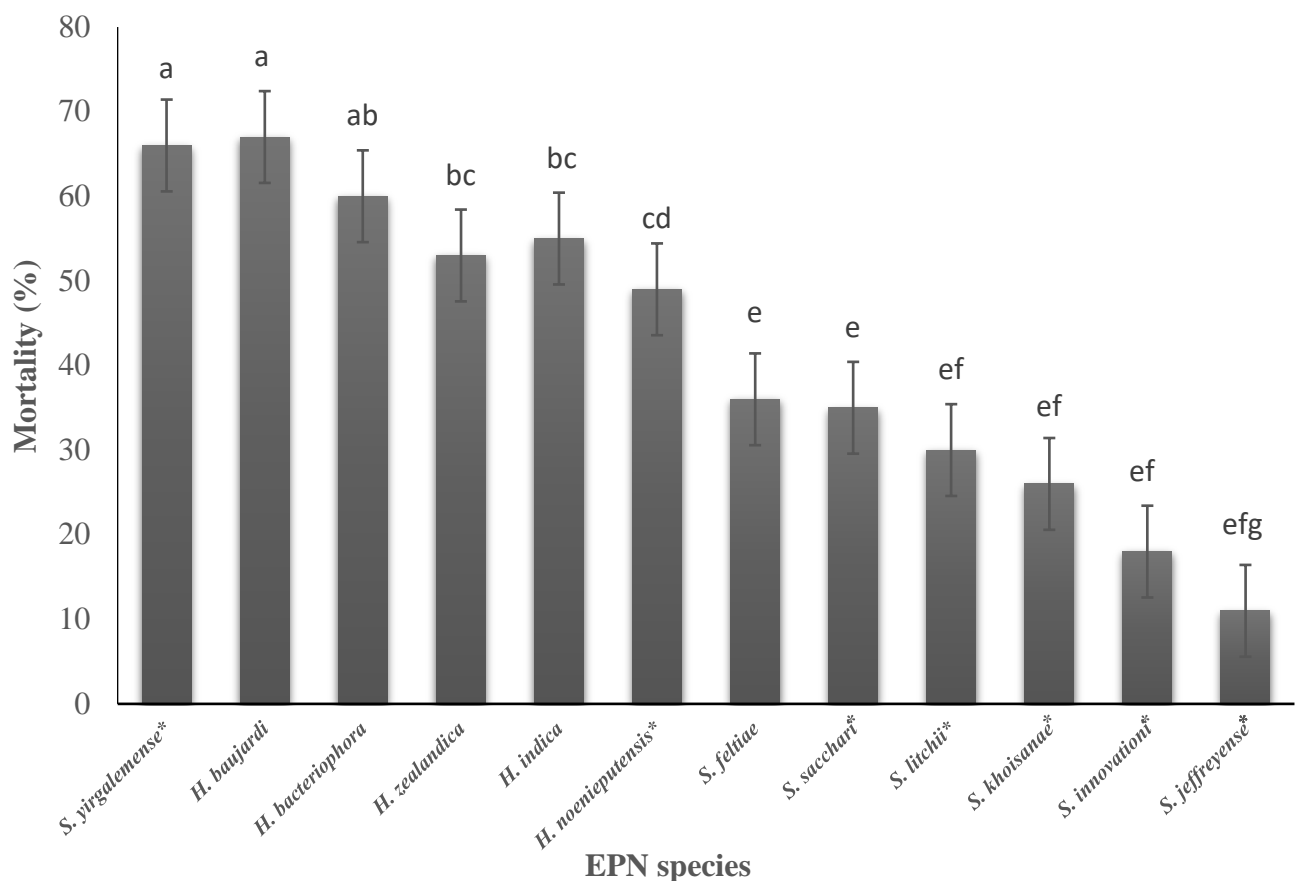
Fig. 3.1. Mean percentage mortality (95 % confidence level) for second instar *Frankliniella occidentalis* larvae treated with different species of entomopathogenic nematodes at a concentration of 100 IJs/insect (one-way ANOVA; $F_{(12, 104)}$ = 99.03, p < 0.001). An asterisk (*) denotes South African EPN species. The different letters above the error bars denote significant differences between treatments (p < 0.05).

3.3.2   EPN efficacy against different life stages of WFT

The results of the two-way ANOVA (treatments and life stages) on the mortality through infection showed no significant differences ($F_{(4, 81)}$ = 3.4929, p = 0.01106) between the

different treatments (*S. yirgalemense, H. baujardi* and *S. jeffreyense*) at the different developmental stages: larva, pupa and adult (Fig. 3.2). The pupal stage showed higher susceptibility to EPNs than the larval and adult stages. *Heterorhabditis baujardi* had the highest efficacy against the pupal stage (72.0 % ± 4.04 %) of WFT, followed by *S. yirgalemense* (65.0 % ± 4.04 %), but not significantly p < 0.05 from each other and, lastly, *S. jeffreyense* (49.0 % ± 4.04 %). *Heterorhabditis baujardi* was also most effective against the larval stage (66.0 % ± 4.04 %), compared with *S. yirgalemense* (43.00 % ± 4.04 %) and *S. jeffreyense* (20.0 % ± 4.04 %). Efficacy of *H. baujardi* against the pupal and larval stages did not differ significantly (p < 0.05).
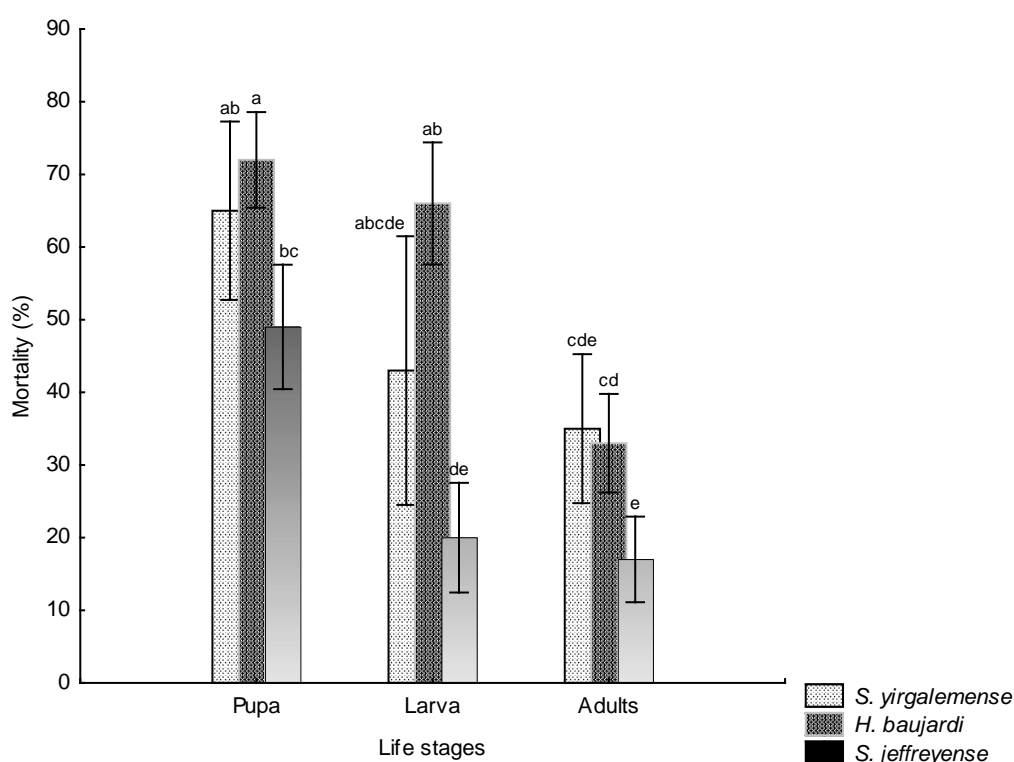
Fig. 3.2. Mean percentage mortality (95 % confidence intervals) 48 h after treatment of three different life stages (second instar larva, pupa and adult) of *Frankliniella occidentalis* with *Steinernema yirgalemense, Heterorhabditis baujardi* and *Steinernema jeffreyense* at a concentration of 100 IJs/insect (two-way ANOVA; $F_{(4, 81)} = 3.4929$, p = 0.01106). The different letters above the error bars denote significant differences between treatments (p < 0.05).

3.3.3. Optimal nematode concentration

For both *S. yirgalemense* and *H. baujardi* an increase in mortality was observed with increasing concentrations of nematodes (Fig. 3.3). However, the response of the two species differed significantly ($F_{(5, 48)} = 1.7971$, p < 0.001), although mortality for the control treatments (0 IJs/larva) did not differ significantly (p < 0.05). The highest mean percentage mortality was

achieved at the highest concentration of 160 IJs/larva with 70.0 % ± 4.80 % for *S. yirgalemense* and 52.0 % ± 4.80 % for *H. baujardi*. For both *S. yirgalemense* and *H. baujardi*, mortality at 80 and 160 IJs/larva did not differ significantly.
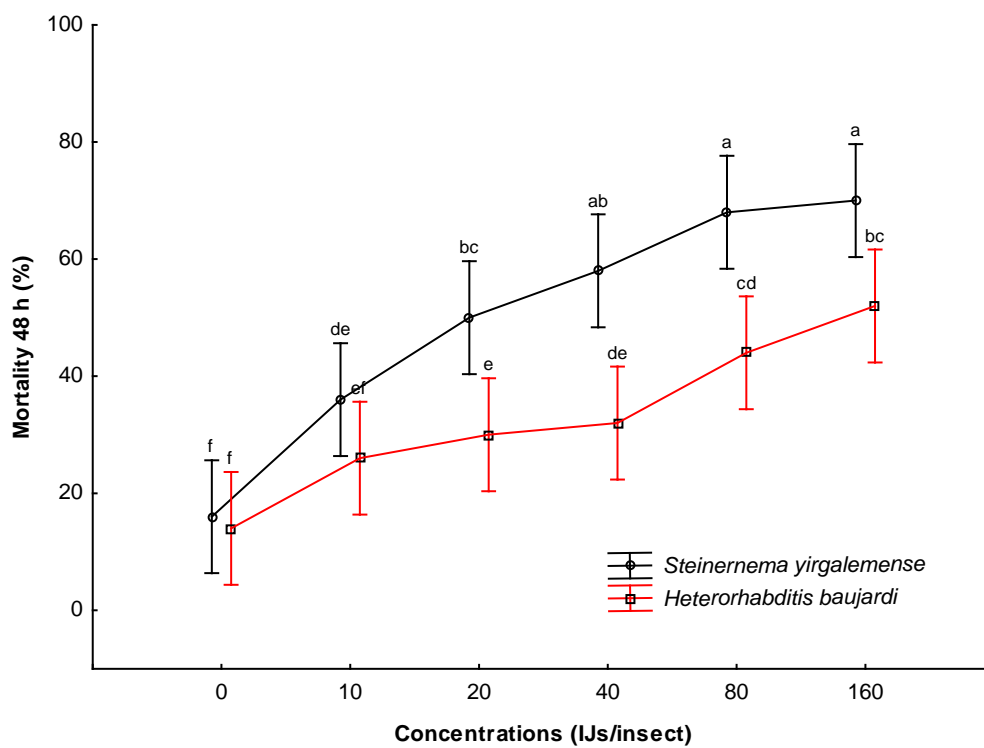
Fig. 3.3. Mean percentage mortality (95 % confidence interval) after treatment of second instar larvae of *Frankliniella occidentalis* with two nematode species, *Steinernema yirgalemense* and *Heterorhabditis baujardi,* at different concentrations (0, 10, 20, 40, 80, and 160 IJs/larva) for 48 h (one-way ANOVA: ($F_{(5, 48)}$ = 1.7971, p < 0.001). The different letters above the error bars denote significant differences between treatments (p < 0.05).

The probit analysis showed that the lethal dosage for *S. yirgalemense* and *H. baujardi* differed. The probit regression line for *S. yirgalemense* was $y = 0.833\,x + 3.56$ and for *H. baujardi* it was $y = 0.833\,x + 2.99$ (Fig. 3.4). *Steinernema yirgalemense* was more potent than was *H. baujardi* with a potency of 5.49, which implies that the former was almost five times more potent than the latter. The LD_{50} and LD_{90} were determined for *S. yirgalemense* and *H. baujardi*, with the LD_{50} for *S. yirgalemense* being the lowest at 45.24 IJs/larva (90 % fiducial limits: 23.60-80.52) and the LD_{90} being 1349.6 IJs/larva (90 % fiducial limits: 447.38-20884.00). The LD_{50} for *H. baujardi* was 258.62 IJs/larva (90 % fiducial limits: 118.77-2469.20), with the LD_{90} being 8920.40 IJs/larva (90 % fiducial limits: 1340.10-0.2 E +08).
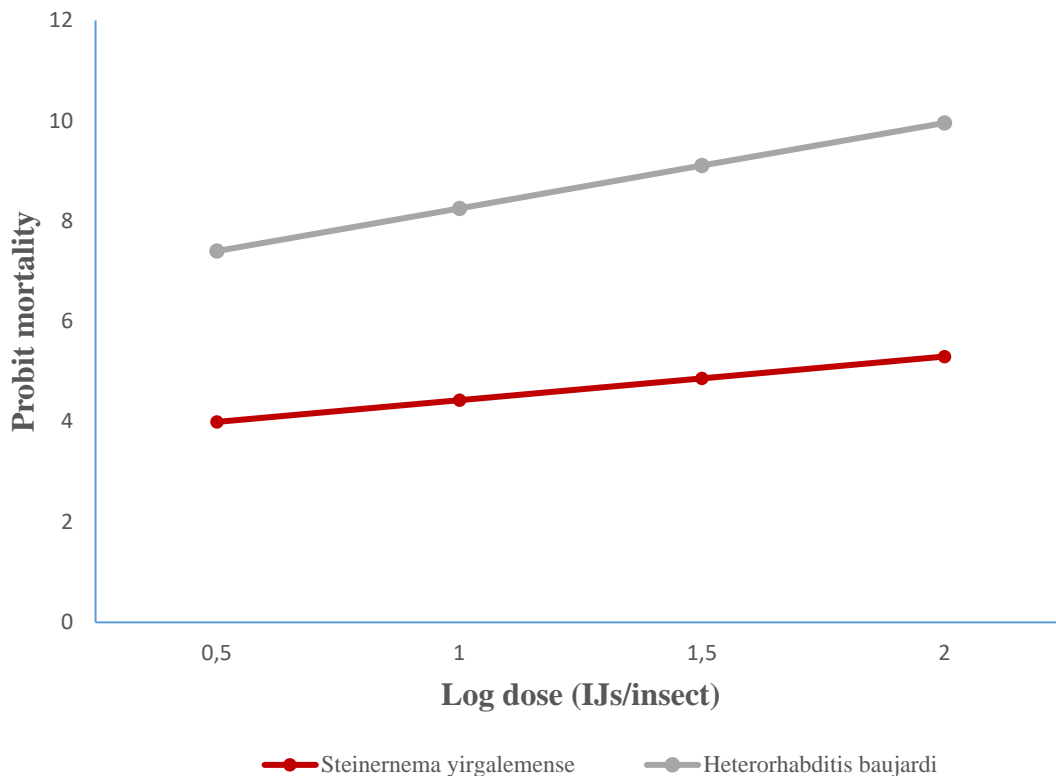
Fig. 3.4. Probit mortality of second instar *Frankliniella occidentalis* larvae 48 hrs after exposure to *Steinernema yirgalemense* and *Heterorhabditis baujardi* at different concentrations (0, 10, 20, 40, 80, and 160 IJs/larva).

