**University of KwaZulu-Natal**

# *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) population dynamics, biological control, and the characterisation of *Bracharoa mixta* (Snellen) (Lepidoptera: Erebidae) and wind in scarring avocado, *Persea americana* Miller (Lauraceae)


By

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## Thesis abstract

Avocado (*Persea americana* Miller) production is a growing, multibillion rand, export oriented industry in South Africa employing tens of thousands of people and contributing significantly to the local GDP. To remain competitive in the global market, the South African avocado industry needs to consistently produce high quality fruit. However, poor quality fruit remains one of the biggest challenges to export. The aim of this study was to identify, describe and quantify the role played by biotic and abiotic factors in scarring avocado fruit, as well as to propose mitigatory approaches. The lack of current, updated and scientifically evidenced documentation is a shortcoming addressed in this thesis with a goal to enable policy makers, growers and academics to minimize scarring losses.

Using a modified beat cup method, the avocado thrips spectrum during flowering and early fruiting was found to include *Frankliniella occidentalis* (Pergande), *Scirtothrips aurantii* Faure, *Thrips gowdeyi* (Bagnall), *Thrips pusillus* Bagnall, *Thrips tenellus* Trybom, *Haplothrips gowdeyi* (Franklin), *Haplothrips bedfordi* Jacot-Guillarmod and *Megalurothrips sjostedti* (Trybom). Tea filter paper (74 µm pore size) and coffee filter paper (53 µm pore size) were determined to be effective thrips screen material for use in thrips exclusion trials. Fruit sampling using naturally infested fruitlets confirmed that avocado is a natural host to *S. aurantii* (the South African citrus thrips). This is the first study to demonstrate that avocado is a natural host to this pest. The confirmation of *S. aurantii,* an established and well-studied pest of citrus, in South African avocado fruit, forms the basis for tailor-made IPM programs in avocado fruit and allows for parallels and comparisons to be drawn on its biology, ecology and management from the citrus industry.

To monitor the presence and abundance of *S. aurantii* in avocado orchards during early fruiting, several different coloured sticky traps and placements (border vs interior) were trialled. Yellow sticky traps were found to be effective, with the incidence and distribution of the pest tending to be random, with numbers high during the spring flush, declining thereafter and picking up in summer. This means that when monitoring for *S. aurantii*, yellow sticky traps can be set-up randomly in an avocado orchard during early fruiting. The spring flush and early fruiting period present a small window of opportunity when control measures can be implemented (if necessary), to prevent economic scarring damage on the young, developing fruit.

Several pre-harvest diseases are known to inflict serious economic damage to avocado fruit, among them avocado scab (*Sphaceloma perseae* Jenkins). Having been confirmed in South

Africa previously, *S. perseae* was initially suspected as the cause of a scarring injury called "wind damage" by growers. However, morphological and DNA fingerprinting did not confirm its presence. *Cladosporium* spp. were repeatedly isolated from typical scars on avocado fruit but they were neither directly infectious, nor infectious in wounds created by mechanical abrasion (to simulate thrips and wind damage), and pathogenicity was not demonstrated.

Thrips and wind abrasion were identified as major quality constraints accounting for 30 % scarring damage, a loss factor of 13.72 % and a combined revenue loss of 5.57 %. Revenue losses in the order of 1.49 % are incurred annually due to *S. aurantii* downgrading (3.86 % loss factor). Cultivar differences were observed, with 'Pinkerton' and 'Carmen®-Hass' being the most susceptible cultivars. Proximity to macadamia trees was also found to increase incidence of *S. aurantii* scarring damage. Avocado growers are therefore advised to take steps to minimise scarring damage by siting avocado orchards away from prevailing and dominant winds, macadamia trees, as well as putting in place suitable windbreaks.

Towards the end of the 2018/2019 avocado season, unidentified Lepidoptera larvae were observed scarring avocado fruits and defoliating leaves in Wartburg, KwaZulu-Natal, South Africa. Using morphological and molecular techniques, *Bracharoa mixta* Snell. (Lepidoptera: Erebidae) (tussock moth) was identified, DNA barcoded (GenBank MN527963) and voucher specimens (voucher I.D AcP 9636) were deposited for future referencing. This is the first report of tussock moths scarring avocado fruit. Potential revenue losses of up to ZAR 1352.90/t (2.26 % revenue loss) through downgrading are possible because this insect is capable of causing economic loss, in sporadic, isolated epizootics. The implications of these findings are that the insect has been identified, characterized and its biology studied, and this will aid in rapid detection and implementation of IPM should there be a further outbreak.

Dispersal/Emergence (D/E) traps and fruit infestation indices were used to monitor *S. aurantii* populations during the critical early fruiting period and soil insecticide applications were applied to control below-ground life stages. Use of fruit infestation indices is recommended ahead of D/E traps for monitoring. Control using soil drenches of biologicals and synthetic insecticides was not demonstrated, probably because only a small percentage of the thrips pupated in the ground (18.40 % under laboratory conditions). The low percentage of *S. aurantii* pupating on the ground means that soil application of non-systemic insecticides and biocontrol agents is not a viable control option.

## Declaration

I, **Gracian Takudzwa Bara**, declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

iii. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed: [redacted] Date: 09.07.20……

(Student) GT Bara

Signed: . [redacted] …… Date: ……10 July 2020……...

(Supervisor) MD Laing

## List of publications

- BARA, G.T. & LAING, M.D. 2020. First report of tussock moths (*Bracharoa mixta* (Snellen)), scarring avocado fruit in KwaZulu-Natal, South Africa. *African Entomology* **28**: 115-124. DOI: 10.4001/003.028.0115.
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- BARA, G.T. & LAING, M.D. 2020. Susceptibility of avocado fruit to *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and wind scarring damage in Limpopo and KwaZulu-Natal Provinces of South Africa. *Acta Phytopathologica et Entomologica Hungarica* **55**: 63-76**.** DOI: 10.1556/038.55.2020.006.
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- BARA, G.T & LAING, M.D. Biological and chemical control of the soil-dwelling stages of the South African citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae), in avocado, *Persea americana* Mill. (Lauraceae). *African Entomology* (*Under review*).

## Conference contributions

- BARA, G.T. & LAING, M.D. 2020. Susceptibility of avocado fruit to *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and wind scarring damage in Limpopo and KwaZulu-Natal Provinces of South Africa. Combined Congress January 20-23, 2020, University of the Free State, Bloemfontein, South Africa.

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- BARA, G.T. & LAING, M.D. 2019. Determination of the natural host status of avocado fruit to pestiferous thrips (Thysanoptera: Thripidae) in KwaZulu-Natal, South Africa. *Proceedings of the 21st Congress of the Entomological Society of Southern Africa. 8 - 11 July 2019, Coastlands Umhlanga Hotel & Conference Centre, Umhlanga, South Africa.* Entomological Society of Southern Africa, Umhlanga, p. 7 [Congress abstract]. ISSN: 1010-2566.
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- BARA, G.T. & LAING, M.D. 2019. Determination of the natural host status of avocado fruit to pestiferous thrips (Thysanoptera: Thripidae) in KwaZulu-Natal, South Africa. Levubu SAAGA study group, 6 August 2019, Levubu, South Africa.
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Finally, the Lord Almighty, for who He is.

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## Thesis introduction

Avocado fruit (*Persea americana* Miller) is an economically important subtropical fruit contributing ZAR 1.75 billion to the gross domestic product of South Africa in 2018 (http://www.worldstopexports.com/avocados-exports-by-country/). During the same year, 6.4 million tonnes were produced globally with the Americas producing 72.7 %, Africa 12.5 %, Asia 11.9 %, Europe 1.5 % and the Oceania 1.4 % (FAOSTAT 2020). South Africa is the 12$^{th}$ largest producer of avocado fruit in the world and in Africa, is the second largest avocado exporter after Kenya. On the African continent, Zimbabwe, Tanzania, Cameroon, the Democratic Republic of Congo and Rwanda also produce significant volumes of avocado fruit. In 2018, South Africa produced 170,000 tonnes of avocado fruits, of which 51 % (86,000 tonnes) were exported, 10-15 % were processed into oil and purée with the rest being sold at fresh produce markets Donkin (2019).

The European market (France, Netherlands, Spain and the UK) is the primary destination for the majority of avocado fruit exported from South Africa. DAFF (2017) reported that South Africa exported 57,867 tonnes of avocado fruit (1.7 % of international market share) with a total value of ZAR 1,061 million in 2016. The European market is highly competitive, with Peru and New Zealand vying for the same market. The South African avocado industry needs to consistently produce fruits of high quality to remain competitive in the global market. However, poor fruit quality remains one of the biggest challenges to exports (Nelson 2014).

Several pests and diseases are major limiting factors to production and export. Diseases caused by *Phytophthora cinnamomi* Rands (phytophthora root rot) (Reeksting *et al.* 2014), *Sphaceloma perseae* Jenkins (avocado scab), *Colletotrichum gloeosporioides* (Penz.) Sacc. (anthracnose) (Sanders & Korsten 2003), and *Pseudocercospora purpurea* (Cooke) Deighton (cercospora spot) are recognized as global production constraints (Darvas & Kotze 1987). Insect pests such as thrips (Thysanoptera: Thripidae) (Dorantes *et al.* 2004), false codling moth (*Thaumatotibia leucotreta* Meyrick) (Van den Berg *et al.* 2001), heart shaped scale (*Protopulvinaria pyriformis* (Cockerell)) (Du Toit *et al.* 1991) and avocado scale (*Fiorinia fioriniae* (Targioni)) (Erichsen & Schoeman 1992) are among some of the pests which commonly reduce the quality of avocado fruit in South Africa.

With a rapidly expanding global avocado market, thrips pose a significant threat by causing damage to plant epidermal tissues, premature fruit drop and malformation of the fruits (González-Hernández *et al.* 1999). Feeding on the fruit epidermal layer by both larvae and

adults causes permanent, superficial scarring of the fruit that can turn the entire fruit surface brown and visually unattractive. Whilst the internal quality is unaffected, the scarred appearance of fruit may cause customers to shy away from buying the fruit. As Yahaya *et al.* (2014) noted, the external appearance of fruit is an important quality attribute that determines consumer attraction, acceptance and purchase of the fruit. The market generally tolerates a small amount of thrips scarring, but as Stevens *et al.* (1999) pointed out, damage in excess of 2 $cm^2$ fruit area will result in the fruit being downgraded from premium export grade to local market or processing grade.