3.3.4. Temporal development

Temporal development of EPNs and visual changes in *F. occidentalis* were observed for five days after inoculation with either *S. yirgalemense* or *H. baujardi*. Food from the cadaver became exhausted within this period because the host is so small. Both nematodes were able to complete their life cycle in the host larvae within five days (Table 3.2). The mean number of IJs that penetrated the host was 2 to 2.2 and 1 to 1.9 IJs/insect for *H. baujardi* and *S. yirgalemense,* respectively, while the mean number of IJs recovered from the host larvae was 17.1 for *S. yirgalemense* and 19.6 for *H. baujardi* (Table 3.3). After 4 and 5 days, respectively, IJs of both *S. yirgalemense* and *H. baujardi* started to emerge from the cadavers (Fig. 3.5 B). Once the nematodes had infected the WFT larva, colour changes were observed from the second day onwards, being reddish for *H. baujardi* (Fig. 3.5 A) and yellowish to brownish for *S. yirgalemense*.

Table 3.2. Temporal development of *Steinernema yirgalemense* and *Heterorhabditis baujardi* in second instar larvae of *Frankliniella occidentalis* after inoculation with 100 infective juveniles (IJs) per larva.

EPN species	Days	EPN growth stage	Observation
S. yirgalemense	1	Immature/recovered IJs	
	2	First generation females and males	Yellowish to brown colour, slimy
	3	IJs	
	4	IJs	
H. baujardi	1	Immature/recovered IJs	
	2	Immature/recovered IJs	
	3	Adult hermaphrodites with eggs	Reddish colour
	4	Adult hermaphrodites with eggs	
	5	IJs	

Table 3.3. Average penetration rate and production of infective juveniles in second instar larvae of *Frankliniella occidentalis* for *Steinernema yirgalemense* and *Heterorhabditis baujardi.*

EPN species	# of IJs penetrated	Recovered IJs
S. yirgalemense	1.9	17.1
H. baujardi	2.2	19.6

79
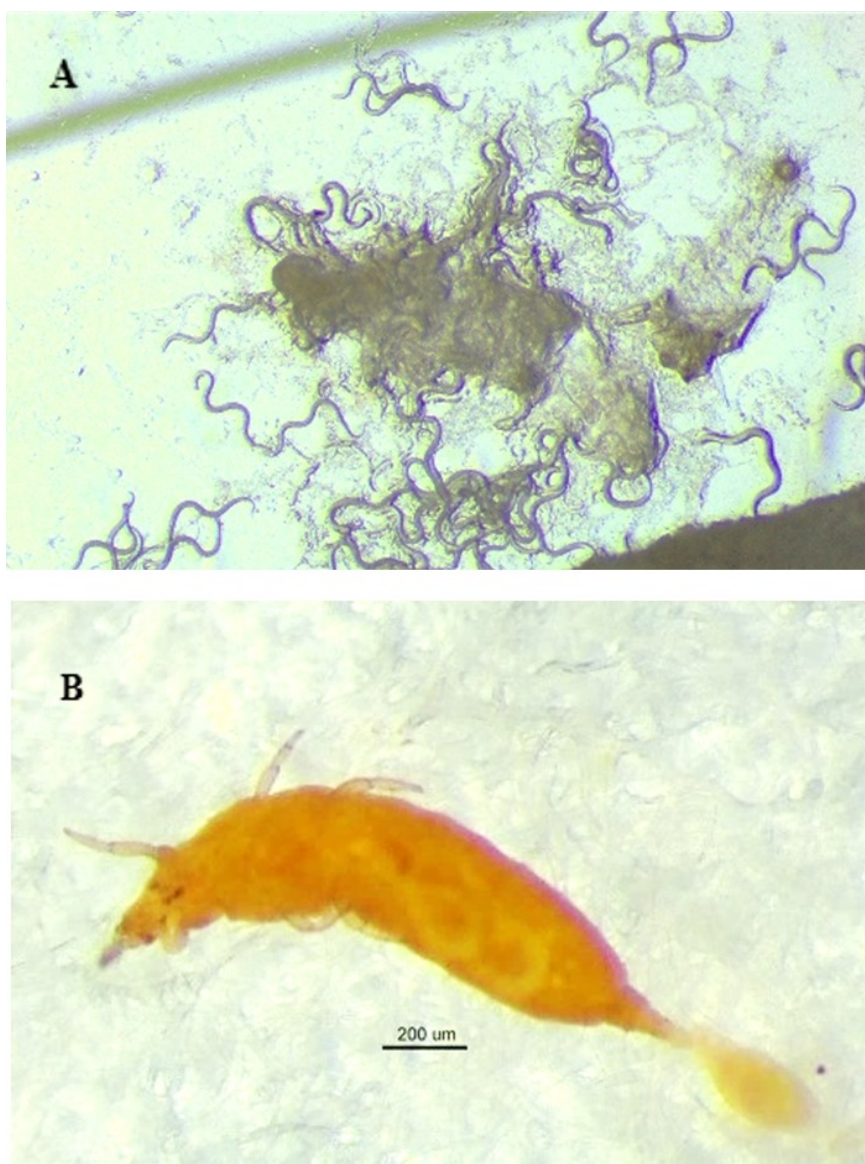
Fig. 3.5. (A) Second instar larva of *Frankliniella occidentalis* infected with IJs of *Heterorhabditis baujardi*. (B) IJs of *Steinernema yirgalemense* emerging from an infected larva.

3.4   DISCUSSION

For EPNs to complete their life cycle in an insect host, they must invade and kill the host and be able to develop into adults and produce IJs which will emerge from the cadaver in search of a new host (Bastidas *et al*. 2014). Laboratory pre-screening of local (five *Heterorhabditis* and seven *Steinernema*) EPNs and one exotic EPN, at a concentration of 100 IJs/insect against second instar larva of *F. occidentalis* showed varying mortality between 11 and 67 %. The variation is attributed to a combination of factors that include host size and host defence mechanism, as well as nematode size and foraging behaviour. *Heterorhabditis baujardi* outperformed the others, although it did not differ significantly from *S. yirgalemense*. This

80

difference was attributed to nematode body size, as *H. baujardi* (± 551 µm) is smaller than *S. yirgalemense* (± 635 µm). The lowest mortality was recorded with *S. litchii, S. khoisanae, S. innovationi*, and *S. jeffreyense*, which all belong to the *Khoisanae*-group with IJ > 1 mm. Since the second instar larvae of *F. occidentalis* are approximately 1.49 mm to 1.79 mm in body length, the poor infectivity of these EPNs was not unexpected. Research with *Bradysia impatiens*, another important undercover pest insect, also demonstrated that the size of the nematode relative to the size of the insect to be controlled has a large impact on pathogenicity (Katumanyane *et al*. 2018a, b, c). In the current study *Heterorhabditis* (57 % mortality) outperformed *Steinernema* (32 % mortality). This suggested that second instar WFT was more susceptible to *Heterorhabditis* spp. than to *Steinernema* spp., as observed by Bedding *et al.* (1993) and Griffin *et al.* (2005). The lowest mortality of thrips occurred with *S. jeffreyense* in all the developmental stages because of its big size. In addition to the effect of nematode size, this can also be attributed to the fact that *Heterorhabditis* species possess a dorsal tooth that enables them to penetrate directly through the cuticle of the host insect, while *Steinernema* species lack the dorsal tooth and can only penetrate via the host's natural openings, e.g. anus. Our results are consistent with the finding of Ebssa *et al*. (2004), who screened six strains of *Heterorhabditis* and 11 strains of *Steinernema* against WFT and recorded mortality ranging from 3-60 %, and with Belay *et al*. (2005) who also concluded that *Heterorhabditis* spp. were more effective (76 %) against WFT than *Steinernema* spp. (37 %).

A significant difference in WFT mortality was found between the best performing nematodes and *S. feltiae,* which is an exotic, commercially produced nematode used mostly to control *F. occidentalis* overseas. *Steinernema feltiae* has dominated in terms of the biological control of insect pests in Europe and the results obtained by Laznik & Trdan (2008) showed very high mortality (82 %) for *S. feltiae* at the lowest concentration of 500 IJs/ml, which was five times higher than the concentration that was used in the current study. However, it has biological limitations in terms of tolerance of high temperatures, since it is a low-temperature-active nematode, which might influence infectivity in field environments encountered in undercover production. The same limitation might apply to other nematodes that have previously been used for the control of WFT, hence, the conclusion by Kashkouli *et al*. (2014) that EPN origin can greatly influence the nematode pathogenicity against insect pests. This confirms why it is important to screen local EPNs adapted to local conditions for use against pests.

Two of the most virulent (*S. yirgalemense* and *H. baujardi*) and one of the least virulent (*S. jeffreyense*) EPN species from the pre-screening were tested further for their virulence against the different developmental stages of *F. occidentalis.* The finding show that pupae of WFT were more sensitive to infection by all three above-mentiond EPN species than adults and larvae. The above result differs with the findings of LeBeck *et al.* (1993) that EPNs are most efficient against all the pre-imaginal stages of insects, as they can enter the latter's bodies with relative ease. This could be explained by the low mobility of the pupal stages, which facilitates the attachment of nematodes to them (Koppenhofer *et al.* 2003). An experiment on banded greenhouse thrips conducted by Trdan *et al.* (2007) showed that the soil-dwelling life stages were more sensitive to EPNs than the adults. Kashkouli *et al*. (2014) observed higher susceptibility of the pre-pupae and pupae of *Thrips tabaci* to EPNs, compared to the susceptibility of the second instar larvae. The larval stages of WFT, which are very active and mobile, show evasive behaviour towards the nematodes (Buitenhuis & Shipp 2005; Kashkouli *et al*. 2017). Contradicting the current results were those obtained by Lim *et al.* (2001), who found low parasitism rates of WFT by the entomoparasitic nematode *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae) on the older life stages (i.e. pupae and adults). *Heterorhabditis baujardi* showed the highest infectivity against the larval and pupal stage of WFT in comparison with *S. yirgalemense,* which can be ascribed to other reasons than only size difference. The WFT adults showed low parasitism for all the EPN species and this was attributed to the hardening of the cuticle as thrips age and the fact that they are very active, which makes penetration difficult.

Thrips mortality increased with increased EPN concentration from 10 IJs/larva to 160 IJs/larva for both *S. yirgalemense* and *H. baujardi*. This also held true with studies on EPN concentration by Chyzik *et al*. (1996), Ebssa *et al.* (2004) and Kashkouli *et al*. (2014) against WFT, with different EPN species. A positive relationship is evident between the concentration of the EPN species, *S. yirgalemense* and *H. baujardi*, and the percentage mortality of *F. occidentalis*. However, the level of increase was significantly different between the two species, as indicated by the significantly different slopes of the linear regression coefficients. The probit regression line for *S. yirgalemense* was greater than was that for *H. baujardi*, which contradicts the conclusions drawn by Ebssa *et al*. (2004) that three *Heterorhabditis* spp. had significantly greater slopes than did two *Steinernema* spp., meaning that *Heterorhabditis* responded better to the increase in concentrations than did *Steinernema*. The calculated LD_{50} and LD_{90} for *S. yirgalemense* showed that it was more potent than *H. baujardi*, with a potency

of 5.49. The above implies that *S. yirgalemense* was relatively pathogenic against the second instar larva, as fewer IJs per larva were required in comparison to the number that were required with *H. baujardi*. Surprisingly, the performance of *H. baujardi*, in terms of pathogenicity to *F. occidentalis*, did not correspond to the screening results obtained, but the differences were not significant even in the screening tests and is ascribed to batch effects. The LD_{50} and LD_{90} of *S. yirgalemense* in the current study for the control of *F. occidentalis* was higher than those for other insects, such as for the vine mealybug, *Planococcus ficus* (Signoret)*,* which had an LD_{50} of 36 and LD_{90} of 555 IJs/insect, according to Le Vieux & Malan (2013). The above means that *F. occidentalis* is not as susceptible to *S. yirgalemense* as are other insects, thus indicating that they require higher concentrations of the nematode to effect control.

Heterorhabditis baujardi showed greater penetration ability than *S. yirgalemense* in the temporal development study. This corresponds with the screening results (Chapter 2), which showed the superiority of *H. baujardi* to *S. yirgalemense*. Relatively few IJs were found to penetrate the insect, due to the small size (approximately 1 mm) of the insect and the number of IJs recovered from the host were relative to the number of IJs that penetrated. This concurs with observations by Bastidas *et al*. (2014).

The production of IJs is dependent on nematode and host species, and usually in big hosts there can be many IJs produced regardless the size of the nematode. *Steinernema yirgalemense* can produce about 75 IJs in a single cadaver of the vine mealybug, *Planococcus ficus* (Signoret) (Le Vieux & Malan 2013). Lim *et al*. (2001) found that the maximum number of *T. nicklewoodi* that entered one host of WFT, when exposed for a day, was five nematodes. On the second day after infection in the temporal development study, the second instar WFT larva infected with *H. baujardi* turned reddish, whereas those infected by *S. yirgalemense* became yellowish to brown in colour, and were slimy when dissected. In addition, on the second day, *S. yirgalemense* were fully developed, having both males, but mostly females, in their population. Hence, they had a relatively short life cycle, with IJs emerging on the fourth day. *Heterorhabditis baujardi* developed into hermaphrodites with eggs on the third day, and IJs started emerging on the fifth day. The number of eggs laid by the female nematode depends on the size of the host, and on the presence of other nematodes competing in the same host (Lim *et al.* 2001), which was the main reason for the relatively few IJs recovered, in terms of the observations made. Lim *et al.* (2001) found a maximum of eleven ovoid-shaped first generation nematodes in a female WFT, and six in a male WFT. The nematodes emerging from the egg developed into IJs due to the lack of food supply in the host. The late second nematode larval

phase is known to stop feeding, and to incorporate the bacteria into the bacterial chamber or vesicle, then transforming into pre-infective and infective larvae, retaining the cuticle of the second larval phase as a sheath (Gaugler 2002). In this study some insects were observed to contain nematodes that only developed to a certain stage, and disappeared without finishing their cycle. The cycle finished within five days for both EPNs, as the IJs were observed leaving the cadaver in search of another host. The current results show that the nematodes were able to complete their short life cycle in *F. occidentalis,* reproducing a single sexual generation. The ability of *S. yirgalemense* and *H. baujardi* to complete their life cycles in second instar WFT holds out a promise of persistence for use as biocontrol agents, although the new cohort of IJs was not recovered from some WFT larvae due to the lack of sufficient food in the small hosts.In conclusion, the results from the current study showed that some local EPNs have outstanding potential for biological control of WFT, particularly in its soil-dwelling pupal stages. Under optimum conditions, mortalities > 60 % of WFT pupae were recorded with locally isolated *S. yirgalemense*, *H. baujardi* and *H. bacteriophora.* Some success was also achieved against second instar WFT larvae.

This is a breakthrough for the use of EPNs in biocontrol, since this is the first study of its kind in terms of EPN control against *F. occidentalis* in South Africa. The fact that *S. yirgalemense* not only showed potential in terms of virulence against WFT, but also against a number of other insect pests, including codling moth, mealybugs, fruit flies, and the sugar cane stalk borer (De Waal *et al*. 2010; Malan *et al*. 2011; Ferreira & Malan 2014; Malan & Hatting 2015; Odendaal *et al.* 2016a,b) is very important for potential commercialisation. *Steinernema yirgalemense* was also shown to very effective against *Bemesia impatiens* (Katumanyane *et al*., 2018b, c) which occurs alongside thrips as important pests in undercover crop production. The potential of *S. yirgalemense* as a biocontrol agent is further supported by the fact that it has already been successfully cultured *in vitro* (Ferreira *et al*. 2015) and its use is not restricted, while importation of exotic EPN species into South Africa is prohibited. Laboratory conditions are not necessarily representative of the field performance of nematodes and future emphasis should be on conducting studies under field conditions, possibly combined with the use of other biological agents and insecticide–pathogen synergistic interactions in IPM systems.

3.5   REFERENCES

ABATE, A.A., SLIPPERS B., WINGFIELD, M.J., MALAN, A.P. & HURLEY, B.P. 2018. Diversity of entomopathogenic nematodes and their symbiotic bacteria in South African plantations and indigenous forests. *Nematology* DOI 10.1163/15685411-00003144.

BASTIDAS, B., PORTILLO, E. & SAN-BLAS, E. 2014. Size does matter: the life cycle of *Steinernema* spp. in micro-insect hosts. *Journal of invertebrate pathology* **121**:46-55. https://doi.org/10.1016/j.jip.2014.06.010

BEDDING, R.J., AKHURST, R. & KAYA, H. (eds.). 1993. *Nematodes and the biological control of insect pests*. East Melbourne: CSIRO Publication.

BELAY, D., EBSSA, L. & BORGEMEISTER, C. 2005. Time and frequency of applications of entomopathogenic nematodes and their persistence for control of western flower thrips *Frankliniella occidentalis*. *Nematology* **7**: 611–622. DOI: 10.1163/156854105774384822

BUITENHUIS, R. & SHIPP, J.L. 2005. Efficacy of entomopathogenic nematode *Steinernema feltiae* (*Rhabditida*: *Steinernematidae*) as influenced by *Frankliniella occidentalis* (*Thysanoptera*: *Thripidae*) developmental stage and host plant stage. *Journal of Economic Entomology* **98**: 1480–1485.DOI: https://doi.org/10.1603/0022-0493-98.5.1480

CAMPOS-HERRERA, R. & GUIERREZ, C. 2008. Screening Spanish isolates of steinernematid nematodes for use as biological control agents through laboratory and greenhouse microcosm studies. *Journal of Invertebrate Pathology* **100**: 100–105. DOI: 10.1016/j.jip.2008.11.009

CHYZIK, R., GLAZER, J. & KLEIN, M. 1996. Virulence and efficacy of different entomopathogenic nematode species against western flower thrips (*Frankliniella occidentalis*). *Phytoparasitica* **24**: 103–110.

DE WAAL, J.Y., MALAN, A.P., LEVINGS, J. & ADDISON, M.F. 2010. Key elements in the successful control of diapausing codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in fruit bins with a South African isolate of *Heterorhabditis zealandica* (Rhabditida: Heterorhabditidae). *Biocontrol Science and Technology* **20**: 489–502.DOI: https://doi.org/10.1080/09583151003599708

DUAN, H., ZHANG Y., GUO, D., TAO, Y. & CHU, D. 2013. Sudden widespread and distribution of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in Shandong Province, China. *Florida Entomologist* **96**: 932–940. DOI: https://doi.org/10.1653/024.096.0330

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & POEHLING, H.M. 2001. Efficacy of entomopathogenic nematodes against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Invertebrate Pathology* **78**: 119–127. DOI: 10.1006/jipa.2001.5051

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & 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. DOI: https://doi.org/10.1016/S1049-9644(03)00132-4

EFRON, B. & TIBSHIRANI, R. 1993. *An Introduction to the Bootstrap*. Chapman & Hall, London.

EHLERS, R. 2001. Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Technology* **56**: 623–633. DOI: 10.1007/s002530100711

FERREIRA, T. & MALAN, A.P. 2014. Potential of entomopathogenic nematodes for the control of the banded fruit weevil, *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae). *Journal of Helminthology* **88**: 293–301. DOI: https://doi.org/10.1017/S0022149X13000175

FERREIRA, T., ADDISON, M.F. & MALAN, A.P. 2015. *In vitro* liquid culture of a South African isolate of *Heterorhabditis zealandica* for the control of insect pests. *African Entomology* **22**: 80–92. DOI: https://doi.org/10.4001/003.022.0114

FINNEY, D.J. 1971. *Probit Analysis*. Cambridge University Press, New York. DOI: https://doi.org/10.1002/jps.2600600940

GAUGLER, R. 2002. *Entomopathogenic Nematology*. CAB International, Wallingford.

GILIOMEE, J.H. 1989. First record of Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) from South Africa. *Journal of Entomological Society of Southern Africa* **52**: 179–180.

GRIFFIN, C.T., BOEMARE, N.E. & LEWIS, E.E. 2005. Biology and behaviour. In: Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (Eds) *Nematodes as Biocontrol Agents*. 47–75. CABI Publishing, Wallingford.

HOMINICK, W.M. 2002. Biogeography. In: Gaugler, R. (Ed.) *Entomopathogenic Nematology*. 115–143. CABI Publishing, Wallingford.

JAGDALE, G., CASEY, M., GREWAL, P. & LINDQUIST, R. 2004. Application rate and timing, potting medium, and host plant effects on the efficacy of *Steinernema feltiae* against the fungus gnat, *Bradysia coprophila*, in floriculture. *Biological Control* **29**: 296–305. DOI: 10.1016/S1049-9644(03)00164-6

KASHKOULI, M., KHAJEHALI, J. & POORJAVAD, N. 2014. Impact of entomopathogenic nematodes on *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) life stages in the laboratory and under semi-field conditions. *Journal of Biopesticides* **7**: 77–84.

KASHKOULI, M., KHAJEHALI, J. & POORJAVAD, N. 2017. Combination of entomopathogenic nematodes and chemical insecticides for controlling the onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae) in the laboratory condition. *Applied Entomology and Phytopathology* **84**: 1–10. DOI: 10.22092/JAEP.2017.107591

KATUMANYANE, A., FERREIRA, T., MALAN, A.P., 2018a. *Bradysia species* (Diptera: Sciaridae) as pests of covered crops, with special reference to biological control using entomopathogenic nematodes. *African Entomology* **26**: 1–13. DOI: 10.4001/003.026.0001

KATUMANYANE, A., FERREIRA, T., MALAN, A.P., 2018b. Greenhouse application of *Steinernema yirgalemense* to control fungus gnats, *Bradysia impatiens (*Diptera: Scaridae). *BioControl.* https://doi.org/10.1007/s10526-018-9895-3

KATUMANYANE, A., FERREIRA, T., MALAN, A.P., 2018c. Potential use of local entomopathogenic nematodes to control *Bradysia impatiens* (Diptera: Sciaridae) under laboratory conditions. *African Entomology* (In Press).

KAYA, H.K. & STOCK, S.P. 1997. Techniques in insect nematology. In: Lacey, L.A. (Ed.) *Manual of Techniques in Insect Pathology*. 281–301. Academic Press, London. DOI: https://doi.org/10.1016/B978-012432555-5/50016-6

KAYA, H.K., AGUILLERA, M.M., ALUMAI, A., CHOO, H.Y., DE LA TORRE, M., FODOR, A., GANGULY, S., HAZAR, S., LAKATOS, T., PYE, A., WILSON, M., YAMANAKA, S., YANG, H. & EHLERS, R.U. 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* **38**: 134–155. DOI: 10.1016/j.biocontrol.2005.11.004

KIRK, W.D. & TERRY L.I. 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology* **5**: 301–310. DOI: https://doi.org/10.1046/j.1461-9563.2003.00192.x

KOPPENHOFER, A.M., COWLES, R.S., COWLES, E.A., FUZY, E.M. & BAUMGARTNER, L. 2003. Comparison of neonicotinoid insecticides as synergists for entomopathogenic nematodes. *Biological Control* **24**: 90–97. DOI: 10.1016/S1049-9644(02)00008-7

LAZNIK, Z. & TRDAN, S. 2008. Entomopathogenic and entomoparasitic nematodes as biological control agents of thrips. *Acta Phytopathologica et Entomologica Hungarica* **43**(2): 317–322. DOI: https://doi.org/10.1556/APhyt.43.2008.2.15

LEBECK, L.M., GAUGLER, R., KAYA, H.K., HARA, A.H. & JOHNSON, M.W. 1993. Host stage suitability of the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Journal of Invertebrate Pathology* **62**: 58–63. DOI: https://doi.org/10.1006/jipa.1993.1074

LE VIEUX, P.D. & MALAN, A.P. 2013. The potential use of entomopathogenic nematodes to control *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *South Africa Journal of Enology and Viticulture* **34**(1): 296–306. DOI: 10.21548/34-2-1109

LEWIS, T. 1997. *Flight and Dispersal. Thrips as Crop Pests*. CAB International, Wallingford.

LIM, U.T., VAN DRIESCHE, R.G. & HEINZ, K.M. 2001. Biological attributes of the nematode, *Thripinema nicklewoodi*, a potential biological control agent of western flower thrips. *Biological Control* **22**: 300–306. DOI: https://doi.org/10.1006/bcon.2001.0979

MALAN, A.P., NGUYEN, K.B. & ADDISON M.F. 2006. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from the south western parts of South Africa. *African Plant Protection* **12**: 65–69.

MALAN, A.P. & HATTING, J.L. 2015. Entomopathogenic nematode exploitation: Case studies in laboratory and field applications from South Africa. In: Campos-Herrera, R. (Ed.) *Nematode Pathogenesis of Insects and Other Pests*. 477–508. Springer International, Basel.

MALAN, A.P., KNOETZE, R. & MOORE, S.D. 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology* **108**: 115–125. DOI: 10.1016/j.jip.2011.07.006

MANNERS, A.G., DEMBOWSKI, R.B. & HEALEY, M.A. 2013. Biological control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in gerberas, chrysanthemums and roses. *Australian Journal of Entomology* **52**: 246–258. DOI: https://doi.org/10.1111/aen.12020

NAVON, A. & ASCHER, K.R.S. 2000. *Bioassays of Entomopathogenic Microbes and Nematodes*. CAB International, Wallingford. DOI: 10.1079/9780851994222.0000

ODENDAAL, D., ADDISON, M.F. & MALAN, A.P. 2016a. Evaluation of above-ground application of entomopathogenic nematodes for the control of diapausing codling moth (*Cydia pomonella* L.) under natural conditions. *African Entomology* **24**: 61–74. DOI: https://doi.org/10.4001/003.024.0061

ODENDAAL, D., ADDISON, M.F. & MALAN A.P. 2016b. Control of diapausing codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) in wooden fruit bins, using entomopathogenic nematodes (Heterorhabditidae and Steinernematidae). *Biocontrol Science and Technology* **26**: 1504–1515. DOI: https://doi.org/10.1080/09583157.2016.1217393

StatSoft Inc, 2016. STATISTICA (Data Analysis Software System) Version 13.0.

TARASCO, E., TRIGGIANI, O., SAI, K. & ZAMOUM, M. 2009. Survey of entomopathogenic nematodes in Algerian soils and their activity at different temperatures. *Frustula Entomologica* **32**: 31–42.

TRDAN, S., ZNIDARCIC, 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.