Control of *S. aurantii* is particularly challenging owing to several factors such as their small size, short generation time, polyphagy and high fecundity (Gilbert & Bedford 1998). In addition, control using foliar applications of insecticides has also been poor, owing to the thrips cryptic behaviour (Thöming 2005). Several thrips species are known to have ground dwelling life stages, including *Frankliniella occidentalis* (Pergande) in eggplant (Zhang *et al.* 2019) and *S. aurantii* in citrus (Gilbert & Samways 2018). Presently, no soil-applied insecticides for the control of thrips in avocado are currently registered in South Africa. Biological control using entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin applied as a soil drench may disrupt the thrips lifecycle, preventing thrips populations from reaching economically damaging levels. The ability of *B. bassiana* to regulate insects has been demonstrated on soil-dwelling pupal phases of *F. occidentalis* in tomatoes (Lee *et al.* 2017) and eggplant (Zhang *et al.* 2019).

In the 2018/2019 avocado season, unidentified caterpillars were observed scarring avocado fruits and defoliating avocado leaves in Wartburg, KwaZulu-Natal, South Africa. This was the first time that this insect was reported causing economic damage in South Africa. The scarring damage and the scale of defoliation warranted a study to identify, characterize and document the biology of the pest as well as to determine the level of damage caused. Accurate identification of agricultural pests is a critical step in the successful implementation of an integrated pest management (IPM) program.

The findings outlined in this study will contribute to the body of knowledge in the avocado industry, particularly, how to manage the biotic and abiotic factors causing avocado fruit scarring.

The Harvard referencing system following the *African Entomology* journal (Entomological Society of Southern Africa) style is used in this thesis, with each chapter structured as a stand-alone research paper.

**Specific objectives**

The specific objectives of the thesis, organized in the respective chapters, are:

1) Chapter 1: Literature review (Entomopathogens: potential to control thrips of avocado with special reference to *Beauveria bassiana*).
    i) Review literature on entomopathogens, for potential inclusion in the management of thrips of avocado.
2) Chapter 2: Determination of the natural host status of avocado fruit to pestiferous thrips (Thysanoptera: Thripidae) in KwaZulu-Natal, South Africa.
    i) To determine the spectrum of thrips species found in avocado flowering panicles;
    ii) To establish the effectiveness of different fruit bagging material as thrips exclusion screens;
    iii) To identify the thrips species responsible for the scarring damage observed in avocado fruit in KwaZulu-Natal Province, South Africa.
3) Chapter 3: Attractiveness of different coloured sticky traps to the South African citrus thrips (*Scirtothrips aurantii* Faure) in avocado, KwaZulu-Natal, South Africa.
    i) To determine the colour of sticky traps that would be most suitable for monitoring *S. aurantii*;
    ii) To establish the distribution of thrips;
    iii) To monitor the populations of thrips during the critical infestation period in avocado orchards in KwaZulu-Natal Province, South Africa.
4) Chapter 4: An investigation into the role played by endophytic *Cladosporium* spp., wind abrasion and thrips (Thysanoptera: Thripidae) in scarring avocado fruit.
    i) To confirm damage signs exhibited by the abrasion damage (simulated thrips and wind);
    ii) To identify pathogenic agents associated with scarring on avocado fruits in KwaZulu-Natal Province of South Africa.
5) Chapter 5: Susceptibility of avocado fruit to *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and wind scarring damage in Limpopo and KwaZulu-Natal Provinces of South Africa.
    i) To quantify losses occurring in two avocado production areas of South Africa;
    ii) To describe the susceptibility of different avocado cultivars to damage by wind abrasion and *S. aurantii* in Limpopo and KwaZulu-Natal Provinces of South Africa.

6) Chapter 6: First report of tussock moths (*Bracharoa mixta* (Snellen) (Lepidoptera: Erebidae)) scarring avocado fruit in KwaZulu-Natal, South Africa.
    i) To identify the caterpillar using morphology and DNA barcoding;
    ii) To establish the damage caused by *B. mixta* in avocado orchards of KwaZulu-Natal Province, South Africa.
7) Chapter 7: Biological and chemical control of soil-dwelling stages of the South African citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae), in avocado, *Persea americana* Mill. (Lauraceae).
    i) To monitor *S. aurantii* population dynamics during the critical early fruiting period by means of dispersal/emergence traps and fruit infestation indices;
    ii) To determine the field efficacy of biologicals (*B. bassiana* and *Steinernema feltiae* Filipjev) the pyrethroid, beta-cyfluthrin, and the semi-synthetic spinosyn, spinetoram, against soil-dwelling stages of *S. aurantii*;
    iii) To establish the pupation sites of mature *S. aurantii* larvae.

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# Chapter 1: Literature review – The potential of entomopathogens to control thrips in avocado, with special reference to *Beauveria bassiana*


## [1]Abstract

The South African avocado industry is an export-orientated, multi-billion rand industry, with an international market share of 2.1 %, and with an average production over five years of 118,000 tonnes produced on 17,500 ha of orchards. Forty percent of the harvested fruit is exported, 50 % is consumed domestically and the remaining 10 % is processed into oil and purée for both the domestic and export market. Being a predominately export-oriented industry, there is a commercial imperative to optimise the exportable percentage of avocado fruit. Scarring damage by thrips results in corky tissue development of the skin, making the fruit unsuitable for export. This review presents an assessment of the potential of a range of entomopathogens, including nematodes, viruses, fungi and bacteria, but especially the entomopathogenic fungus *Beauveria bassiana*, for the biological control of thrips. *B. bassiana* is a natural-occurring soil fungus that has been commercialised for the biocontrol of several insect species such as whitefly, weevils, aphids, thrips, and mealybugs on different vegetable (potato, cabbage, kale), fruit (citrus, mango) and berry (coffee) crops. The use of *B. bassiana* for biological control is cost-effective and many strains are commercially available. The objective of this review is to highlight the role fungi play in insect control, to discuss commercially available mycoinsecticides, and to analyse the potential of entomopathogenic fungi as an alternative to synthetic chemicals in controlling thrips in avocado.



**Keywords:** South Africa, avocado, biological control, *Beauveria bassiana*, thrips.


[1] *This chapter has been slightly modified and published as*:

BARA, G.T. & LAING, M.D. 2020. Entomopathogens: potential to control thrips in avocado, with special reference to *Beauveria bassiana*. *Horticultural Reviews* **47**: 325-368. DOI: 10.1002/9781119625407.ch7.

## 1.1 Introduction

In 2018, world avocado production was 6.4 m tonnes, with Mexico accounting for 34 % of the total production (FAOSTAT 2020). Approximately 73 % of avocado fruit are produced in the Americas, 13 % in Africa, 11 % in Asia, and 2 % in Europe and in the South Pacific. Mexico is the largest producer of avocado fruit in the world, followed by Indonesia, the Dominican Republic and USA (FAOSTAT 2020). South Africa is ranked low in terms of production; however, it is among the top ten exporters of avocado fruit in the world. Within Africa, South Africa is the second largest avocado exporter after Kenya, with Rwanda, the Democratic Republic of Congo, Cameroon and Zimbabwe also producing significant quantities. The South African avocado industry is export oriented, with approximately 40 % of the total production volume being exported (Blakey & Wolstenholme 2014), 50 % consumed domestically and the remaining 10 % being processed into oil and purée for both the domestic and export market (for cosmetic or culinary use).

Of the 40 % export crop, 95 % is shipped to Europe, and the rest is exported to the Middle East and other southern African countries. South Africa's production is concentrated mainly in the subtropical areas of Limpopo (60 %), Mpumalanga (29 %), KwaZulu-Natal (9 %) and parts of the Cape provinces (2 %). Approximately 70 % of the trees grown in South African avocado nurseries are 'Hass', and the remaining 30 % is comprised mostly of 'Fuerte', 'Ryan' and 'Pinkerton' (Blakey & Wolstenholme 2014). Production is anchored on 340 commercial growers and 78 emerging growers with the industry employing 8,200 permanent and 7,300 seasonal workers (Donkin 2018).

The South African avocado season extends from mid-March to September. Due to climatic differences between growing regions, most of the major cultivars are available over an extended period during the season (Vorster 2001). For example, 'Fuerte' is harvested from mid-March to May in the northern regions, and in July to August in KwaZulu-Natal.

Avocado production is one of the fastest-growing agricultural sectors in South Africa. However, various insect pests and diseases reduce production quantities and quality. Thrips are a significant concern for avocado exporters (Bara & Laing 2019). They cause losses through direct damage to fruits, resulting in quality loss. This drives research for sustainable thrips management practices. The introduction of strict maximum residue level (MRL) regulations for pesticides in fruits being imported into the European Union has compounded the existing thrips problem and jeopardises the future exports of fresh avocado fruit.

Worldwide, current pest management strategies largely rely on synthetic chemical pesticides for agricultural productivity and efficiency. However, adverse effects on beneficial

organisms, accumulation of pesticide residues in the environment and food products, insecticide resistance and gene erosion are major concerns (Kumar 2012), giving greater impetus to the development of more sustainable insect management technologies. Pimentel & Levitan (1986) noted that less than 0.1 % of crop-sprayed pesticide reaches the target pest, with the remaining 99.9 % entering the environment. Dabrowski *et al.* (2002), in a water quality study of the Lourens River in the Western Cape, detected high levels of pesticides downstream of a farming area, exceeding both the national water quality standards and those established by the US Environmental Protection Agency (EPA). Endosulfan, chlorpyrifos, iprodione and fenvalerate are highly toxic bio-accumulating chemicals frequently collected in water sources in the Western Cape region (Ochieng *et al.* 2013).

At least 586 insect species are resistant to at least one of 325 chemical insecticides (Sparks & Nauen 2014) and revelations by van der Sluijs *et al.* (2013) that neonicotinoids were responsible for bee population decline has further increased the need to develop biological methods to manage insect pests. Kessler *et al.* (2015) showed that bees cannot control their exposure to neonicotinoids in their food and, in fact, preferred to consume neonicotinoid-contaminated foods, thus exposing them to considerable hazard. Discovery of new synthetic pesticides has also become increasingly difficult and costly, with companies having to screen at least 150,000 chemicals to find a new, commercially acceptable, synthetic pesticide. According to McDougall (2016) this requires an investment of US$250m, taking at least 10 years to develop a new product. By comparison, microbial biopesticides require $1–2 m to develop and about 3–5 years to get to market. These time and cost incentives have aided in the steady growth of the global biopesticide market (Marrone 2014). The fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae), unlike viruses, protozoans, and bacteria (which require ingestion), provides one option for biocontrol because it can infect arthropods by direct penetration of the host cuticle, and thus functions mainly as a contact pathogen (Hajek & St. Leger 1994). After killing the insect host, the fungus releases millions of new infective spores into the environment, facilitating a secondary epizootic, and long-term biological control.

### 1.2 Commercial production in South Africa

Commercial avocado production in South Africa is an intensive monoculture cropping system. Approximately 70 % of the trees produced by avocado nurseries are of the cultivar ‘Hass’, and the remaining 30 % are mostly of the cultivars ‘Fuerte’, ‘Ryan’ and ‘Pinkerton’. Avocado nurseries are currently producing 1,500 ha plantings annually. These trees are mainly being

used to replace old orchards, but there are also some new orchards being planted (Donkin 2019).

Crop monoculture has many advantages, including the economic advantages of specialization, flexibility to intensively grow a high-value, cash crop, and the means to assure adequate supplies (Cook & Weller 2004). However, monocultures generally provide less biological diversity and thus tend to be less ecologically stable than polycultures. Large blocks of avocado trees in perennial plantations allow for a build-up of pests with few predators or pathogens to challenge their growth (Wetzel *et al.* 2016). Generalist natural enemies, primarily predators, are usually less abundant in monocultures because they have fewer alternative hosts to sustain them before the pests on which they can feed build up to damaging levels on the crop (Bianchi *et al.* 2006).

Thrips are difficult to control using a "chemicals only" approach because they are a rebound pest in an over-simplified agricultural ecosystem. To improve biocontrol efficacy, genetic biodiversity could be enhanced by the planting of mixtures of ryegrass and clover in orchards, to increase soil organic matter by providing groundcover residues and rhizosphere decomposition (Atucha *et al.* 2013). This, in addition to providing greater biodiversity and reducing excessive runoff, may also provide a more suitable environment for biological control agents to thrive.

### 1.3 Requirements for export and local quality

The South African avocado industry, being predominantly export-oriented, needs to ensure that the quality of fruit that is produced meets international standards. The South African Avocado Growers Association (SAAGA), in association with the National Department of Agriculture, determines the quality standards for export. Instruments such as the Agricultural Product Standards Act of 1990, provide guidelines and recommended procedures in South Africa for avocado exports (Eksteen 1999). DAFF (2010) recognises that the European Union Commission has provided several regulations governing the quality of produce deemed acceptable, such as EC 1935/2004 and 94/62/EC, which concern packaging of foodstuffs and EC 178/2002, relating to food safety aspects. Quality inspections are carried out by a parastatal organisation, the Perishable Products Export Control Board (PPECB), on a consignment basis prior to shipping, to ensure that factors such as fruit maturity, size, and blemish levels of the meet the standards of the intended destination. All growers producing export fruit are bound by Good Agricultural Practice standards that are laid down by the Department of Agriculture. More than 95 % of the industry is EuropGAP accredited as a minimum standard (DAFF 2012).

In general, avocado fruit for export should be clean, intact, disease-free and in a condition that can withstand transport and handling.

### 1.4 Economics of avocado production

The South African avocado industry is export-orientated, with approximately 2.1 % of international market share in 2019 (http://www.worldstopexports.com/avocadoes-exports-by-country/). During that year, South Africa exported 86,000 tonnes (51 % of 170,000 tonnes produced locally) of avocado fruit, contributing US$116.7 million (ZAR 1.75 billion) to the country's GDP (Donkin 2019). In 2014, approximately 51 % of total avocado fruit produced in South Africa were exported, 21 % were sold through the National Fresh Produce Markets (NFPMs), 15 % was sold to the informal markets (hawkers and vendors), 4 % was processed, while the remaining 8 % was delivered directly to retailers (DAFF 2015).

### 1.5 Pests and diseases of avocado

One of the major challenges to avocado production in many areas of the world, including South Africa, is root rot caused by *Phytophthora cinnamomi* Rands (Hardham & Blackman 2018). High summer rainfall (>1000 mm p.a.), and warm temperatures favour the spread of the disease. This disease is effectively controlled through phosphorous acid trunk injections, integrated with practices that promote root health, such as the addition of compost and mulches. The majority of plantings since the early 1980s have been on phytophthora-tolerant rootstocks such as 'Duke 7'. In recent years, a growing number of trees have been planted with the resistant rootstock 'Merensky II' (Dusa®), which is higher yielding than 'Duke 7'. Approximately 60 % of current nursery trees are on 'Merensky II'. Other commonly used resistant rootstocks include 'Bounty' and 'Velvick' (Donkin 2007).

Several other pests and diseases provide major challenges to the South African avocado industry. Among those insect pests are fruit flies (Diptera: Tephritidae), thrips (Thysanoptera: Thripidae), false codling moth (*Thaumatotibia leucotreta* Meyrick) (Erichsen & Schoeman 1992) and the heart shaped scale (*Protopulvinaria pyriformis* (Cockerell)) (Du Toit *et al.* 1991). Diseases other than phytophthora root rot (*P. cinnamomi*) include stem canker (*Phytophthora cinnamomi*) (Lonsdale *et al.* 1988), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Sacc.) and Cercospora spot (*Pseudocercospora purpurea* (Cooke) Deighton) (Darvas & Kotze 1987).

## 1.6 Thrips of avocado

Thrips are fringe winged insects in the order Thysanoptera. A study in Mexico by Johansen *et al.* (1999) revealed 38 thrips species on avocado, but only six of them were recognized as primary pests of this crop. Worldwide, some of the species recorded on avocadoes include *Liothrips persea* (Watson), *Scirtothrips aceri* (Moulton), *Frankliniella cephalica* (Crawford), *Heliothrips haemorrhoidalis* (Bouche) (Dorantes et al. 2004), *Selenothrips rubrocinctus* (Giard) (Denmark & Wolfenbarger 2010) and *Scirtothrips perseae* Nakahara (Hoddle *et al.* 2012). These cosmopolitan, polyphagous species survive on foliage by scraping and sucking the superficial cells, in the process leaving silver-white discoloured spots, which later turn dark. The damage is mostly observed on leaves and fruits. However, thrips damage can also be found on tender shoots, buds, and flowers. Species of thrips occupy widely disparate niches, resulting in the manifestation of a diverse array of life styles. Phytophagous thripids, such as *Scirtothrips* spp., can exploit immature foliage, whereas mature leaves support *H. haemorrhoidalis*, and senescing leaves can be utilized by *S. rubrocinctus* (Morse & Hoddle 2006).

Thrips cause malformation of fruit, induce premature fruit drop and also create lesions that become entry points for microorganisms such as *Sphaceloma perseae* (Jenkins) (González-Hernández *et al.* 1999). While minor thrips damage can be tolerated, any damage covering an area of more than about 2 $cm^2$ will result in the fruit failing to meet the premium export standards (Stevens *et al.* 1999). According to Steyn *et al.* (1993), the red-banded thrips, *S. rubrocinctus*, and the greenhouse thrips, *H. haemorrhoidalis*, are the most common thrips species of avocado fruit in South Africa. A recent study by Bara & Laing (2019) revealed that *Scirtothrips aurantii* Faure, the South African citrus thrips, is also an economic pest of avocado fruit in South Africa.

### 1.6.1 Classification

Thrips are insects in the Kingdom: Animalia, Phylum: Arthropoda and Class: Insecta (CABI 2018). The order Thysanoptera includes over 8800 species (Kumar *et al.* 2013) and is sub-divided into two sub-orders Terebrantia and Tubulifera. Tubulifera has a single family (Phlaeothripidae), whilst Terebrantia has four families (Aleolothripidae, Merothripidae, Thripidae and Heterothripidae) (Lewis 1973). The most damaging thrips (>90 %), belong to the family Thripidae (sub-order Terebrantia). In this family belong the damaging *Frankliniella occidentalis* (Pergande), *Thrips tabaci* (Lindeman), *Heliothrips haemorrhoidalis* (Bouche), *Thrips simplex* (Morison), *Taeniothrips dianthi* (Priesner), *Thrips fuscipennis* (Haliday),

*Parthenothrips dracaenae* (Heeger), *Thrips palmi* (Karny), and *Hercinothrips femoralis* (Reuter). In the family Phlaeothripidae the common pests include *Haplothrips cottei* (Vuillet) and *Liothrips vaneeckei* (Priesner) (Tommasini 2003).

### 1.6.2 Biology of thrips

*Heliothrips haemorrhoidalis*, *S. rubrocinctus* and *S. aurantii* are all members of the sub-order Terebrantia and have similar life cycles (**Figure 1.1**). The development of thrips includes six stages: egg, two larval stages, two pupal stages, and an adult stage. Members of the Thripidae exhibit high female fecundity. After an initial pre-oviposition period, a female can oviposit throughout her lifetime (Reitz 2009) and, under optimum conditions of temperature and food, produce up to seven progeny per day (Robb & Parrella 1991). Robb & Parrella (1991) reported an average total lifetime fecundity exceeding 200 per female. This high level of fecundity can lead to high intrinsic rates of population increase which can result in the rapid development of outbreaks of this pest on suitable hosts (Hulshof *et al.* 2003).

Thrips lay extremely small eggs, about 0.2 mm long, by cutting slits in plant tissue with their ovipositors, and inserting their eggs, one per slit (Gullan & Cranston 2010). The eggs take up to four days to hatch. Sex determination is through haplodiploidy. The haploid males are produced from unfertilized eggs whereas the diploid females are produced from fertilized eggs (Moritz 1997). Terry & Kelly (1993) argued that mated females do not allocate the sex of their progeny; rather the adult sex ratios resulted from their dispersal and distribution in response to host quality and longevity.

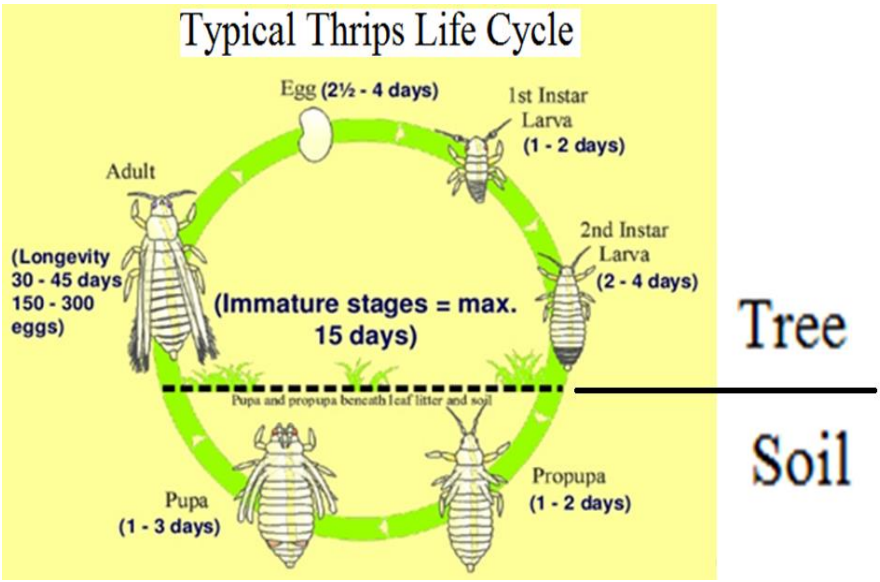


**Figure 1.1.** Typical (generalized) thrips life cycle.

(Adapted from https://www.discoverlife.org/nh/tx/Insecta/Thysanoptera/images/thrips10-300x261.gif html, © Mark S. Hoddle, all rights reserved).