Chapter 4

Greenhouse application of *Steinernema yirgalemense* to control *Frankliniella occidentalis* (Thysanoptera: Thripidae) in blueberry production

Abstract

The need for biological control of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is on the rise for blueberry production in South Africa because of the rapid growth of the industry. WFT is regarded as one of the economically important and prevalent insect pests in undercover production. Resistance to spinosad, the most commonly used insecticide for WFT control in South Africa, has already been reported for WFT. Most biological control agents do not achieve effective control of WFT, because of the cryptic habits of WFT. The endemic entomopathogenic nematode, *Steinernema yirgalemense*, was found to be pathogenic against WFT in the laboratory. The effect of different concentrations of *S. yirgalemense* in controlling *F. occidentalis* on commercial blueberries grown in Haygrove tunnels was investigated, targeting all stages of WFT. Two trials, one with lower concentrations of 4.3, 8.6 and 17.2 IJs/cm^2 and the other with higher concentrations of 25, 50 and 100 IJs/cm^2, were conducted. WFT only reached < 50 % mortality with the highest concentration of 100 IJs/cm^2. This is ascribed to suboptimal temperatures during the trial period which restricted *S. yirgalemense* establishment and sustainability. *Steinernema yirgalemense* was, however, still persistent at mean substrate temperatures < 15 °C. The application of *S. yirgalemense* to the soil/growth substrate of blueberries in Haygrove tunnels when warmer temperatures prevail, should be investigated further. Further studies should target WFT on the new growth, just after pruning, when populations peak and cause significant economic damage. Weekly follow-up applications to increase efficacy of EPNs should also be investigated.

Keywords: Greenhouse, western flower thrips, *Steinernema yirgalemense,* blueberry, *Frankliniella occidentalis*

4.1.   INTRODUCTION

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most important thrips species encountered in greenhouse production, as it feeds on a wide range of crops and ornamentals, reducing crop value, and transmitting viral diseases such as tomato spotted wilt virus (Moritz *et al*. 2004). WFT is regarded as a serious pest that is capable of extensive economic damage worldwide (Siguna 2007). The control of WFT is challenging, because of its cryptic behaviour, rapid reproductive rate, and potential to develop resistance to insecticides (Gouli *et al*. 2008). The use of insecticides for controlling *F. occidentalis* is unsustainable, because of the high costs involved and because heavy reliance on them has led to development of resistance (Cloyd 2015).

The use of several biological control agents, which are on the market for use against all stages of WFT in greenhouses, has been accepted as a management strategy worldwide, mainly as an alternative to prevent insecticide resistance of the insect. Biological control agents, including predatory mites and bugs are released inundatively against *F. occidentalis* (Brødsgaard 2004; Shipp & Ramakers 2004), while the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) (Hypocreales: Cordycipitaceae), has also been used as a biological control agent (Murphy *et al*. 2014). However, WFT's cryptic habits reduces the efficacy of the biocontrol agents, as these are unable to invade tightly enclosed areas, like apical meristems and flower buds, preferred by WFT (Premachandra *et al.* 2003; Berndt *et al.* 2004). Entomopathogenic nematodes (EPNs) are lethal pathogens of insects that occur naturally in the soil (Griffin *et al*. 2005; Wright *et al*. 2005), where they are able to locate their host via their carbon dioxide secretions, vibrations, and other chemical cues (Kaya & Gaugler 1993). EPNs of the genera *Steinernema* and *Heterorhabditis*, in the families Steinernematidae and Heterorhabditidae (order Rhabditida), respectively, are among the alternative biocontrol strategies that can be implemented in an integrated pest management (IPM) programme (Grewal *et al*. 1994). Their use against many pests, including WFT, has been intensively investigated in other parts of the world.

The combination of EPNs and other control agents has proved to be synergistic, yielding higher mortality than the use of a single agent (Lacey & Georgis 2012). Studies on the use of EPNs for the biological control of *F. occidentalis* for undercover production are on the rise worldwide. The commercial nematode *Steinernema feltiae* Wouts, Mráček, Gerdin & Bedding, is used internationally against WFT and other insect pests. Previous studies have shown a high

susceptibility of the soil-dwelling stages of WFT to EPNs (Ebssa *et al*. 2006; Buitenhuis & Shipp 2005). *Steinernema feltiae* has been studied against the prepupae and pupae of *F. occidentalis*, in petri dishes, with up to 70 % mortality being recorded on potted chrysanthemums (*Dendranthema grandiflora*) at 10 000 IJs/ml. However, foliar applications showed minimal efficacy, even at twice the recommended rates (20 000 to 40 000 IJs/ml). The same experiment in field conditions showed minimal mortality but still better control of the pupal stages (Buitenhuis & Shipp 2005).

The South African blueberry industry is growing rapidly, due to high demand from the United Kingdom (UK) and the European Union (EU) for blueberries (*Vaccinium corymbosum*, Ericaceae) and sudden domestic interest. The area planted to blueberry, which was estimated to 1300 hectares in 2016/17, is projected to grow to about 2000 hectares by 2020, with the Western Cape Province leading with 60 % of the production (Sikuka 2017). The production of blueberries in Haygrove tunnels is gaining popularity, because of the higher yields and the improved marketability of fruit. WFT cause economic damage to blueberries when they lay eggs and feed on the ovaries, resulting in scarring that renders berries unmarketable (Arévalo *et al*. 2009). Growers in the Western Cape report that WFT can cause severe damage to the buds and new growth after pruning (D. Ngadze, Berryworld SA, pers. communication). As the blueberry industry in South Africa expands, the thrips problem is most likely to increase too. Commercial farmers of blueberry crops are already experiencing the persisting WFT problem, despite the use of chemical control, combined with biological control agents like predatory mites and predatory bugs (*Orius* spp). Food safety certification required for export restricts the use of chemical control by producers close to and during harvesting, therefore they have to rely on biological control. WFT is economically harmful in blueberry production in South Africa during the blooming period, which occurs during the winter months (May and June), and during the flush in the summer months (between December and February) after plants have been pruned during November/December (D. Ngadze, Berryworld SA, personal communication). Both these periods can be the targeted for the release of biological agents.

The efficacy of EPNs depends on the selection of the most virulent nematode for the target host (Gaugler & Georgis 1991). In laboratory screenings (Chapter 3), the local nematode *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams showed great potential for the control of the soil-dwelling pupal stages of WFT (65 % mortality) and also for control of second instar larvae (43 % mortality) at 100 IJs/insect, therefore it was selected for

further testing in field trials. *Steinernema yirgalemense* has not only shown potential for WFT control in South Africa, but also for the control of other insect pests, like codling moth (De Waal *et al*. 2011; Odendaal *et al*. 2016a,b), mealybugs (Le Vieux & Malan 2015; Platt *et al*. 2017), fruit flies (James *et al*. 2018), the banded fruit weevil (Dlamini *et al*. 2018) and *Bradysia* spp. (Katumanyane *et al*. 2018). Research on *in vitro* culturing of EPNs for commercialisation is ongoing (Ferreira *et al.* 2016).

The objective of the study was to determine the effect of different concentrations of *S. yirgalemense* for biocontrol of *F. occidentalis* in a commercial blueberry greenhouse. Additionally, the persistence of the IJs in the growth medium was tested.

4.2.   MATERIALS AND METHODS

4.2.1. Trial site

Blueberry plants, grown under a 200-micron poly-plastic cover in Haygrove tunnels in Klapmuts (33°49'41"S 18°33'6.48"E), Western Cape Province, were used in this trial. Plants were naturally infested with *F. occidentalis* that were assumed to have migrated from adjacent fields that previously housed infested plants. The thrips species was confirmed to be *F. occidentalis* by means of morphological and molecular characterisation.

Two-and-a-half-month-old blueberry plants (var. Dazzle), originally imported from the Mountain Blue orchards in Australia, were grown in pots (30- cm-diam.), with the potting medium consisting of pit coconut husk, imported from Holland. In the Western Cape the peak flowering period for this blueberry variety is during May/June and the main harvesting period is during August.

The experiments were conducted during peak flowering season (May-June 2018). The trial with low nematode concentrations was carried out between 16 May and 13 June 2018 and the trial with high nematode concentrations from 30 May to 27 June 2018. The last pesticide treatment (active ingredient spinosad) was applied a month before the beginning of the first trial. The commercial predatory mite *Amblydromalus limonicus* (Acari: Phytoseiidae), sold as LIMONICA[®] by Koppert, was applied a week and three weeks after the start of the first and second trials, respectively.

4.2.2. Source of nematodes

In vitro cultured *S. yirgalemense*, according to the procedure adopted by Ferreira *et al.* (2016), were used in this study. The equation developed by Navon & Ascher (2000) was used to calculate the nematode concentrations used in the various experiments.

4.2.3. Monitoring of environmental parameters

Climatic conditions, i.e. ambient temperature, growth substrate temperature and relative humidity (RH), were monitored using ColdChain Thermodynamics I-buttons. The weekly cycle showed an average of the environmental parameters for each week, with daily maximum and minimum values.

4.2.4. High and low EPN concentration applications

Steinernema yirgalemense was applied to a natural infestation of *F. occidentalis* at low IJ concentrations of 0, 4.3, 8.6, and 17.2 IJs/cm^2 on 16 May 2018, and at higher concentrations of 0, 25, 50, and 100 IJs/cm^2 on 30 May 2018. The higher concentrations were targeted at control of WFT larvae, based on recommendations of 50 IJs/cm^2 for soil applications and 25 IJs/cm^2 for leaf applications of *S. feltiae* in the commercial product ENTONEM® by Koppert, for the control of the larvae of sciarid flies (Sciaridae), WFT and leaf miners, .

Both experiments were arranged in a complete randomised design, using eight pots for each treatment ($n = 32$ pots), with one buffer row and four buffer plants between treatment pots (Fig. 4.1). The pots were irrigated before treatment to ensure 100 % saturation of the growth medium at the time of application. To reduce EPN sensitivity to desiccation and UV radiation, an adjuvant was used, WETCIT® (Borax and orange oil), was added to all nematode suspensions at the rate of 0.5 ml/L. WETCIT® is registered for use in undercover blueberry production in South Africa. Treatments were applied using a 2-L handheld sprayer. Each plant received 600 ml of nematode suspension and the control treatment received water with the adjuvant only. Each plant was first sprayed from above, from a distance of approximately 30 cm, to ensure good coverage of the foliage and flowers, with limited run-off. The rest of the nematode suspension was drenched evenly over the growth medium surface. Growth medium moisture level was kept at a constant 100 % by means of daily drip irrigation.

Fig.4.1. Experimental layout in a completely randomised design, under Haygrove tunnels in blueberry production, with eight pots for each treatment, the buffer row, and the plants.

After treatment application, HORIVER® blue sticky cards (25 cm) (Koppert) were placed in each of the pots, at about 5 cm from the surface of the substrate, to attract and trap any emerging adult thrips. The blue sticky cards are the most widely used method for monitoring thrips (Trdan & Jenser 2003). Each of the experimental pots was isolated, using white thrips-proof fabric that was tightly wrapped around the base of the pot and secured with packaging tape (Fig. 4.2), and tied onto a 1-m stick at the top with a plastic string. Double-sided tape was used to seal the sides of the cylindrical enclosures. These enclosures prevented the adult thrips from escaping, while leaving them enough space in which to move around freely.

After the plants had been left undisturbed for 7 days, the number of adult *F. occidentalis* on each sticky card was counted, under a Zeiss stereo Discovery V8 Microscope fitted with Axiocam ERc 5s. Data was recorded weekly over a 4-week period and a fresh sticky trap was inserted each week when removing a trap to count WFT.

Fig. 4.2. Treatment pot enclosed with thrips-proof fabric and a blue sticky trap attached to trap emerging adult *Frankliniella occidentalis*.

4.2.5. Persistence of EPNs

EPN persistence was evaluated concurrently with the nematode concentration experiments. Last-instar mealworm larvae, *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) were used to assess nematode persistence. Five perforated 0.2-ml Eppendorf tubes were tied together with cotton thread, with a single larva placed in each tube (Fig. 4.3). A set of five tubes was buried randomly in the substrate of each pot, with the thread extending above the soil. The tubes were retrieved after 7 days and a fresh set of tubes with mealworm larvae was buried in the same pot. Mortality was recorded, and the dead mealworms were dissected to confirm infection, whereas the live individuals were rinsed in running water through a sieve and placed on moist filter paper in a Petri dish. The dish was sealed with Parafilm® and left to incubate for another week at 25 °C. After the time period had elapsed, the larvae were dissected to confirm mortality by nematode infection.

Fig. 4.3. Treatment pot isolated with thrips-proof fabric mounted on a 1-m stick showing Eppendorf tubes tied together with cotton thread for persistence testing (circled).

4.2.6   Statistical analysis

All statistical analyses were performed using STATISTICA 13.2 software (StatSoft Inc. 2008). The data were analysed using analysis of variance (ANOVA), with *post hoc* comparison of means using Bonferroni's method, or a bootstrap multi comparison, if the residuals were not evenly distributed (Efron & Tibshirani 1993). Significant differences were determined on a 95 % probability level. Mean percentages were given along with the standard error of means.

4.3.   RESULTS

4.3.1.   Low nematode concentrations

4.3.1.1 Environmental conditions

The mean ambient temperature for the four-week evaluation period (16 May to 13 June 2018) was 16 °C, which ranged between a minimum of 4 °C and a maximum of 32 °C (data not shown). The IJs can only survive for 24 hours on the foliage, and the mean ambient temperature on the day of nematode application was 21 °C, with a minimum of 17 °C, which is well below the optimum temp of 25 °C for *S. yirgalemense*. Infection of leaf-dwelling WFT stages was therefore not expected to contribute much to reducing WFT numbers, indicating that only the substrate temperature would have played a significant role in the trapping of adult thrips on the blue sticky traps. As the plants were drip irrigated, the humidity of the substrate was always

close to 100 %. The average temperature of the growth substrate ranged between a minimum of 8 °C and a maximum of 39 °C, with the mean average for the duration of the experiment being 17 °C, which is still below the optimum temperature of 25 °C for *S. yirgalemense* (Fig. 4.4).
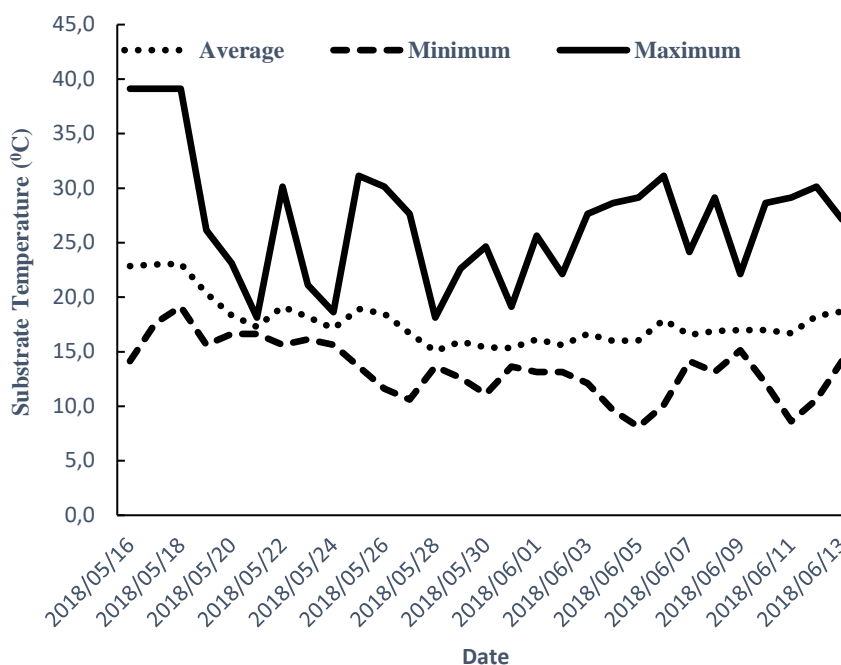
Fig. 4.4. Growth substrate temperature (minimum, maximum, average) in potted blueberries in Haygrove tunnels from 16 May 2018 until 16 June 2018.