The Terebrantia pupate in soil or in the tree canopy (Mound & Walker 1982), and the resting stages can last three to five days before adults emerge (Chin & Brown 2008). During this pupal stage, the insect's body organs are reshaped, wing-buds develop and genitalia are formed (Gullan & Cranston 2010). In warm weather, the adult stage can be attained in three weeks (Mound & Walker 1982) and can last 45 days, with several generations in a single year being possible (Chin & Brown 2008).

Development is temperature and host dependent. At 25-30°C, egg to adult development time can be as brief as 9-13 days. Eggs hatch in two to four days under optimum temperatures, followed by the two nymphal stages (Reitz 2008), after which feeding stops and the thrips drop to the ground to pupate. Buitenhuis & Shipp (2008) reported that whilst pupation in the ground does occur, there are also significant numbers that remain on the host plants. The first pupal stage (prepupa) is non-feeding and is followed another non-feeding pupal stage known as the pupa. Winged adults usually appear one to three days later (Reitz 2009).

## 1.7 Management of thrips

### 1.7.1 Thrips monitoring

Early detection of thrips infestation is crucial for successful control. Newly flushed leaves can be examined to get an indication of whether thrips are abundant enough to be a likely problem later, when young fruit are present. Visual inspection methods are often used by tapping plant organs onto a tray, or checking flowers at regular time intervals (Pearsall & Myers 2000). Bashir *et al.* (2014) noted the cost effectiveness of coloured sticky traps as tools for monitoring various insect pests. Thrips are known to be highly attracted to blue and yellow coloured sticky cards (Blumthal *et al.* 2005; Muvea *et al.* 2014).

### 1.7.2 Cultural control

Sanitary practices such as removing weeds, old plant material and debris from orchards are essential cultural practices contributing to the control of thrips (Northfield *et al.* 2008). Adding coarse organic mulch beneath trees and maintaining a mulch layer 15 cm thick in avocado orchards may reduce the survival of thrips that drop from trees to pupate (Jensen *et al.* 2002).

### 1.7.3 Silicon fertilization

In some agricultural systems, silicon fertilizers are applied as a crop protection treatment. Silicon in sugarcane has been found to enhance plant defence against spittlebug (*Mahanarva fimbriolata* Stål) herbivory (Korndörfer *et al.* 2011). A number of studies have shown the

benefits of soil and/or foliar silicon applications (Korndorfer *et al.* 2004; Redmond & Potter 2006; Massey *et al.* 2007).

Silicon deposition was believed to contribute to the increased rigidity and abrasiveness of plant tissues, creating a mechanical barrier and reducing their palatability and digestibility to invertebrate herbivores (Goussain *et al.* 2005; Massey & Hartley 2009). However, Stanley *et al.* (2014) disputed this mode of action, suggesting that other mechanisms may be involved, and specifically silicon-mediated plant resistance. It has been suggested that the priming of plant defence responses, alterations in phytohormone homeostasis, and interaction with defence signalling components are all potential mechanisms involved in regulating silicon-triggered resistance responses (Van Bockhaven *et al.* 2012). Gatarayiha *et al.* (2010) hypothesized that silicon applications primed biochemical defences in plants, which indirectly made insect pests more susceptible to entomopathogens. In addition, silicon-treated, arthropod-attacked plants have been demonstrated to display increased attractiveness to natural enemies, as reflected by elevated biological control in the field, in tritrophic interactions (Reynolds *et al.* 2016).

### 1.7.4 Chemical control

Currently, several insecticides are available for the control of avocado thrips (*Scirtothrips perseae*) in avocado such as sabadilla (e.g. Veratran®), spinosad (e.g. Success®), and abamectin (e.g. Agri-Mek®) (Morse *et al.* 2006). Stevens *et al.* (2001) also noted that carbaryl (Carbaryl 80 W) and diazinon (Diazinon 50 % WP) are effective against greenhouse thrips, *H. haemorrhoidalis,* but are not recommended under the current AvoGreen IPM program in New Zealand because they have moderate toxicity to beneficial insects (AvoGreen 2010). Chemical control is complicated by the fact that most of the developmental stages in thrips are shielded from chemicals: the eggs are laid within leaf tissue, pupae in the soil/leaf litter, larval and adult stages hide in buds or between leaves and flower structures). In addition, due to their rapid reproductive cycle, with several generations possible in the same year or season, thrips are able to continuously evolve resistance to pesticides (Jensen 2000). Only a few crop protection products are registered for avocado fruit and this, coupled with the need to meet food quality and safety standards, creates an urgent need to find novel control products and associated management techniques.

### 1.7.5 Biological control

Larvae of thrips are easy prey for a wide range of general arthropod predators but those more specific to thrips include members of the Aeolothripidae (eg., *Franklinothrips vespiformis* Crawford), the anthocorid genera *Orius* and *Montandoniola*, the Cecidomyiid genus *Thripsobremia* and the Sphecidae genus *Microstigmus* (Mills 1991). Some of these are commercially available and are currently used as biological control agents in a variety of crops (Loomans & van Lenteren 1995; Loomans 2003).

Thrips parasitoids all belong to the superfamily Chalcidoidea. Most of them are solitary endoparasitoids of larvae (Eulophidae) or eggs (Mymaridae, Trichogrammatidae) (Loomans & van Lenteren 1995). The eulophid larval parasitoids *Thripobius semiluteus* and *Ceranisus menes* have been recorded in avocado orchards parasitizing thrips larvae, and *Thripobius* is known to significantly reduce fruit scarring by greenhouse thrips in South Africa (Steyn *et al.* 1993).

Biological control, utilising entomopathogens, provides an approach where resistance to the control agent is unlikely to develop. Entomopathogens are microorganisms that are pathogenic to arthropods such as insects, mites, and ticks (**Table 1.1**). Several species of naturally-occurring bacteria, fungi, nematodes, and viruses infect a variety of arthropod pests and can play an important role in their management (Chandler *et al.* 2011). Some entomopathogens are mass-produced *in vitro* (bacteria, fungi, and nematodes) or *in vivo* (nematodes and viruses) and are sold commercially (Glare *et al.* 2012). Research has been conducted on the use of entomopathogens of thrips in greenhouse crops but very little on perennial crops (as in the case with avocado fruit).

Studies conducted in South Africa have revealed that *S. aurantii* is a recently confirmed economic pest in avocado (Bara & Laing 2019) but no biocontrol studies have been conducted on this pest to date.

## 1.8 Entomopathogens

### 1.8.1 Bacteria

A number of spore-forming bacteria, such as *Bacillus* spp., *Paenibacillus* spp., and *Clostridium* spp, and non-spore-forming ones that belong to the genera *Photorhabdus*, *Pseudomonas*, *Serratia*, *Xenorhabdus* and *Yersinia* have been shown to be entomopathogens. *Bacillus* and *Paenibacillus* are pathogenic mainly to coleopteran, dipteran, and lepidopteran insects. When *Bacillus thuringiensis* Berliner is ingested, alkaline conditions in the insect gut (pH 8 - 11) activate the toxic protein (delta-endotoxin) that attaches to the receptor sites in the midgut and

creates pores in midgut cells (Gill *et al.* 1992). This leads to the loss of osmoregulation, midgut paralysis, and cell lysis. Contents of the gut leak into the insect's body cavity (haemocoel) and the blood (haemolymph) leaks into the gut, disrupting the pH balance, resulting in septicemia and eventual death of the host insect (Vachon *et al.* 2012).

**Table 1.1.** Examples of entomopathogens after Gwynn (2014) and Yashaswini & Kumar (2016).

| Classification /species | Target pests |
|---|---|
| **Bacteria** | |
| *Bacillus thuringiensis* Berliner subsp. *aizawai* | Lepidoptera |
| *B. thuringiensis* Berliner subsp. *israelensis* (serotype H-14) | Fungus gnats |
| *B. thuringiensis* Berliner subsp. *kurstaki* | Lepidoptera |
| *Bacillus firmus* Werner | Nematodes |
| **Fungi** | |
| *Purpureocillium lilacinus* (Thom) Samson (formerly *Paecilomyces lilacinus*) | Root-knot nematodes |
| *Isaria fumosorosea* Wize (formerly *Paecilomyces fumosoroseus* Wize) | Whiteflies, thrips and aphids |
| *Metarhizium* spp. | Beetles, locusts and grasshoppers, hemiptera, spiders |
| *Lecanicillium lecanii* R. Zare & W. Gams | Whiteflies, thrips and aphids |
| *Beauveria bassiana* | Whiteflies, thrips, weevils and aphids |
| *Metarhizium rileyi* (Farl.) Kepler, Rehner & Humber | Foliage feeding caterpillars |
| *Hirsutella thompsonii* Fisher | Citrus rust mite |
| **Viruses** | |
| *Helicoverpa armigera* nuclear polyhedrosis virus | *Helicoverpa armigera* (Hübner) |
| *Spodoptera exigua* nuclear polyhedrosis virus | *Spodoptera exigua* (Hübner) |
| *Spodoptera littoralis* nuclear polyhedrosis virus | *Spodoptera littoralis* (Boisduval) |
| **Nematodes** | |
| *Steinernema* spp. | Thrips, fungus gnat larvae, various orders of soil borne insects |
| *Heterorhabditis* spp. | Thrips, fungus gnat larvae, various orders of soil borne insects |

The most successful microbial pesticide to date is *Bacillus thuringiensis* (Bt), which has dominated the microbial pesticide market worldwide in its use as a biological pesticide (Glare *et al.* 2017). When Bt spores are ingested by susceptible insects, Cry toxins are activated by proteolytic enzymes in the alkaline gut (Bravo *et al.* 2007). The activated toxin causes cell lysis, eventually resulting in paralysis and death of the insect (Soberón *et al.* 2010).

*Bacillus thuringiensis* crops (Bt crops) are plants that have been genetically engineered (modified) to express the endospore (or crystal) toxins of the bacterium to kill specific insect pests if they attack the crop (Abbas 2018). Genetic transformation is achieved by insertion of the target gene, its promoter and termination sequences, and a marker gene into the crop genome using the microprojectile bombardment method ("gene gun") or *Agrobacterium tumefaciens* Smith & Townsend (Peterson *et al.* 2013). Bt cotton produces toxins that target specific caterpillar pests such as beet armyworm (*Spodoptera exigua* (Hübner)), cotton bollworm (*Helicoverpa armigera* (Hübner)), and tobacco budworm (*Heliothis virescens* (Fabricius)) (Abbas 2018), whereas Bt corn is modified to resist the European corn borer (*Ostrinia nubilalis* (Hübner)), the corn earworm (*Helicoverpa zea* (Boddie)), the southwestern corn borer (*Diatraea grandiosella* (Dyar)), the fall armyworm (*Spodoptera frugiperda* (Smith)) and the corn rootworm (*Diabrotica virgifera* (LeConte)) (Peterson *et al.* 2013). Commercially registered Bt products include YieldGard® (Monsanto) and Agrisure® (Syngenta) in maize, and Bollgard® (Monsanto) in cotton (Koch *et al.* 2015).