4.3.1.2 Efficacy of low concentration applications

A two-way ANOVA of the effect of time and the concentrations of *S. yirgalemense* on the numbers of *F. occidentalis* recovered from sticky traps over four weeks showed no significant interaction between the treatments and the weeks ($F_{(9,84)} = 0.40080$, $p = 0.931$) (Fig. 4.5). Over the four weeks no treatment produced significantly fewer thrips than the untreated control. In week 2, the lowest concentration (4.3 IJs/cm^2) had the highest number (9.13 ± 1.54) of adult WFT recovered, which did not differ significantly ($p = 0.931$) from the control treatment (8.38 ± 1.54). The number of thrips recovered for the highest concentration in the fourth week was more than that recovered in the previous weeks (7.63 ± 1.29).
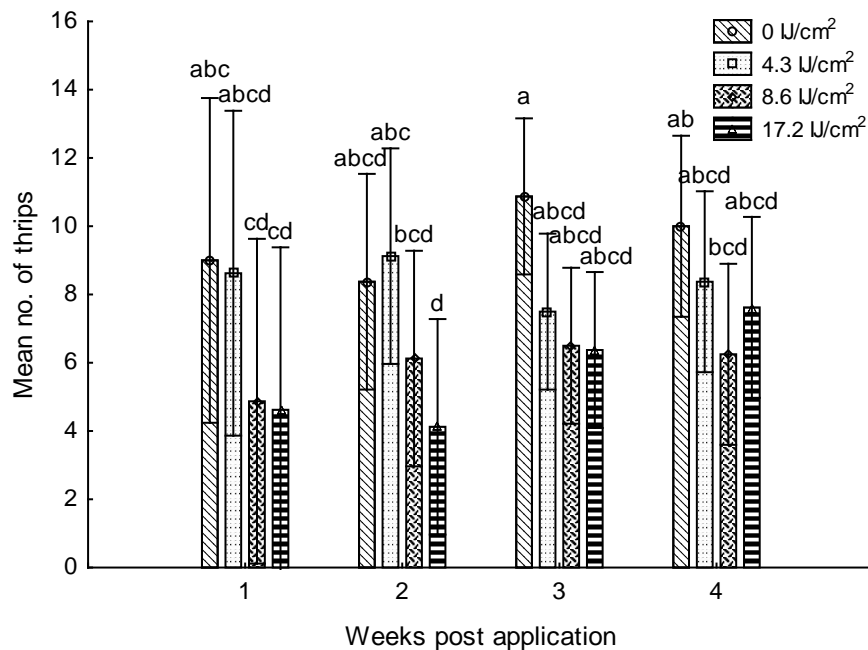
Fig. 4.5. Mean number of adult *Frankliniella occidentalis* recovered from eight blue sticky traps per treatment per week after field treatment of blueberry plants with different concentrations of *Steinernema yirgalemense* (4.3, 8.6, 17.2 IJs/cm^2 and control treated with water only. (Two-way ANOVA: $F_{(9.84)} = 0.40080$, $p = 0.931$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

A one-way ANOVA analysis showed that the cumulative number of adult thrips on the blue sticky traps over four weeks decreased significantly with increasing EPN concentrations ($F_{(3, 28)} = 5.2634$, $p < 0.001$) (Fig. 4.6). Cumulative results show that there were significantly fewer thrips that emerged in the 8.6 and 17.2 IJs/ cm^2 treatments than in the lowest concentration and the untreated control. The highest concentration had the lowest mean number of WFT recovered (5.69 ± 0.826). The lowest concentration (4.3 IJ/cm^2) differed significantly ($p = 0.0053$) from the two higher nematode concentrations applied, which did not differ significantly from each other.

99
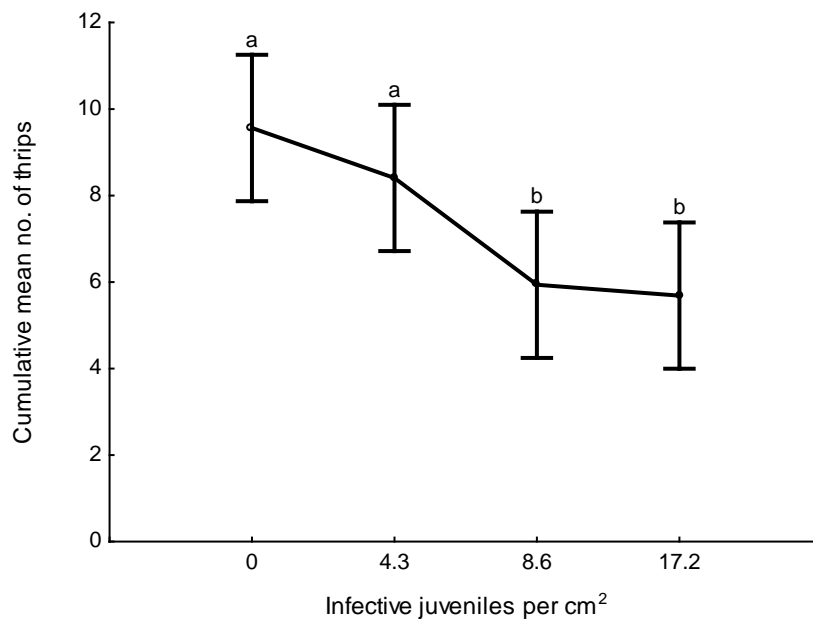
Fig. 4.6. Cumulative mean number of adult *Frankliniella occidentalis* (95 % confidence intervals) recovered from eight blue sticky traps per treatment over four weeks, after application of *Steinernema yirgalemense* at four different concentrations (4.3, 8.6, 17.2 IJ/cm^2 and control treated with water only (one-way ANOVA: $F_{(3, 28)} = 5.2634$, $p < 0.001$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

4.3.1.3 EPN persistence

Analysis using a two-way ANOVA showed no significant difference ($F_{(9, 84)} = 0.41526$, $p = 0.92366$) between the main effects of the IJ concentrations over the course of 4 weeks (Fig. 4.7). No significant differences were observed between any treatments in any of the weeks. During the first week, the highest nematode concentration resulted in the highest mealworm mortality (42.5 % ± 5.94 %), which declined over the 4-week duration. The lowest concentration had the lowest mealworm mortality (25.0 % ± 5.94 %) after the first week, with a decline over the following 3 weeks. The cumulative percentage mortality (Fig. 4.8) showed a significant differences ($F_{(3, 28)} = 12.755$, $p < 0.001$) in persistence, expressed in terms of mealworm mortality, between the control treatment and the three nematode concentrations over the 4 weeks. The highest nematode concentration resulted in the highest mean mortality over 4 weeks (27.4 % ± 2.99 %).

Fig. 4.7. Percentage mortality (95 % confidence interval) of *Tenebrio molitor* larvae buried in soil after exposure to *Steinernema yirgalemense* at different concentrations (4.3, 8.6, and 17.2 IJs/cm^2) over a period of 4 weeks (two-way ANOVA: $F_{(9, 84)} = 0.41526$, $p = 0.92366$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

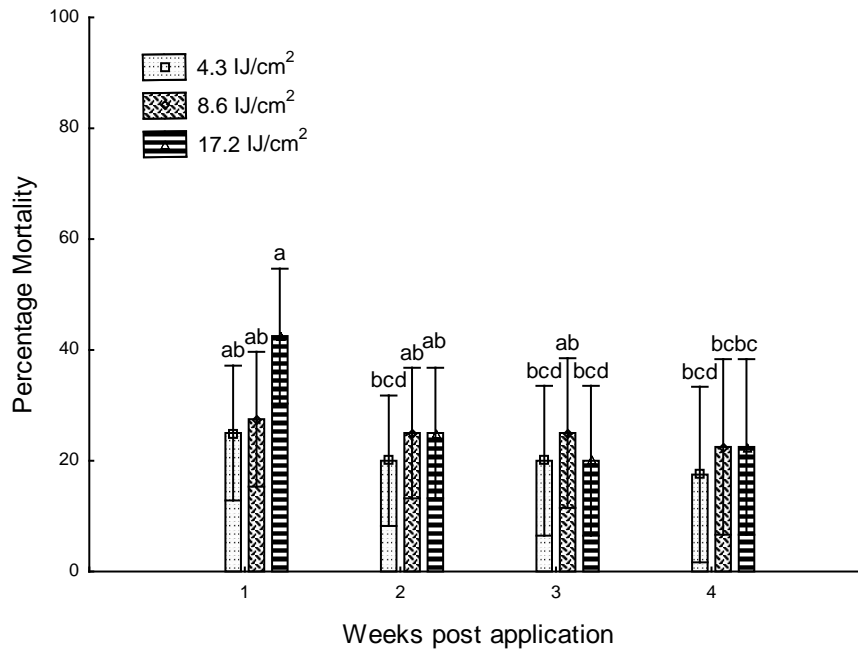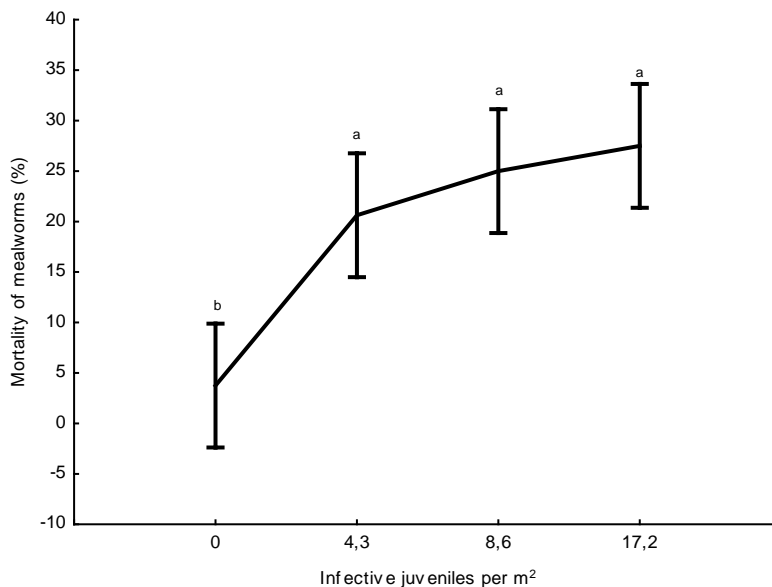**Fig. 4.8.** Cumulative percentage mortality (95 % confidence interval) over four weeks of *Tenebrio molitor* larvae buried in soil after exposure to *Steinernema yirgalemense* at different concentrations (4.3, 8.6, and 17.2 IJs/cm^2) (one-way ANOVA: $F_{(3, 28)} = 12.755$, $p < 0.001$). The different letters above the error bars denote significant differences between treatments ($p < 0.05$).

101

4.3.2 High nematode concentrations

4.3.2.1 Environmental conditions

The mean ambient temperature for the 4-week evaluation period (30 May to 27 June 2018) was 14 °C, which ranged between a minimum of 4 °C and a maximum of 35 °C (data not shown). The IJs can only survive for 24 hours on the foliage, and the mean ambient temperature on the day of nematode application was 14°C, with a minimum of 11 °C, which is considerably below the optimum temp of 25 °C for *S. yirgalemense*. The mean average temperature of the growth substrate during the trial period was 16 °C and ranged between 15 °C and 20 °C, with a minimum and maximum temperature of 8 °C and 31 °C, respectively (Fig. 4.9).
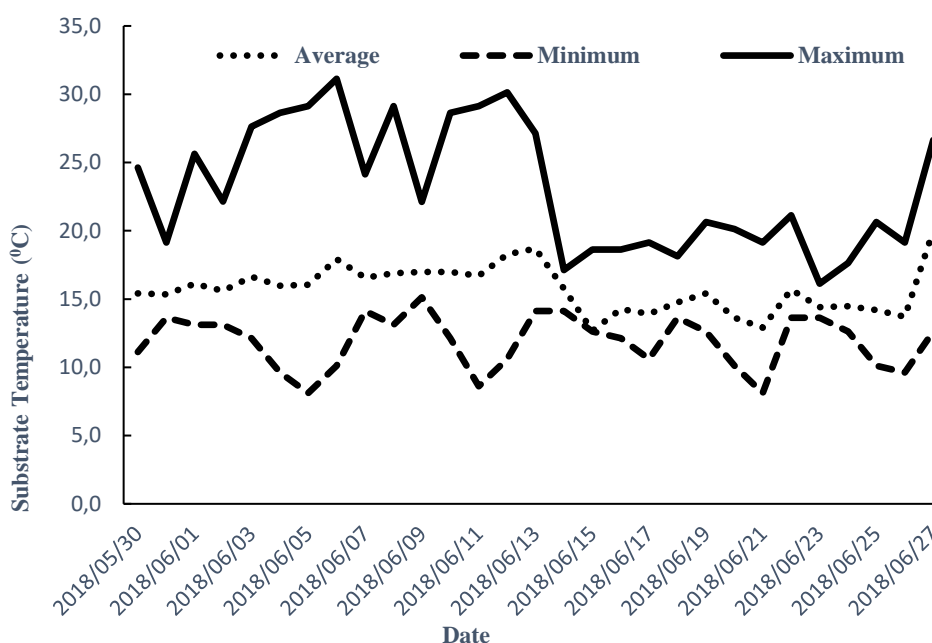
Fig. 4.9. Growth substrate temperature (minimum, maximum, average) in potted blueberries in Haygrove tunnels for a period of 4 weeks.

4.3.2.2 Efficacy of high concentration applications

Analysis of the results using a two-way ANOVA on the main effect of time and concentration of *S. yirgalemense* on the number of *F. occidentalis* on blue sticky traps, over a period of 4 weeks, showed no significant interaction between the treatments and the weeks after treatment ($F_{(9, 84)} = 0.73922$, $p = 0.672$) (Fig. 4.10). Lower number of thrips were observed in the untreated control in all four weeks. The fewest thrips were observed in the first week with the application of the highest nematode concentration (100 IJs/cm^2) (2.25 ± 0.535), but the number did not differ significantly ($p < 0.05$) from the intermediate nematode concentration

102

(50 IJs/cm^2) (3.13 ± 0.535). However, the number differed significantly (p < 0.05) compared to the lowest nematode treatment (25 IJs/cm^2) (4.125 ± 0.535) and the control (5.125 ± 0.535). For the second week, only the control differed significantly from the highest nematode concentration applied. However, in the third and fourth weeks all nematode treatments differed significantly from the control, with the least number of thrips being found on the sticky traps for the highest concentration.
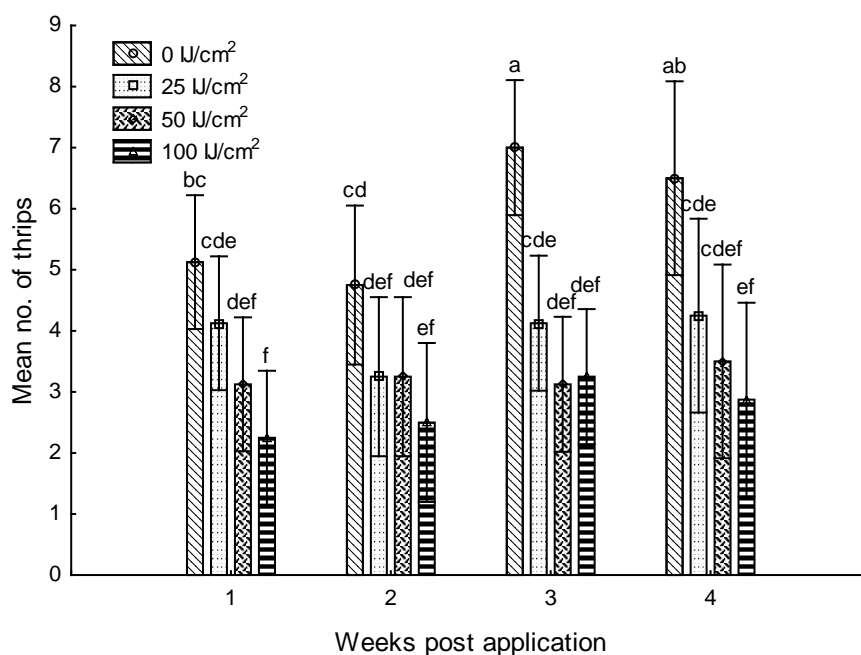
Fig. 4.10. Mean number of adult *Frankliniella occidentalis* recovered from eight blue sticky traps per treatment per week after field treatment of blueberry plants with different concentrations of *Steinernema yirgalemense* (25, 50, 100 IJs/cm^2 and control treated with water only). Two-way ANOVA: $F_{(9, 84)}$ = 0.73922, p = 0.672. The different letters above the error bars denote significant differences between treatments (p < 0.05).

A one-way ANOVA showed that the cumulative number of adult thrips recovered on blue sticky traps over four weeks increased significantly with decreasing EPN concentration ($F_{(3, 28)}$ = 12.990, p < 0.001) (Fig. 4.11). Cumulative results show that there were significantly fewer thrips that emerged in all treatment concentrations, as compared to the untreated control. The highest number of thrips was recovered from the control treatment (5.84 ± 0.378), and significant differences (p < 0.001) were found between the control and the other nematode treatments. No significant difference (p < 0.001) was found between the lowest concentration (3.94 ± 0.378) and the intermediate nematode concentration (3.25 ± 0.378). The intermediate nematode concentration was also not significantly different (p < 0.001) from the highest

concentration (2.72 ± 0.378). The highest concentration treatment had the least number of thrips recovered, only 2.72 ± 0.378.
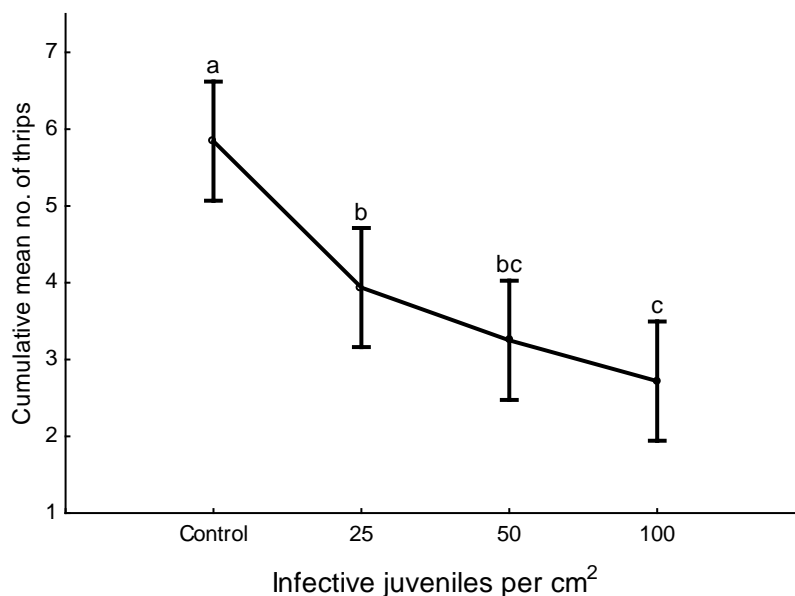
Fig. 4.11. Cumulative mean number of adult *Frankliniella occidentalis* (95 % confidence intervals) recovered from eight blue sticky traps per treatment over four weeks, after application of *Steinernema yirgalemense* at four different concentrations (25, 50, 100 IJs/cm^2 and control treated with water only (one-way ANOVA: (F $_{(3, 28)}$ = 12.990, p < 0.001). The different letters above the error bars denote significant differences between treatments (p < 0.05).

4.3.2.3 EPN persistence

There were no significant interactions (F $_{(9, 84)}$ = 0.55028, p = 0.83348) between the test period, the weeks, and the treatments of the trial (Fig. 4.12). In all the weeks no significant differences were observed between all concentration treatments. From the first to the second week, EPN persistence (expressed in terms of mealworm mortality) declined for all treatments. However, week 3 showed an increase, with no significant difference (p < 0.05) between the concentrations of nematodes applied. The cumulative percentage mortality was significantly different (F $_{(3, 28)}$ = 12.616, p < 0.001) in persistence, expressed in terms of mealworm mortality, between the control and the three nematode concentrations over the 4 weeks. (Fig. 4.13). The intermediate concentration had the highest mortality of mealworms (44.37 % $\pm$ 4.77 %), followed by the highest concentration (42.50 % $\pm$ 4.77 %), and the lowest concentration (38.13

% ± 4.77 %) with the least. The highest nematode concentration showed a slight decline in mortality when compared with the intermediate nematode.

Fig. 4.12. Percentage mortality (95 % confidence interval) of *Tenebrio molitor* larvae buried in soil after exposure to *Steinernema yirgalemense* at different concentrations (25, 50, and 100 IJs/cm^2) over a period of 4 weeks (two-way ANOVA: F $_{(9, 84)}$ = 0.55028, p = 0.83348). The different letters above the error bars denote significant differences between treatments (p < 0.05).

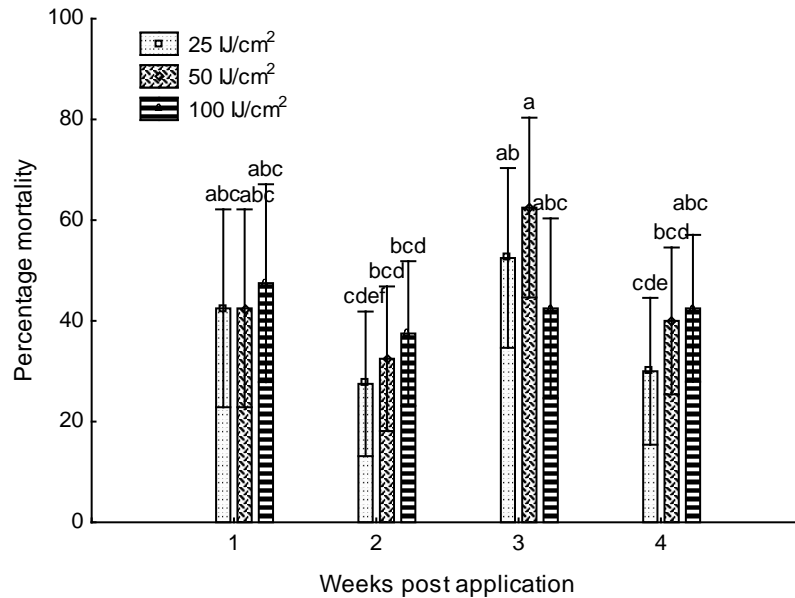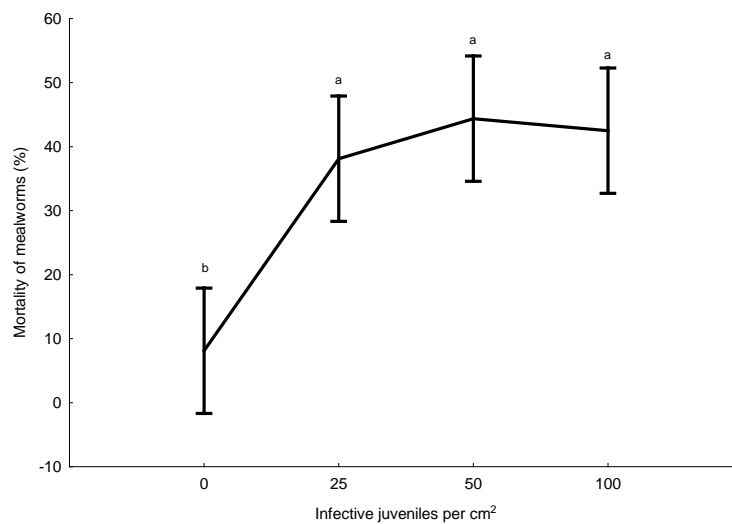**Fig. 4.13.** Cumulative percentage mortality (95 % confidence interval) over four weeks of *Tenebrio molitor* larvae buried in soil after exposure to S*teinernema yirgalemense* at different concentrations (25, 50, and 100 IJs/cm^2) (one-way ANOVA: F $_{(3, 28)}$ = 12.616, p < 0.001). The

different letters above the error bars denote significant differences between treatments (p < 0.05).

4.4   DISCUSSION

The blueberry industry in South Africa is experiencing a period of robust growth. Production mostly takes place in Haygrove tunnels, because growers are able to manipulate the environmental conditions to optimize yields. The short life cycle, as well as overlapping generations during the blueberry flowering cycle, make *F. occidentalis* a key pest that can reach damaging levels in a very short period (Arévalo *et al*. 2009). Blueberry blooming period lasts for approximately 25 days, from the beginning of flower opening to petal drop and attacts more thrips, because of the white colour of the flowers (Arévalo *et al*. 2009). An IPM system seems to be a viable solution for the control of WFT. The field trial was done during the flowering period when economic damage of thrips was observed and when chemical control cannot be applied due to the presence of pollinators and to avoid exceeding minimum chemical residues on the edible crop.

In this study both foliar and soil applications of EPNs were applied simultaneously to target WFT larvae, adults and pupae. Foliar applications of *S. feltiae*, together with a wetting agent, have previously been shown to control WFT adults and larvae successfully in chrysanthemum (Buitenhuis & Shipp 2005). Another reason for including foliar applications is that while pupation mostly occurs in the soil, some thrips pupate on the host plants, especially in complex floral architecture (Broadbent *et al.* 2003) and soil applications therefore target only a portion of the WFT population.

The relatively poor efficacy of *S. yirgalemense* observed in this study is ascribed to the low substrate temperatures (15 °C to 20 °C) prevailing during the study period. These suboptimal temperatures might have affected establishment and sustainability of *S. yirgalemense* negatively, although the data suggest that *S. yirgalemense* was still able to infect and reproduce, resulting in trap catches of WFT of up to 50 % less than the untreated control. Several studies having already demonstrated the influence of temperature and RH on the infectivity of EPNs. Kaya (1990) emphasised that soil temperature has a major impact on nematode behaviour and temperature tolerance for infection and reproduction, and variation might exist among different EPN species and strains (Wright *et al*. 2005). Under the temperature conditions prevailing in this study, the control treatments for both experiments

captured a relatively high number of thrips. The temperature range was below optimum for nematode infection and reproduction. Extreme temperatures of 0 °C and 40 °C are lethal to EPNs, with temperatures below 10 °C to 15 °C restricting their mobility, while temperatures higher than 30 °C to 40 °C can inactivate them (Bedding *et al*. 1993; Grewal *et al.* 1994). In studies conducted by Odendaal *et al.* (2016b) and Platt (2017), *S. yirgalemense* were found not to be active at temperatures < 14 °C, resulting to generally low mortality for codling moth and mealybugs.