Bt toxins are also effective against several other lepidopteran, dipteran and coleopteran pests (Schnepf *et al.* 1998) as well as members of Hymenoptera, Homoptera, Orthoptera and Mallophaga (Crickmore *et al.* 1998). Other commercially available entomopathogenic bacteria include *Lysinibacillus sphaericus* for mosquito control (Charles *et al.* 2000), *Paenibacillus popilliae* Dutky for Japanese beetle control, and gram-negative bacteria in the genus *Serratia* for the control of beetle larvae (Glare *et al.* 2017). Toxins produced by *Photorhabdus temperate* Fischer-Le Saux, Viallard, Brunel, Normand & Boemare strains were found to be effective against *F. occidentalis* and *T. tabaci*, with fecundity in *F. occidentalis* being significantly reduced (Gerritsen *et al.* 2004). *Frankliniella occidentalis* and *T. tabaci* are both terebrantian species and, in addition to having similar biologies, cause damage consistent with that caused by thrips on avocado fruit. It is, therefore, likely to be worthwhile to look at entomopathogenic bacteria currently registered for other insect pests (**Table 1.2**) for efficacy in controlling thrips on avocado fruit.

### 1.8.2 Nematodes

Entomopathogenic nematodes (EPNs) are microscopic, soil-dwelling organisms that are parasitic to insects. Several species of *Heterorhabditis* and *Steinernema* are available in multiple commercial formulations (Ruiu 2018), primarily for managing soil insect pests. Towards the end of the larval stages, some mature terebrantian thrips larvae drop to the ground to pupate (Gilbert & Samways 2018). The below-ground stages, therefore, present an

opportunity for control by EPNs. If the thrips life cycle could be disrupted, subsequent population growth would be curtailed and fruit damage would be minimized.

Infective juveniles of EPNs actively seek out their hosts and enter through natural openings such as the mouth, spiracles, and anus or the intersegmental membrane. Once inside the host body, the nematodes release symbiotic bacteria that kill the host through bacterial septicemia. *Heterorhabditis* spp. carry *Photorhabdus* spp. bacteria and *Steinernema* spp. carry *Xenorhabdus* spp. bacteria (Dara 2017). EPN members of Heterorhabditidae and Steinernematidae families have been reported to be effective against both the adult and nymph stages of onion thrips, *Thrips tabaci*, under field conditions (Azazy *et al.* 2018). Under glasshouse conditions, *Steinernema feltiae* Filipjev and *S. carpocapsae* Weiser have been found to be efficacious against adults and nymphs of the western flower thrips, *Frankliniella occidentalis* (Tomalak *et al.* 2005).

In semi-field experiments, two EPNs, *S. feltiae* and *S. carpocapsae* (Rhabditida: Steinernematidae) caused high levels of mortality of *Thrips tabaci* as prepupae (92 %) and pupae (92.6 %), respectively (Khajehali & Poorjavad 2014). However, in another study, *S. feltiae*, *H. bacleriophora* and *S. carpocapsae* were ineffective against whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), under controlled conditions in peppers (Ruiz-Platt & Cabello 2009).

Research is required to augment what has been established to further understand how EPNs can be effectively used to control thrips in IPM programs. Typically, infective juveniles can be applied at a spray volume of 750 L to 1890 L $ha^{-1}$ using ground spraying equipment such as mist blowers, pressurized blowers and electrostatic blowers, as well as being applied aerially (Georgis 1992). Furthermore, EPNs can also be applied using drip systems, making application convenient as this method is simple, quick and provides good coverage. Microjet systems are the main irrigation systems used in South African avocadoes. However, no EPNs are currently registered specifically for thrips control. Commercial formulations for other problem pests are available (**Table 1.3**), from which further research can be undertaken.

**Table 1.2.** Examples of registered bioinsecticides based on entomopathogenic bacteria (Ruiu 2018; Copping 1998; Sparks et al. 2012).

| Active substances | Trade names* | Main targets | Crops |
|---|---|---|---|
| *Bacillus thuringiensis aizawai* | Able-WG, Agree-WP, Florbac, XenTari | Armyworms, diamondback moth | Maize, cotton, potato, eggplant |
| *Bacillus thuringiensis kurstaki* | Biobit, Cordalene, Costar-WG, Crymax-WDG, Deliver, Dipel, Foray, Javelin-WG, Lepinox Plus, Lipel, Rapax | Lepidoptera | Tomato, leafy vegetables |
| *Bacillus thuringiensis israelensis* | Teknar, VectoBac, Vectobar | Mosquitoes and Black flies | Mosquito, black fly, fungus gnat larvae |
| *Bacillus thuringiensis tenebrionis* | Novodor, Trident | Colorado potato beetle | Potato, eggplant, tomato |
| *Bacillus thuringiensis sphaericus* | VectoLex, VectoMax | Mosquitoes | Mosquitoes |
| *Burkholderia* spp. | Majestene, Venerate | Chewing and sucking insects and mites; nematodes | Maize, wheat, rice, grass, oat, lupine, and coffee |
| *Saccharopolyspora spinose* Mertz & Yao | Tracer120, Conserve, Spinosad | Lepidoptera, Thysanoptera, Diptera, Coleoptera and Hymenoptera | Rape seed, wheat, cotton, avocado, tomato, pepper, eggplant, potato, sweet potato |
| *Chromobacterium subtsugae* Martin, Hirose & Aldrich | Grandevo | Chewing and sucking insects and mites | Alfalfa, asparagus, banana, brassica leaf vegetables, bulb vegetables, cereal grains, citrus, cucurbits |
| *Bacillus firmus* Bredemann & Werner | Bionemagon, Votivo | Nematodes | Tuff grass, tomato |

*Strains may be registered under different trade names

**Table 1.3.** Commercially available bio-insecticides based on entomopathogenic nematodes (Ruiu 2018; Rechcigil & Rechcigil 2000).

| Active substances | Trade names* | Main targets | Crops |
|---|---|---|---|
| *Steinernema carpocapsae* Weiser | Capsanem, Carpocapsae-System, Exhibitline SC, Optinem-C, NemaGard, Nemastar, NemaTrident-T, NemaRed, Nemasys C, Palma-Life, Ecomask, Hortscan | Borer beetles, caterpillars, cranefly, moth larvae, *Rhynchophorus ferrugineus*, Tipulidae, fleas, fungus gnats, termites | Artichoke, banana, tuff grass, cranberries, leafy vegetables, sweet potato, pome fruit |
| *Steinernema feltiae* Filipjev | Entonem, NemaShield, NemaTrident-F, Nemapom, Nemaplus, Nemaflor, NemaFly, Nemafrut, Nemasys F, Nematrip, Nematech-S SP, NemaTrident-S, Nemax-F, Nemycel, Steinernema-System, Optinem-F, Sciarid, Nemasys | *Bradysia* spp., *Chromatomyia syngenesiae*, *Phytomyza vitalbae*, soil dwelling pests, codling moth larvae, sciarids, thrips | Sweet potato, leafy vegetables, pome fruit, other fruit trees, banana |
| *Steinernema kraussei* (Steiner) | Larvanem, Nemaplant, NemaShield-HB, Nematop, Nematech-H NemaTrident-H, NemaTrident-C, Nema-green, Optinem-H | *Otiorhynchus* spp., chestnut moths, black vine weevil and soil-dwelling beetle larvae, *Melolontha melolontha*, caterpillars, cutworms, leaf miners | Cranberry, tree nurseries and soft fruits |
| *Heterorhabditis downesi* Stock, Griffin and Burnell | NemaTrident-CT | Black vine weevil *Otiorhynchus sulcatus* | Forest plantings, berries, ornamentals |

| | | | |
|---|---|---|---|
| *Phasmarhabditis hermaphrodita* (Schneider) | Slugtech-SP | Molluscs | Molluscs |
| *Heterorhabditis megidis* Poinar, Jackson & Klein | Dickmaulrusser-nematoden | Soil insects, primarily *Otiorhynchus sulcatus* | Berries and ornamentals |
| *Heterorhabditis bacteriophora* Poinar (Oswego) | Hetromask, Cruiser, Nematop | Root knot nematodes | Tuff grass, sweet potato, citrus, grapes |
| *Phasmarhabditis hermaphrodita* (Schneider) | Nemaslug | Slugs | Snails and slugs |

*Strains may be registered under different trade names

### 1.8.3 Viruses

The Baculoviridae family includes DNA viruses that form pathogenic relationships with invertebrates, which have potential in biological control (Haase *et al.* 2015). Baculoviruses are ingested orally by insects, with the first infection normally taking place after ingestion of contaminated food (Ruiu 2018). As entomopathogenic viruses need to be ingested by the insect host to be effective, they are therefore ideal for controlling pests that have chewing mouthparts, such as lepidopteran caterpillars (Ruiu 2018), with some strains having been commercialized (**Table 1.4**). All commercial strains, with the exception of the forest control product for sawfly (Hymenoptera: Diprionidae), target Lepidoptera (Rechcigil & Rechcigil 2000). Several lepidopteran pests are important hosts of baculoviruses including nucleopolyhedroviruses (NPV) and granuloviruses (GV). These related viruses have different types of inclusion bodies in which the virus particles (virions) are embedded. Virus particles invade the nucleus of the midgut, fat body or other tissue cells, compromising the integrity of the tissues and liquefying the cadavers (Williams *et al.* 2017). Before death, infected larvae climb higher in the plant canopy, before bursting, which aids in the dissemination of virus particles from the cadavers to the lower parts of the canopy, thus aiding in the spread of the virus to healthy larvae (Dara 2017). Entomopathogenic viruses are very host specific and can cause significant reduction of host populations (Chen *et al.* 2011). However, the widespread use of baculovirus formulations is limited owing to their low stability in the environment and their high production costs in live hosts (Sun 2015). Examples of some commercially-available viruses include *Helicoverpa zea* single-enveloped nucleopolyhedrovirus (HzSNPV), *Spodoptera exigua* multi-enveloped nucleopolyhedrovirus (SeMNPV), and *Cydia pomonella* granulovirus (CpGV) (Dara 2017). Viral pathogens of thrips do exist but, presently, no commercial formulations have been developed (Hauxwell 2008).

**Table 1.4.** Commercially available bioinsecticides based on entomopathogenic viruses (Ruiu 2018; Copping 1998).

| Active substances | Trade names* | Main targets | Crop |
|---|---|---|---|
| *Helicoverpa zea* nucleopolyhedrovirus | Heligen | *Helicoverpa* spp. and *Heliothis virescens* (Fabricius) | Tomato, cotton, tobacco, beans, soybeans, maize, lettuce |
| *Spodoptera litura* nucleopolyhedrovirus | Biovirus–S, Somstar-SL | *Spodoptera litura* (Fabricius) | Cotton |
| *Adoxophyes orana* granulovirus (AoGV) | Capex | Summer fruit tortrix moth (*Adoxophyes orana* (Fischer von Roslerstamm)) | Apple, pear, plum, rose |
| *Cryptophlebia leucotreta* granulovirus | Cryptex | False codling moth (*Thaumatotibia leucotreta* (Meyrick)) | Citrus, avocado, tea, cotton |
| *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) | Biovirus–H, Helicovex, Helitec, Somstar-Ha | African cotton bollworm (*Helicoverpa armigera*), corn earworm (*H. zea*), and other *Helicoverpa* species (*H. virescens*, *H. punctigera*) | Vegetables, fruit |
| *Helicoverpa zea* nuclear Polyhedrosis virus | Gemstar | *Heliothis* and *Helicoverpa* species | Maize, cotton |
| *Plutella xylostella* granulovirus | Plutellavex | *Plutella xylostella* (Linnaeus) | Cruciferous vegetable crops |
| *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) | Littovir | African cotton leaf worm (*Spodoptera littoralis* (Boisduval)) | Vegetables, fruits |

| | | | |
|---|---|---|---|
| *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) | Gypchek | *Lymantria dispar* (Linnaeus) | Apple, poplar, maple |
| *Cydia pomonella* granulovirus (CpGV) | CYD-X, Madex, Carpovirusine, Carposin, Granupom, Madex 3 | *Cydia pomonella* (Linnaeus) | Apple, pear |
| *Neodiprion abietis* nucleopolyhedrovirus (NeabNPV) | Neodiprion abietis NPV | *Neodiprion abietis* (Harris) | Balsam fir |
| *Spodoptera exigua* nucleopolyhedrovirus (SeNPV) | Spexit, Spod-X | *Spodoptera exigua* (Hübner) | Vegetables, fruits |
| *Autographa californica* MNPV | VPN-80 | Lepidoptera larvae | Tomato, tobacco |
| *Anticarsia gemmatalis* AgNPV | Polygen | Velvet bean caterpillar, sugarcane borer | Soybean |
| *Mamestra brassicae* MNPV | Virin- EKS | Lepidoptera larvae | Cabbage, tomato, potato |

*Strains may be registered under different trade names

### 1.8.4 Fungi

Entomopathogenic fungi play an important role in the regulation of a number of different insect populations (Goettel *et al.* 2010). An entomopathogenic fungus (EPF) is a fungus that can act as a parasite of insects, and kills or seriously disables them. The majority of EPFs are found within two groups: the order Hypocreales within the phylum Ascomycota, and the phylum Entomophthoromycota (Humber 2012). Ansari *et al.* (2007) showed that when applied in horticultural growing media, *Metarhizium brunneum* Petch (a member of the Ascomycota), was more effective than chemical insecticides (imidacloprid, fipronil) in killing *F. occidentalis* pupae (70–90 % mortality compared to 20–50 % mortality, respectively). EPFs cause lethal infections and regulate insect and mite populations in nature by epizootics (McCoy *et al.* 1988). *Beauveria bassiana* was found to cause 96 % mortality in *F. occidentalis* (Gao *et al.* 2012), whilst in another study, *M. brunneum* strains V275 and ERL700 caused 85 to 96 % mortality of *F. occidentalis* larvae and pupae (Ansari *et al.* 2008). Several pathogenic fungi based bio-insecticides have been formulated and commercially manufactured (Gul *et al.* 2014). Ramanujam *et al.* (2014) reported that of the 171 EPF products developed, *B. bassiana*-based products accounted for 34 %, while *M. anisopliae* (more recently named *M. brunneum*), *Isaria fumosorosea* and *B. brongniartii* products accounted for 34, 6 and 4 % of total products, respectively. For the control of various insect pests, several commercial fungal entomopathogenic products are available, including: BIO 1020®, Biogreen®, Green Guard®, Green Muscle® and *Metarhizium* 50® (based on *Metarhizium* spp.) and Bb plus® (based on *B. bassiana*) (Bidochka & Small 2005). Research on jalapeño peppers under greenhouse conditions revealed that foliar applied *B. bassiana* (BotaniGard®) reduced chilli thrips (*Scirtothrips dorsalis* Hood) larvae by about 50 %, five days after treatment (Seal & Kumar 2010). A list of commercially available mycoinsecticides, their target pests and related crops is presented in **Table 1.5**.

Entomopathogenic fungi infect insects of almost all orders, including Hemiptera, Diptera, Coleoptera, Lepidoptera, Orthoptera and Hymenoptera (Ramanujam *et al.* 2014). Unlike bacterial, viral, and protozoan pathogens that need to be ingested to be active, the spores of EPFs stick to the insect host's cuticle, after which they germinate, then penetrate the insect. Therefore, entomopathogenic fungi have the potential to be active against non-feeding stages such as pupae. Thrips have a critical non-feeding pupal stage (Gilbert & Samways 2018) that can be targeted in control regimes. Keller & Zimmermann (1989) reported that the order Hyphomycetes typically induce epizootics in populations of soil-dwelling insects. Hyphomycetes, such as *Metarhizium* and *Beauveria*, can infect both adult and larval stages.

This ability to attack multiple life stages is important in disease transmission because winged individuals can assist in the spread of disease (Goettel *et al.* 2010). Commercially developed *B. bassiana* formulations registered for thrips control in orchards include Mycotrol®, Naturalis® and BotaniGard® (Maina *et al.* 2018). The commercially available BotaniGard® was reported to be effective against the Californian citrus thrips, *Scirtothrips citri* (Moulton), and the avocado thrips, *Scirtothrips perseae* Nakahara, under laboratory conditions (Zahn & Morse 2013). Both these thrips species are taxonomically closely related to the South African citrus thrips, *S. aurantii*, and their potential to be controlled by a commercial GHA strain of *B. bassiana* supports efforts to control *S. aurantii* using entomopathogens.

Four steps are usually necessary for infection by an EPF: adhesion, germination, differentiation and penetration. A range of integrated intrinsic and extrinsic factors such as water, ions, fatty acids and nutrients on the cuticle surface, as well as the physiological state of the host, influence spore adhesion and germination (Hassan *et al.* 1989). Successful germination requires the assimilation of utilizable nutrients and a tolerance to any toxic compounds present on the surface of the insect or mite (Latgé *et al.* 1987).

Adhesion is achieved when spores successfully attach or adhere to the external body surface of the insect. Under the right conditions of temperature and (usually high) humidity, these spores germinate, grow as hyphae and colonize the insect's cuticle. Penetration of the cuticle is accomplished by the germ tube itself or by the formation of an appressorium that attaches to the cuticle and gives rise to a narrow penetration peg (Wraight *et al.* 1998). Penetration is both a mechanical and an enzymatic process, the exact mechanism for entry being species specific (McCoy *et al.* 1988). Most terrestrial pathogens are known to penetrate the cuticle directly, and more rarely via wounds, sense organs or spiracles.

Inside the host body cavity, the fungal cells proliferate, usually as walled hyphae or in the form of wall-less protoplasts (depending on the fungus involved). After some time, the insect succumbs to the infection (sometimes by fungal toxins) and new propagules (spores) are formed in or on the cadaver, if environmental conditions are conducive. Conditions such as high humidity are known to provide a conducive environment for sporulation (Shahid *et al.* 2012). In addition to being stable, the development of resistance to entomopathogenic fungi in insect populations has rarely been documented (Goettel *et al.* 2010) and is thus proposed as a sustainable alternative to the use of synthetic insecticides for the control of thrips.

**Table 1.5.** Commercially available mycopesticides with their target pests (Milner 2000; Ruiu 2018; Ramanujam et al. 2014).

| Active substances | Trade names* | Main targets | Crops |
|---|---|---|---|
| *Beauveria bassiana* | Bio-Power, Biorin/Kargar, Botanigard, Daman, Naturalis, Nagestra, Beauvitech-WP, Bb-Protec, Conidia, Naturalis L, Ago Bio, Bassiana | Wide range of insects and mites (eg., whitefly, thrips, aphids, mealybugs) | Berries, maize, fruit, ornamentals, banana, leafy vegetables, pastures and tuff grass |
| *Beauveria brongniartii* (Sacc.) | Bas-Eco, Betel | *Helicoverpa armigera*, berry borer, root grubs, scarab beetle larvae | Sugarcane |
| *Hirsutella thompsonii* Fisher | No-Mite | Citrus rust mite | Citrus |
| *Metarhizium brunneum* Petch | Biomet/Ankush, Bio-Magic, Devastra, Kalichakra, Novacrid, Met52/BIO1020 granular, Pacer, Bioblast, Metaquino, DeepGreen, Metarhizium 50 | Beetles and caterpillar pests, grasshoppers, termites, spittle bugs | Grapes, berries, cabbage, banana, maize, leafy vegetables, bulbous plants |
| | Attracap | *Agriotes* spp. | Potatoes, leafy vegetables |
| *Metarhizium flavoviride* Gams & Roszypal | Biogreen, Green muscle | Scarab larvae, locusts, grasshoppers | Wheat, oilseed rape |
| *Isaria fumosoroseus* (Wize) A.H.S. Br. & G. Sm. | PFR-97, Pae-Sin | Whitefly | Citrus, sugarcane |
| *Paecilomyces lilacinus* (Thom) Samson | Bio-Nematon, MeloCon, Mytech-WP, Paecilo | Plant pathogenic nematodes | Banana, capsicum, tomato, brinjal, onion, chilli, okra, papaya |
| *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. | Bioact WG, No-Fly-WP, Paecilomite, PFR-97 | Insects, mites, nematodes, thrips | Ornamentals, cut flowers, lettuce |
| *Verticillium lecanii* (Zimm.) Viégas | Bio-Catch, Mealikil, Bioline/Verti-Star, Vertalec | Mealy bugs and aphids and sucking insects | Cabbage, cucumber |

| | | | |
|---|---|---|---|
| *Lecanicillium lecanii* (Zimm.) Zare & W. Gams, Nova Hedwigia | Lecatech-WP, Varunastra | Aphids, leaf miners, mealybugs, scale insects, thrips, whiteflies | Cabbage, cucumber |
| *Lecanicillium muscarium* (Petch) Zare & W. Gams, Nova Hedwigia | Mycotal | Whiteflies, thrips | Cucumber, strawberries, sweet pepper, tomato and ornamentals |
| *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar | DiTera | Nematodes | Food, fiber, ornamental crops |
| *Lagenidium giganteum* Couch | Laginex | Mosquitoes | Mosquitoes |
| *Nomuraea rileyi* (Farl.) Samson | Numoraea 50 | Lepidoptera | Soybean, tomato, cotton |
| *Hirsutella thompsonii* | Mycohit | Acari | Citrus |
| *Conidiobolus thromboides* Drechsler | Vektor 25SL | Aphids, thrips, whiteflies | Potato, soybean, cotton |
| *B. bassiana+ M. anisopliae+ I. fumosoroseus* | Tri-Sin | Psyllid | Tomato, potato |

*Strains may be registered under different trade names

## 1.9 *Beauveria bassiana*

In the early 1800s, a white muscardine disease that periodically decimated the European silk industry plagued the silkworm farms of Italy and France. Steinhaus (1956) asserted that the probable causal organism had been reported as early as 2700 BC in China but had never been identified. In 1835, an Italian scientist, Agostino Bassi, demonstrated that the disease was caused by a microbial infection and that it could be controlled by altering the living conditions of the silkworms (*Bombyx mori* L.) to decrease the spread of the disease (Lord 2005). Later the microbe, a filamentous fungus, responsible for the disease was named *Beauveria bassiana* in honour of Bassi's discovery (Alexopoulos *et al.* 1996).