Previous laboratory experiments (Chapter 3) showed that *S. yirgalemense* was able to cause mortality of 66 % of WFT pupae at a constant temperature of 25 °C. Arthurs *et al*. (2003), in their study of the parasitic nematode, *Thripinema nicklewoodi* Siddiqi (Tylenchida: Allantonematidae), against WFT, suggested that under fluctuating temperatures, in comparison to a constant temperature of 20 °C, discontinuous exposure of the nematodes to lower temperatures (10 °C) and higher temperatures (35 °C) still allowed for development and reproduction. Such development and reproduction occurred when the nematodes were allowed periodic 10-hour daily exposure to a suitable temperature range from 20 °C to 30 °C. A study undertaken by Kung *et al*. (1990), on the effects of soil temperature, moisture and RH on EPN persistence, showed that *Steinernema carpocapsae* Poinar, a temperate nematode, was persistent at low temperatures from 5 °C to 25 °C, whereas only poor persistence was observable at a temperature of 35 °C. In contrast, the subtropical origin of *Steinernema glaseri* enabled it to perform optimally at high temperatures (15−35 °C), with it having poor persistence at 5 °C.

WFT also reproduces rapidly at higher temperatures, as compared to the rate at which they reproduce at low temperatures (McDonald *et al.* 1998), with higher infestations at temperatures above 30 °C being more likely than at lower temperatures (Arthurs *et al.* 2003). Some days during the trial experienced relatively high temperatures, and although such temperatures were prevalent for only a few hours, they could still have enhanced the rapid reproduction of WFT. Both *S. yirgalemense* and the WFT were present in the same ecological niche for a short time, but because of the rapid reproduction rate of WFT at higher temperatures, EPN efficacy was reduced. The rapid rate of development at higher temperatures could be an escape mechanism for WFT against EPNs.

The increased EPN concentrations resulted in lower numbers of adult WFT captured on sticky traps. With due consideration to the possibility that the number of insects on the traps might not have accurately reflected the actual WFT population level, it can be concluded that

the best results were obtained with an EPN concentration of 100 IJs/cm^2. This concurs with Ebssa *et al.* (2006), who found that the WFT mortality caused by *H. bacteriophora* and *Steinernema abbasi* Elawad, Ahamad & Reid at 100 IJs/cm^2 was not significantly different from the mortality in the water-treated control, and that the highest mortality was recorded at 1000 IJs/cm^2.

The reduction in WFT numbers at the low EPN concentrations did not differ significantly from the reduction obtained with high concentrations, which could be attributed to the fact that the mean temperatures recorded during the first trial were higher than were the mean temperatures recorded during the second trial. Another possible reason for the lack of variation was that *A. limonicus* was released in the tunnel a week before the start of the first experiment with low EPN concentrations. This might have contributed to supressing the thrips populations, overlapping the start of the experiment. Koppert-Biological Systems (2013) recommends that *A. limonicus* be released at 2-weekly intervals, implying that their persistence beyond 2 weeks is limited. The second experiment, implemented 3 weeks after the release of *A. limonicus*, would not have been much affected by their release, and the reduction in WFT numbers could be attributed to the introduction of *S. yirgalemense.*

Persistence trials indicate that EPNs persisted and were able to infect the mealworms for at least 4 weeks post application. Generally, the mortality of the mealworms in both the experiments was very low, leading to the conclusion that the low substrate temperatures limited the ability of *S. yirgalemense* to infect the mealworms, which is a good host for EPNs. De Waal *et al*. (2011) recorded low levels of mortality (< 3 %) for the local EPN isolates, including *S. yirgalemense*, against codling moth, *Cydia pomonella*, in winter temperatures between 12 $^{\circ}$C and 17 $^{\circ}$C. Le Vieux and Malan (2015) showed a 6-month laboratory persistence for *S. yirgalemense* against the codling moth larva of > 80 %, which was not significantly different for all the months concerned, showing the potential of this species to persist.

In conclusion, although the level of WFT suppression by *S. yirgalemense* in both field trials was low, particularly compared to the levels of mortality achieved in laboratory trials, the results indicate the potential for the biological control of *F. occidentalis* using *S. yirgalemense.* The results also suggest that applying the EPNs when greenhouse temperatures are higher will allow for optimum nematode infection and better WFT suppression. The application of *S. yirgalemense* as soil and foliar treatments for WFT control on the new growth of blueberries (after post-harvest pruning), therefore, warrant further investigation. Persistence studies should

also preferably be conducted in separate pots, because as putting mealworms in the pots where WFT control is evaluated, might diverge the EPN's focus from the WFT. The frequency of follow-up EPN applications for optimum control should also be investigated.

4.5. REFERENCES

ARTHURS, S., HEINZ, K.M., THOMPSON, S. & KRAUTER, P.C. 2003. Effect of temperature on infection, development and reproduction of the parasitic nematode *Thripinema nicklewoodi* in *Frankliniella occidentalis. BioControl* **48**: 417–429. DOI https://doi.org/10.1023/A:1024798928406

ARÉVALO, H.A., FRAULO, A.B. & LIBURD, O.E. 2009. Management of flower thrips in blueberries in Florida. *Florida Entomologist* **92**: 14–17. https://doi.org/10.1653/024.092.0103

BEDDING, R.J., AKHURST, R. & KAYA, H. (Eds). 1993. *Nematodes and the Biological Control of Insect Pests*. CSIRO Publication, Melbourne.

BERNDT, O., POEHLING, H.M. & MEYHOFER, R. 2004. Predation capacity of two predatory laelapid mites on soil-dwelling thrips stages. *Entomologia Experimentalis et Applicata* **112**: 107–115. https://doi.org/10.1111/j.0013-8703.2004.00185.x

BROADBENT, A.B., RHAINDS, M., SHIPP, L., MURPHY, G. & WAINMAN, L. 2003. Pupation behaviour of western flower thrips (Thysanoptera: Thripidae) on potted chrysanthemum. *Canadian Entomology* **135**: 741–744. DOI: https://doi-org.ez.sun.ac.za/10.4039/n03-007

BRØDSGAARD, H.F. 2004. Biological control of thrips on ornamental crops. *Journal of Economic Entomology* **98**: 253–264.

BUITENHUIS, R. & SHIPP, J.L. 2005. Efficacy of entomopathogenic nematode *Steinernema feltiae* (*Rhabditida*: *Steinernematidae*) as influenced by *Frankliniella occidentalis* (*Thysanoptera*: *Thripidae*) developmental stage and host plant stage. *Journal of Economic Entomology* **98**: 1480–1485. https://doi.org/10.1093/jee/98.5.1480

CLOYD, R.A. 2015. Western flower thrips management in greenhouse production systems in the 21st century: alternative strategies need to be considered. *Acta Horticulture* **1104**: 381–393. DOI10.17660/ActaHortic.2015.1104.57

DE WAAL, J.Y., MALAN, A.P. & ADDISON, M.F. 2011. Efficacy of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) against codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) in temperate regions. *Biocontrol Science and Technology* **21**: 1161–1176. https://doi.org/10.1080/09583157.2011.607922

DLAMINI, B.E. 2018. Biological control of the banded fruit weevil, *Phlyctinus callosus*, on deciduous fruit and grapevine, using entomopathogenic nematodes and fungi. *Austral Entomology*, (submitted).

EBSSA, L., BORGEMEISTER, C. & POEHLING, H.M. 2006. Simultaneous application of entomopathogenic nematodes and predatory mites to control western flower thrips *Frankliniella occidentalis*. *Biological Control* **39**: 66–74. https://doi.org/10.1016/j.biocontrol.2006.02.005

EFRON, B. & TIBSHIRANI, R. 1993. An introduction to the bootstrap. Chapman and Hall, London.

FERREIRA, T., ADDISON, M.F. & MALAN, A.P. 2016. Development and population dynamics of *Steinernema yirgalemense* and growth characteristics of its associated *Xenorhabdus* symbiont in liquid culture. *Journal of Helminthology* **90**: 108−112.

GAUGLER, R. & GEORGIS, R. 1991. Culture method and efficacy of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). *Biological Control* **1**: 269–274. https://doi.org/10.1016/1049-9644(91)90077-D

GOULI, S., GOULI, V., SKINNER, M., PARKER, B., MARCELINO, J. & SHTERNSHIS, M. 2008. Mortality of western flower thrips, *Frankliniella occidentalis*, under influence of single and mixed fungal inoculations. *Journal of Agricultural Technology* **4**: 37–47.

GREWAL, P.S., SELVAN, S. & GAUGLER, R. 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* **19**: 245–253.

GRIFFIN, C.T., BOEMARE, N.F. & LEWIS, E.E. 2005. Biology and behaviour. In: Greval, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (Eds) *Nematodes as Biocontrol Agents*. 47–64. CABI, Wallingford.

HARRIS, M.A., OETTING, R.D. & GARDNER, W.A. 1995. Use of entomopathogenic nematodes and a new monitoring technique for control of fungus gnats, *Bradysia coprophila* (Diptera: Sciaridae), in floriculture. *Biological Control* **5**: 412–418. https://doi.org/10.1006/bcon.1995.1049

HIGGINS, C.J. 1992. Western flower thrips (Thysanoptera: Thripidae) in greenhouses/population-dynamics, distribution on plants, and associations with predators. *Journal of Economic Entomology* **85**: 1891–1903. https://doi.org/10.1093/jee/85.5.1891

JAMES, M., MALAN, A.P. & ADDISON, P. 2018. Surveying and screening South African entomopathogenic nematodes for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Crop Protection* **105**: 41–48. https://doi.org/10.1016/j.cropro.2017.11.008

KATUMANYANE, A., FERREIRA, T., MALAN, A.P., 2018. Potential use of local entomopathogenic nematodes to control *Bradysia impatiens* (Diptera: Sciaridae) under laboratory conditions. *African Entomology* 26: 1–13. DOI:https://doi.org/10.4001/003.026.0001

KAYA, H.K. 1990. Soil ecology. In: Gaugler, R. & Kaya, H.K. (Eds) *Entomopathogenic Nematodes in Biological Control*. 93–116. CRC Press, Boca Raton, FL.

KAYA, H.K. & GAUGLER, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* **38**: 181–206. https://doi.org/10.1146/annurev.en.38.010193.001145

KOPPERT-BIOLOGICAL SYSTEMS. 2013. Info-sheet LIMONICA. http://www.koppert.com/products/products-pests-diseases/products/detail/limonica/. 01.08.2018.

KUNG, S., GAUGLER, R. & KAYA, H.K. 1990. Effects of soil temperature, moisture and relative humidity on entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* **57**: 242–249. https://doi.org/10.1016/0022-2011(91)90123-8

LACEY, L.A. & GEORGIS, R. 2012. Entomopathogenic nematodes for control of insect pests above and below with comments on commercial production. *Journal of Nematology* **44**: 218–225.

LE VIEUX, P.D. & MALAN, A.P. 2015. Prospects for using entomopathogenic nematodes to control the vine mealybug, *Planococcus ficus*, in South African vineyards. *South African Journal of Enology and Viticulture* **36**: 59–70. http://www.scielo.org.za/scielo.php?script=sci_arttext&pid=S2224-79042015000100012&lng=en&tlng=en.

MCDONALD, J. R., BALE, J. S. & WALTERS, K. F. A. 1998. Effect of temperature on development of the Western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *European Journal of Entomology* **95**: 301–306.

MORITZ, G.S., KUMM, S. & MOUND, L. 2004. Tospovirus transmission depends on thrips ontogeny. *Virus Research* **100**: 143–149. https://doi.org/10.1016/j.virusres.2003.12.022

MURPHY, G., FERGUSON, G. & SHIPP, L. 2014. Thrips in greenhouse crops – biology, damage and management. *Ontario Ministry of Agriculture, Food and Rural Affairs Factsheet* 03–077.

NAVON, A. & ASCHER, K.R. 2000. Bioassays of *Bacillus thuringienis* products used against agricultural pests. In: Navon, A. & Ascher, K. R. S. (Eds) *Bioassays of Entomopathogenic Microbes and Nematodes*. 1–24. Wallingford, UK: CABI Publishing.

ODENDAAL, D., ADDISON, M.F. & MALAN, A.P. 2016a. Control of codling moth (*Cydia pomonella*) (Lepidoptera: Tortricidae) in South Africa with special emphasis on using entomopathogenic nematodes. *African Entomology* **23**: 259–274. http://hdl.handle.net/10520/EJC176580

ODENDAAL, D., ADDISON, M.F. & MALAN, A.P. 2016b. Evaluation of above-ground application of entomopathogenic nematodes for the control of diapausing codling moth (*Cydia pomonella* L.) under natural conditions. *African Entomology* **24**: 61–74. http://hdl.handle.net/10520/EJC185856

PLATT, T. 2017. Investigating the above-ground application of EPNs for the control of the vine mealybug *Planococcus ficus* (Doctoral dissertation, Stellenbosch: Stellenbosch University). http://hdl.handle.net/10019.1/102729

PREMACHANDRA, W.T., BORGEMEISTER, C., BERNDT, O., EHLERS, R.U. & POEHLING, H.M. 2003. Combined releases of entomopathogenic nematodes and the

predatory mite *Hypoaspis aculeifer* to control soil-dwelling stages of western flower thrips *Frankliniella occidentalis*. *Biocontrol* **48**: 529–541. DOI https://doi.org/10.1023/A:1025703512113

SHIPP, J.L. & RAMAKERS, P.M. 2004**.** Biological control of thrips on vegetable crops, pp. In: Heinz, K.M., Van Driesche, R.G. & Parella, M.P. (Eds) *Biocontrol in Protected Culture*. 265–276. Ball, Batavia, IL.

SIGUNA, A.D. 2007. Cultural and botanical methods for the management of thrips on French beans *Phaseolus vulgaris*. Thesis M.Sc. (Agricultural Entomology), School of Pure and Applied Sciences, Kenyatta University, Nairobi. http://hdl.handle.net/123456789/137

SIKUKA, W. 2017. *The Budding Blueberry Industry in South Africa*. https://gain.fas.usda.gov/RecentGAINPublications/TheBuddingBlueberryIndustryinSouth Africa. 08.06.2018.

StatSoft Inc, 2016. STATISTICA (Data Analysis Software System) Version 13.0.

TRDAN, S. & JENSER, G. 2003. Monitoring of western flower thrips (*Frankliniella occidentalis* Pergande) in the vicinity of greenhouses in different climatic conditions in Slovenia. *IOBC/WPRS Bulletin* **26**: 25–31.