In addition to being an entomopathogen, *B. bassiana* (formerly *Tritirachium shiotae*) commonly occurs as a saprophyte in soil and as a plant endophyte (Bruck & Lewis 2002). It is a naturally occurring soil-borne fungus that parasitizes various arthropod species, causing white muscardine disease. It is cosmopolitan and is pathogenic to a wide spectrum of arthropods spanning most orders of class Insecta (Zimmermann 2007), with a host range of up to 700 insect species (Goettel *et al.* 2000). Rehner & Buckley (2005) described *B. bassiana* as a species complex of morphologically similar and closely related isolates.

Just before entering the second moult, larvae of many thrips species drop to the soil litter beneath the plant or move to some protected place on the plant (crevices, such as bark scales, hollow twigs, bases of leaf stalks, leaf sheaths, and leaf spaces) to pupate (Gilbert & Samways 2018). This stage is generally protected because the pupae are shielded from predators and do not feed, only exhibiting slight mobility when disturbed. With full coverage spraying of *B. bassiana* onto the soil and leaf litter (pupation sites), high infection rates and high mortality levels can be attained. For example, Ansari *et al.* (2007) noted that the application of entomopathogenic fungi through soil drenches or pre-mixing with potting compost is an effective control strategy against *F. occidentalis.* Similarly, Zhang *et al.* (2019) reported that granules impregnated with *B. bassiana* were 70 % effective against *F. occidentalis* on eggplants when applied as a soil treatment against the soil-dwelling pupal phases.

After pupation, the adults may escape predation by jumping or flying to refuge. Adult and larval thrips feed by sucking plant cell contents of epidermal cells in host plants. Deposition of infective *B. bassiana* spores on plant surfaces increases the likelihood of spores being picked-up during feeding and spread during dispersal (Hajek 1997).

Alexopoulos *et al.* (1996) described endophytes as being non-pathogenic fungi that live inside healthy plants and Bacon (1993) suggested that the mutualistic association provides nutrients and moisture for the fungus, and imparts stress tolerances to the plant. *Beauveria bassiana* has been shown to be an endophyte (Moonjely *et al.* 2016) in maize (Poaceae) (Lewis & Bing 1991), cassava (Euphorbiaceae) (Greenfield *et al.* 2016) and potatoes (Solanaceae) (Jones 1994). Lewis & Bing (1991) reported that once established in the plant, *B. bassiana* reduced tunnelling by larval European corn borer, *Ostrinia nubilalis* (Hübner) in maize. Injection of conidial suspensions has also been used as a means for inoculation of maize plants (Leckie 2002). Studies conducted by Wagner & Lewis (2000) in maize, revealed that when *B. bassiana* conidia were applied as a foliar application, the spores germinated and penetrated the plant through natural openings (such as stomata) and through small holes (facilitated by enzymatic activity and mechanical pressure produced by the fungus). Once inside the plant, the mycelium branched and grew throughout the epidermal regions and into the palisade parenchyma but did not cause any adverse effects on the plants. Interestingly, saprophytic EPFs (such as *B. bassiana* and *M. anisopliae*) can establish colonies in plant roots, even in the absence of insect hosts (Barelli *et al.* 2016). In the plant, *B. bassiana* produces toxins as metabolites such as beauvericin, bassianolide, and the red pigmented toxin oosporein, which may build up in the plant and deter insect herbivory (Leckie 2002).

Avocado thrips (*S. perseae*) and the Californian citrus thrips (*S. citri*) were found to be very susceptible to the commercial *B. bassiana* strain GHA (Zahn & Morse 2013). Granules impregnated with *B. bassiana* were also reported to be 70 % effective against *F. occidentalis* on eggplants when applied as a soil treatment against soil-dwelling, pupal phases (Zhang *et al.* 2019). These terebrantian thrips exhibit the same life histories as the thrips spectrum of avocado in South Africa. Whilst no biocontrol tests have been conducted on thrips on South African avocadoes, there is good reason to support such studies. Commercially developed formulations registered for general thrips control include (*M. anisopliae*) Met52®, (*Isaria fumosorosea*) NoFly™ (Kivett *et al.* 2016), (*B. bassiana*) Mycotrol® and BotaniGard® (Maina *et al.* 2018), and (*Lecanicillium longisporum*) Vertalec® (Rechcigil & Rechcigil 2000).

### 1.9.1 Classification of *Beauveria bassiana*

*Beauveria bassiana* is in the Kingdom: Fungi, Phylum: Ascomycota, Class: Sordariomycetes, Order: Hypocreales and Family: Cordycipitaceae (CABI 2018). Several genera are included in the family, including the entomopathogenic genera *Cordyceps*, *Isaria*, *Lecanicillium* and

*Beauveria*. Species within the genus *Beauveria* are typically differentiated from other fungi by specific morphological characteristics. *Beauveria bassiana* is a filamentous fungus that produces colourless (hyaline) aerial conidia from conidiogenous cells freely on the mycelia (CABI 2018).

### 1.9.2 Morphology of *Beauveria bassiana*

*Beauveria bassiana* is characterized morphologically by its sympodial to whorled clusters of short-globose to flask-shaped conidiogenous cells, which give rise to a succession of one-celled, hyaline, holoblastic conidia that are borne on a progressively elongating sympodial rachis (Saranraj & Jayaprakash 2017). Conidia are the principal morphological feature used for species identification in *Beauveria*. Conidia may be globose, ellipsoidal, cylindrical, or comma shaped, and range in size from 1.7 to 5.5 mm. Petch (2006) highlighted the complexity of species identification owing to the proliferation of new species described between the late 19th to mid-20th centuries, few of which are morphologically distinct from previously described species. In all, 49 species have been placed in *Beauveria* and 22 epithets are currently valid. Presently most environmental isolates of *Beauveria* are classified in either *B. bassiana* or *B. brongniartii* (Tanada & Kaya 2013).

In culture, *B. bassiana* grows as a white mould. The conidiogenous cells of *B. bassiana* are short, ovoid and terminate in a narrow apical extension called a rachis. The rachis elongates after each conidium is produced, resulting in a long zig-zag extension (Humber 2007). The conidia are single-celled, haploid and hydrophobic (Saranraj & Jayaprakash 2017).

### 1.9.3 Physiology and life cycle

*Beauveria bassiana* is considered to be the anamorph of *Cordyceps bassiana*, an ascomycete in the order Clavicipitales, and both are considered to be endoparasitic pathogens of insects and other arthropods (Nikoh & Fukatsu 2000). All life stages of the fungus appear to be infectious, including hyphae, aerial conidia, single-cell blastospores, and submerged conidia (specialized cells produced in minimal liquid media). However, the asexually produced (aerial) conidia are considered to be the main dispersal and infectious structures, capable of resisting various abiotic stresses, to a greater extent than hyphae and blastospores (Holder *et al.* 2007).

Disease development is strongly influenced by a pathogen's ability to disperse (Anderson & May 1981). Infective propagules of entomopathogenic fungi in the Hypocreales are passively dispersed by wind and rain (Shah & Pell 2003), as well as by the migration and subsequent death of infected hosts (Hajek 1997).

*Beauveria bassiana* is a polymorphic fungus whose life cycle includes both single and multicellular stages. In soil or decaying plant material, it grows as multicellular mycelia (St Germain & Summerbell 2006) and reproduces and disperses as asexual conidia. Akbar *et al.* (2004) reported that *B. bassiana* conidia are smaller than most other fungal spores, measuring only 2 - 4 μm wide and, when released into the environment, remain dormant or in a non-vegetative state until appropriate conditions activate germination. Under optimum temperature and high humidity, these spores germinate, grow as hyphae and colonize the insect's cuticle (**Figure 1.2**) (Boucias *et al.* 2008). *Beauveria bassiana* conidia require a temperature range of between 0 and 40°C, with an optimum temperature of 20 – 30°C (Benz 2015) and a relative humidity above 97 % for germination (Saranraj & Jayaprakash 2017). At 25°C, germination takes between 10 and 20 hours and usually takes place on thinner, non-sclerotised areas of the cuticle, like joints, between segments or the mouthparts (Zimmermann 2007).

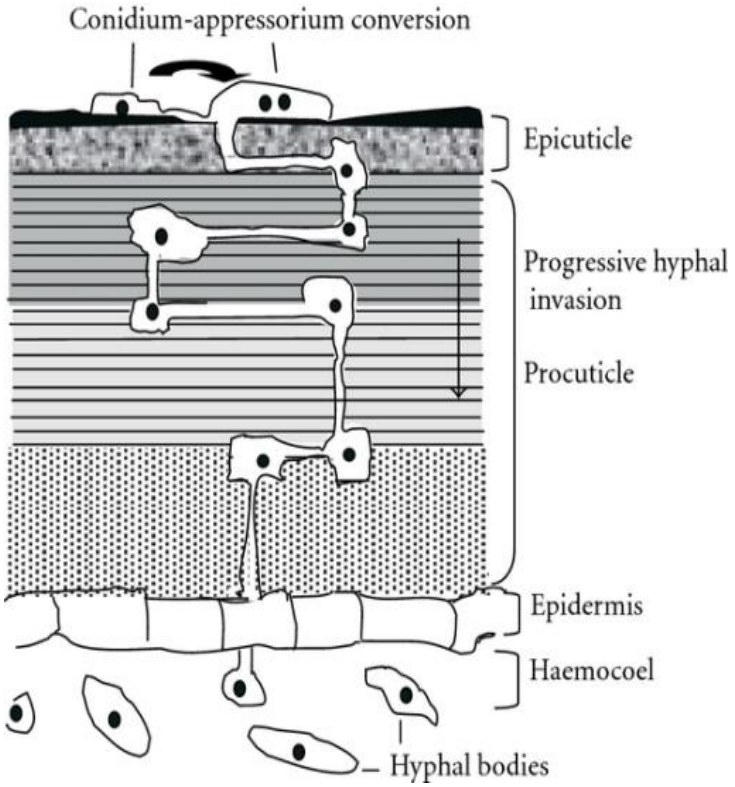


**Figure 1.2.** Infection by *Beauveria bassiana* (Sandhu et al. 2012).

The interactions between the penetrating fungus and the insect immune system are complex and comprise many molecular and cellular reactions (Vilcinskas & Götz 1999). During the infection process, *Beauveria* spp. produce proteolytic enzymes and toxins, while the host insects respond with cellular and humoral defence reactions. These reactions consist

of the production of antifungal compounds, inducible protease inhibitors and proteins, which detoxify fungal toxins in the insect.