WRIGHT, D.J., PETERS, A., SCHROER, S. & FIFE, J.P. 2005. Application technology. In: Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (Eds) *Nematodes as Biocontrol Agents*. 91–106. CABI, Wallingford.

Chapter 5

5.1   CONCLUSION

Frankliniella occidentalis, western flower thrips (WFT), is considered to be a major pest in undercover crop production worldwide (Cloyd 2009). The South African blueberry industry is growing rapidly and production in Haygrove tunnels is gaining popularity, because of the relatively higher yields and improved marketability of fruit. Since the introduction of *F. occidentalis* into South Africa the 1980s, the pest has spread rapidly to become one of the most prevalent insect pests in blueberry production. The efforts to control WFT so far have mainly been through the use of insecticides, but this is complicated by the fact that WFT has developed resistance to most insecticide classes. Chemical control is also restricted for use on food crops, especially during harvesting, due to food safety concerns, leaving farmers with biological control as the best alternative option. Biological control agents that are commonly used in blueberry production are predatory mites and predatory bugs (*Orius* spp.). Their efficacy is limited by the cryptic behaviour of the WFT, as their relatively large size makes it difficult for them to penetrate the enclosed spaces like flower buds favoured by WFT. Entomopathogenic nematodes (EPNs), however, are able to enter these spaces in search of insect host and therefore insect pest control using EPNs is starting to gain popularity in South Africa. *Steinernema feltiae* is a common EPN species that has been commercially used in the USA and in European countries against *F. occidentalis* with great success. However, as *S. feltiae* has not been isolated in South Africa, its importation and use is prohibited in the country. This sparked interest in the use of locally isolated EPN species, which may work best in local environments. The efficiency of locally isolated EPN species against different insect pests, and their mass culturing for commercialisation are still being explored.

Successful implementation of an IPM programme requires accurate identification of pests. WFT are often confused with other species, as approximately 7500 species of thrips are known to exist (Mound 2009). Morphological identification is difficult, due to the small, fragile nature of the pest. It requires well-prepared microscope slides to be viewed under a high-quality compound microscope and considerable expertise is required to identify key morphological features for species identification. During the current study, the focus was on identifying *F. occidentalis*, using both morphological and molecular techniques. Key morphological characteristics were observed fitted well with the descriptions given for *F. occidentalis*, and with the three colour morphs of *F. occidentalis*, the intermediate brown colour was found to be

114

the dominant colour morph in the locations sampled. Given that taxonomic expertise is getting scarce and not always readily available, it is a valuable alternative to use molecular identification. The analysis of molecular data of DNA sequences has been suggested as a complementary approach to the classical methods to identify insects. Molecular identification fundamentally requires the identification of suitable molecular markers, as they tend to vary in respect of the precise identification of specific insects. PCR using the mtCOI gene for differentiation of thrips species was used for morphological identification. The length of the amplicon, depends on the choice of primers used, and for our sequences was < 400 bp. The use of molecular identification in the study represented an alternative to morphological identification, which proved to be difficult, not only for *F. occidentalis*, but also the other thrips species that were found sporadically in the study areas. The *F. occidentalis* populations collected were compared to other populations in the GenBank database and showed 100 % identity. Little variation was found between the populations collected from the two locations of the study. Molecular identification is often quicker, but the costs can be prohibitive. The use of a combination of PCR and morphological characteristics seems to be a better option, especially using morphological keys for day-to-day identification, augmented by regular molecular identification as a kind of quality control.

Biological experiments require a constant and reliable culture of test organisms, and establishing and maintaining such a culture often is laborious and time-consuming. It is especially difficult for small insects like thrips, and previous studies have attributed failures in the rearing of thrips to factors like high vulnerability to contamination, environmental conditions, host quality, and crowding (Loomans & Murai 1997). Green beans are a commonly used host plant for culturing thrips, while chrysanthemums are known to be very attractive to WFT. Chrysanthemum leaflets and green bean pods were selected as host plants to study the life-history of WFT, as this would provide insight into which life stages are suitable to target with EPNs. The fact that more first instar larva hatched on chrysanthemums, faster larval developmental rate and a higher survival rate on chrysanthemums indicate that chrysanthemum is a more attractive and more suitable host than green bean. The preference for chrysanthemums over green beans was attributed to the morphological and chemical traits associated with the host, which were not evaluated in the study. Generally, there was reduced number of eggs that hatched for both host plants, because artificially grown thrips tend to be more sensitive than are those in natural environments, especially when the daylight occurring in natural environments is compared, with the artificial lights provided in rearing cages. Microclimate

conditions are important for successful rearing of thrips, especially adequate daylight, temperature and ventilation have been recognised as key factors affecting egg hatching as they are linked to diapause in thrips. When rearing WFT for laboratory bio-assays it is important to use the most suitable host plant that is practical to obtain as many insects as possible and to ensure sufficient light and ventilation, which is key for egg hatching. Usually, flowers are the best parts of the plant for *F. occidentalis* feeding and oviposition (Lewis 1973), although their limitation is that they cannot be kept for long.

Laboratory pre-screening results for 11 local EPN species and one exotic *S. feltiae* revealed highly variable mortality, ranging between 11 % and 67 %, against second instar larvae of *F. occidentalis*. This variation is attributed to a combination of factors related to host size and defence mechanism and also the nematode size and foraging behaviour. EPN size in relation to host size is fundamental in determining the susceptibility of insects to infection by EPN species, and is therefore a key factor to determine the choice of an EPN species against a particular micro-insect pest. This study concluded that the relatively small nematodes, *H. baujardi and S. yirgalemense* in particular, were able to infect the WFT, with no infection occurring with nematodes > 1mm. The foraging behaviour of the EPNs also play a role in pathogenicity of the nematode against a particular host. WFT was observed to be more susceptible to the *Heterorhabditis* spp. than *Steinernema* spp., which besides the difference in size, was also attributed to the fact that *Heterorhabditis* possess a dorsal tooth that enables them to penetrate the host insect directly through the cuticle of the host, whereas *Steinernema* spp. lack the dorsal tooth and can only penetrate through natural openings, e.g. the anus. *Steinernema feltiae* did not perform well in comparison with the local EPN isolates in the laboratory. The fact that it was outperformed by the local *S. yirgalemense* may be because the latter is slightly smaller and thus better able to infect and multiply in the second instar WFT larvae.

The results of the bioassays also confirmed results of other researchers (Chyzik *et al*. 1996; Ebssa *et al*. 2001; Premachandra *et al*. 2003; Pundt 2011), that the pupae of WFT are more susceptible to nematode infection than the adults and larvae. This is attributed to the low mobility of the pupal stages, which facilitates the attachment of nematodes to them (Koppenhöfer *et al.* 2003). The low level of parasitism shown by the adults for all the EPN species is attributed to the hardening of the cuticle as thrips age, and to the fact that they are very active, thus making penetration difficult. Thrips mortality increased with increased concentration from 10 IJs/larva to 160 IJs/larva for both *S. yirgalemense* and *H. baujardi*. The

LD$_{50}$ and LD$_{90}$ shows that *S. yirgalemense* was five times more potent than was *H. baujardi*. Generally, *F. occidentalis* seems to require more *S. yirgalemense* IJs for optimum control, under the same conditions, in comparison to the findings of other studies on other insect pests like mealybugs, fungus gnats and banded fruit weevil.

For EPNs to complete their life cycle in an insect host, they must invade and kill the host and be able to develop into adults and produce IJs which will emerge from the cadaver in search of a new host (Bastidas *et al*. 2014). The temporal development studies showed that *S. yirgalemense* and *H. baujardi* were able to complete one life cycle in *F. occidentalis*, meaning that the nematodes developed into hermaphrodites in the case of *H. baujardi*, and males and female in the case of *S. yirgalemense*, and their eggs developed into infective juveniles (IJs). Relatively few IJs penetrated each of the host insects, because of their small size and the EPN life cycle was short, taking approximately five days to produce a new cohort of nematodes. The IJs recovered from the host were relative in number to the number of IJs that had penetrated the host. The number of eggs laid by the female depends on the size of the host as well. The nematodes emerging from the egg developed into IJs, due to the lack of food supply in the host. The late second larval phase is known to stop feeding, and to incorporate the bacteria into the bacterial chamber or vesicle, then transforming into pre-infective and infective larvae, retaining the cuticular of the second larval phase as a sheath. It was also observed that in some insects the IJs were able to infect host insects but the nematodes only developed to a certain stage, and disappeared without finishing their cycle. The phenomenon is ascribed to a lack of food in the micro-insect. The small size of WFT can be a limiting factor in the potential of EPNs to multiply in a production environment and this indicates that follow-up applications of EPNs may be required to achieve effective biocontrol of WFT.

EPN performance under optimum laboratory conditions is not necessarily representative of the performance under field conditions, as was evident in the field trials conducted with different concentrations of *S. yirgalemense*. The field trial was aimed at targeting all developmental stages of WFT, therefore foliar and soil applications were made simultaneously during the flowering period of blueberries in May/June. The level of WFT suppression achieved with all the concentrations of *S. yirgalemense* was less than what was achieved in the laboratory screening studies. Concentrations below 100 IJs/cm^2, in combination with low substrate temperatures of < 15 $^{\circ}$C, proved to limit the efficacy of *S. yirgalemense*. Previous studies have demonstrated that temperature greatly influences the infectivity, development, reproduction, and survival of EPNs (Kaya (1990). Studies by Odendaal *et al.* (2016) and Platt

117

(2017), showed that *S. yirgalemense* is inactive at temperatures < 14 $^\circ$C. The low-to-moderate suppression of *F. occidentalis* in the field trial does, however, indicate that the EPNs were still able to survive the daily fluctuations in temperature. This was also corroborated by the persistence trials which showed that *S. yirgalemense* was able to persist in the substrate for four weeks, despite the sub-optimal temperatures. Persistence studies should preferably be conducted in separate studies, because putting mealworms in the pots where WFT control is evaluated, might reduce the effect of the EPNs on WFT, thereby underestimating the efficacy of the EPNs. For successful biocontrol using EPNs it is necessary to know the temperature tolerance of the particular EPN species, and to apply the EPNs when the micro-climate is most suitable to ensure optimum results. The components of an IPM system states that any method used might not significantly reduce the pest population, but combining the different methods should give adequate reduction to prevent economic losses.

The bioassays and field experiments furthered understanding of the potential of EPNs for use as biocontrol agents against *F. occidentalis* under local conditions. The current study established the basis for further applied studies to search for environmentally compatible strategies that allow for the enhancement of *S. yirgalemense* as a biocontrol agent for *F. occidentalis*. Future field trials should focus on EPN applications to target WFT on the new growth flush after post-harvest pruning, usually between December and February, when WFT populations peak and temperatures in the tunnels are closer to the optimum for *S. yirgalemense.* The feasibility of applying *S. yirgalemense* in conjunction with other biological agents, and the insecticide–pathogen synergistic interactions in IPM systems, should be investigated. Further work is also required on the application of EPNs in various formulations with conventional spray equipment. Succsessful mass rearing of *S. yirgalemense* has been achieved and paves the way for commercialisation. The demonstrated potential of *S. yirgalemense* as a biocontrol agent for *F. occidentalis* should not preclude the continued testing of additional locally isolated EPN species for pathogenicity against *F. occidentalis*.

5.2   REFERENCES

BASTIDAS, B., PORTILLO, E. & SAN-BLAS, E. 2014. Size does matter: the life cycle of *Steinernema* spp. in micro-insect hosts. *Journal of invertebrate pathology* **121**:46-55. https://doi.org/10.1016/j.jip.2014.06.010

CHYZIK, R., GLAZER, J. & KLEIN, M. 1996. Virulence and efficacy of different entomopathogenic nematode species against western flower thrips (*Frankliniella occidentalis*). *Phytoparasitica* **24**: 103–110.

CLOYD, R.A. 2009. Western flower thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: have we reached an impasse? *Pest Technology* **3**: 1–9.

EBSSA, L., BORGEMEISTER, C., BERNDT, O. & POEHLING, H.M. 2001. Efficacy of entomopathogenic nematodes against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Invertebrate Pathology* **78**: 119–127. DOI: 10.1006/jipa.2001.5051

KAYA, H.K. 1990. Soil ecology. In: Gaugler, R. & Kaya, H.K. (Eds.) *Entomopathogenic Nematodes in Biological Control*. 93–116. CRC Press, Boca Raton, FL, U.S.A.

KOPPENHÖFER, A.M., COWLES, R.S., COWLES, E.A., FUZY, E.M. & BAUMGARTNER, L. 2003. Comparison of neonicotinoid insecticides as synergists for entomopathogenic nematodes. *Biological Control* **24**: 90–97. https://doi.org/10.1016/S1049-9644(02)00008-7

LEWIS, T. 1973. Thrips. Their biology, ecology and economic importance. Academic Press, London.

LOOMANS, A.J. & MURAI, T. 1997. Culturing thrips and parasitoids. In: Lewis, T. (Ed.) *Thrips as Crop Pests*. 639–666. CAB International, Wallingford.

MOUND, L.A. 2009. *Thysanoptera* (Thrips) of the world-a-checklist. www.ento.csiro.ou/thysanoptera /worldthrips.html.

ODENDAAL, D., ADDISON, M.F. & MALAN, A.P. 2016. Evaluation of above-ground application of entomopathogenic nematodes for the control of diapausing codling moth (*Cydia pomonella* L.) under natural conditions. *African Entomology* **24**: 61–74. http://hdl.handle.net/10520/EJC185856

PLATT, T. 2017. Investigating the above-ground application of EPNs for the control of the vine mealybug *Planococcus ficus* (Doctoral dissertation, Stellenbosch: Stellenbosch University). http://hdl.handle.net/10019.1/102729

PREMACHANDRA, W.T., BORGEMEISTER, C., BERNDT, O., EHLERS, R.U. & POEHLING, H.M. 2003. Laboratory bioassays of virulence of entomopathogenic nematodes against soil-inhabiting *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). *Nematology* **5**: 539–547. DOI: 10.1163/156854103322683256

PUNDT, L. 2011. Biological control: using beneficial nematodes. https://ag.umass.edu/greenhouse-floriculture/fact-sheets/. 15.03.2018.