The fungi proliferate inside the haemocoel, producing hyphal bodies that are distributed passively in the haemolymph, depleting nutrients and producing biologically active metabolites such as beauvericin (Wang & Xu 2012), 2-pyridone tenellin, bassianolide, beauverolides, bassianin, dibenzoquinone oosporein (Vey *et al.* 2001) and bassiacridin (Quesada-Moraga & Alain 2004), which account for its toxicity to insects. Beauvericin is thought to be the most important toxin, exhibiting insecticidal, antimicrobial, cytotoxic, and apoptotic activity (Klarić & Pepeljnjak 2005).

Zimmermann (2007) noted that the incubation period is dependent on the host, host stage, temperature and virulence of the fungal strain. During incubation, the fungus induces behavioural and feeding changes in the insect resulting in reduced feeding and reduced fecundity (Ouedraogo *et al.* 2003). Eventually, the insect succumbs to the infection (sometimes by fungal toxins) and new propagules (spores) are formed in or on the cadaver. Under humid conditions, the fungus grows saprophytically, emerging out of the host body, producing conidia on the exterior surface of the cadaver and setting the stage for future infections. Under very dry conditions, the fungus may also persist in the hyphal stage inside the cadaver. Zimmermann (2007) noted this phenomenon in locusts in Africa, where the fungus produced conidia inside the locust body.

Villamizar *et al.* (2018) further showed that *B. bassiana* produced resilient, overwintering propagules called microsclerotia that enable the fungus to survive unfavourable environmental conditions and the absence of hosts. Microsclerotia are tolerant to desiccation and are able to produce infective conidia under nutritionally poor conditions. This makes these structures promising stages to develop as formulated propagules for use as mycoinsecticides for soil or aquatic applications. The microsclerotia propagules form either microsclerotia or mycelial pellets which are generated by liquid culture fermentation (Song *et al.* 2016; Jackson & Payne 2016). Further, Huarte-Bonnet *et al.* (2019) demonstrated that the microsclerotial structures produced viable conidia upon rehydration. These features make the fungus attractive as a crop protection tool under variable environmental conditions, especially for soil dwelling stages such as *S. aurantii* pupae.

### 1.9.4 *Beauveria bassiana* in insect vectored technology

Insects may function as a mechanical carrier or vector for *B. bassiana*, aiding in the transportation of the fungal spores. They have been used in the auto-dissemination of the fungus to pest populations (Zimmermann 2007). Dowd & Vega (2003) noted that sap beetles (Coleoptera: Nitidulidae), contaminated with *B. bassiana* by means of an autoinoculative device, successfully transferred the fungus to the beetles' overwintering sites. Bumble bees have been used to deliver *Beauveria* conidia to target western flower thrips (*F. occidentalis*), greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood)), and green peach aphid (*Myzus persicae* (Sulzer)), in greenhouse crops (Shipp *et al.* 2006). Bee-vectored *B. bassiana* was reported to cause substantial mortality of lygus, whiteflies, thrips and aphids (up to 80 % mortality) in greenhouse cage trials on tomato and sweet pepper. The main advantage of using bees is that they deliver the fungal spores directly to the flowers and leaves where the thrips are feeding (Mascarin & Jaronski 2016).

### 1.9.5 Effects of *Beauveria bassiana* on non-target organisms

Several interactions of *Beauveria* spp. with hyperparasitic, antagonistic and especially, phytopathogenic fungi have been reported (Zimmermann 2007). The ascomycete *Syspastospora parasitica* (Tulasne), formerly known as *Melanospora parasitica*, is a known hyperparasitic fungus that attacks *B. bassiana* (Posada *et al.* 2004) whilst Krauss *et al.* (2004) noted that *Clonostachys* spp. and *Trichoderma* spp. may suppress or overgrow *B. bassiana in vitro*. This interaction has been exploited in crop protection because *Beauveria* spp. have been shown to be antagonistic to various plant pathogenic fungi. Vesely & Koubova (1994) reported that under greenhouse conditions, *B. bassiana* was antagonistic to *Pythium ultimum* Trow, *P. debaryanum* R. Hesse and *Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley & Crous. However, *Pythium irregular* Buisman, *Neocamarosporium betae* (Berl.) Ariyaw. & K.D. Hyde, *Phoma exigua* var. *foveata* (Foister) Boerema and *Rhizoctonia solani* J.G. Kühn showed resistance to *B. bassiana*.

The effect of *B. bassiana* on plants has also been investigated and Zimmermann (2007) concluded that no known side-effects or phytopathogenic activity had been reported. The plant may affect the infectivity and persistence of *B. bassiana* but no phytopathogenic reactions have been recorded. Poprawski *et al.* (2000) observed that nymphs of the greenhouse whitefly, *T. vaporariorum*, were highly susceptible to *B. bassiana* on cucumber plants, while insects reared on tomato plants were significantly less susceptible. This was thought to be as a result of the

inhibitory effect that tomatine (a glycoalkaloid found in the stems and leaves of tomato plants) has on *B. bassiana.*

*Beauveria bassiana* is a soil-borne fungus and findings by Zimmermann (2007) suggest that there were no or very limited detrimental effects on the soil-dwelling collembolans and mites that were tested. Vestergaard *et al.* (2003) noted that data from field investigations did not indicate any adverse effects on honeybees (Vandenberg 1990; Copping 2004), non-target arthropods (Brinkman & Fuller 1999), earthworms (Hozzank *et al.* 2003), fish (Copping 2004), birds (Althouse *et al.* 1997), or vertebrates and plants (Zimmermann 2007). In humans, conidia of *Beauveria* species have been identified to have allergenic potential (Westwood *et al.* 2005).

### 1.9.6 *Beauveria bassiana* in integrated pest management (IPM)

Fungal biopesticides, such as *B. bassiana*, are suitable for incorporating into IPM programs and can play a key role in resistance mitigation, evolution from synthetic pesticides and reduction in reliance on chemicals. *Beauveria bassiana* has been successfully incorporated in the IPM of coffee berry borer (*Hypothenemus hampei* Ferrari) in monocropping systems. Coffee berry borer (CBB) is a serious pest of coffee worldwide (Velmourougane *et al.* 2010). Biological control is achieved when formulations are applied as a foliar spray targeting CBB female founders as they migrate from refuges or parchment coffee areas during the peak flight activity, and as a ground treatment, targeting fallen, infested berries on the ground. Aristizábal *et al.* (2016) reported that these treatments resulted in a decrease in CBB infestation of up to 75 % in Colombia. Other control techniques, such as sanitization of the fallen berries and use of mass capturing with methanol-ethanol traps, also reduced coffee berry losses. The same technique holds promise in avocado against thrips. The above-ground and below-ground stages could be successfully targeted using *B. bassiana*, with other control techniques such as mulching being integrated into an IPM program to aid thrips suppression and reduce fruit quality losses. The strategy requires the substitution of chemical pesticides with microbial control agents but complemented with cultural control and habitat management. The cultural practices may include mulching and provision of a suitable environment that enhances the reproduction, survival, and efficacy of natural enemies (conservation biological control), combined with silicon fertilisation to increase host resistance and attractiveness of the avocado trees to natural enemies (Reynolds *et al.* 2016), and to enhance fungal efficacy (Gatarayiha *et al.* 2010).

### 1.9.7 Limitations to the use of *Beauveria bassiana* as a biocontrol agent

The effectiveness of *Beauveria bassiana* as a biocontrol agent is moderated by a range of abiotic (Fernandes *et al.* 2015) and biotic factors (Mascarin & Jaronski 2016). Fargues & Luz (2000) noted that when environmental conditions are unsuitable, the conidia are easily inactivated, reducing their capacity to regulate pest populations. Foliar application of fungal conidia may be inactivated by sunlight and ultraviolet light, or conidia may simply die due to rapid drying (Skinner *et al.* 2012). Moisture is an important factor affecting spore germination, mycelial growth and the pathogenicity of *B. bassiana* (Richter & Fuxa 2004). Whilst soil moisture may be higher and more constant than on the plants, spore movement is, however, more restricted in the soil, resulting in reduced infection levels (Goettel *et al.* 2010). The level of organic matter and pH can be important in the eventual infection levels, as well as in the survival of propagules.

In soil, other microbes, such as the hyperparasitic fungus *S. parasitica* (Posada *et al.* 2004), and small invertebrates such as springtails (Broza *et al.* 2001) have been shown to consume the fungi or to be directly or indirectly antagonistic to entomopathogens. In addition, microbes such as bacteria resident within insect hosts can compete within infected cadavers and, if the fungus does not have mechanisms to exclude these microbes, the fungus will be unable to colonize the cadaver and sporulate prolifically. These limitations may explain why *B. bassiana* and *Cladosporium oxysporum* Berk. & M.A. Curtis were ineffective in field trials against the citrus pests citrus psylla (*Trioza erytreae* (Del Guercio)), the black citrus aphid (*Toxoptera citricida* (Kirkaldy)) and the false codling moth (FCM; *Thaumatotibia leucotreta* (Meyrick)), yet they were promising in laboratory assays (Moore 2002).

Effective IPM requires that *B. bassiana* be compatible with several pesticides. To date, the efficacy of *B. bassiana* has been tested for compatibility with various pesticides, and some agrochemicals are now known to modify conidial survival (Benz 2015). For example, under laboratory conditions, chlorpyriphos was of low toxicicity; spinosad, Econeem (azadirachtin), quinalphos, acetamprid, endosulfan and thiodicarb were moderately toxic; imidacloprid and triazophos were moderately toxic; and profenofos, indoxacarb and methyldemeton were highly toxic to the fungus (Amutha *et al.* 2010). Kahn *et al.* (2012) reported that the popular fungicides mancozeb and copper oxychloride were not compatible with *B. bassiana*, and caused complete or strong inhibition of vegetative growth as well as sporulation.

Commercial production and use of *B. bassiana* are limited by its cost-effectiveness because considerable quantities of conidia have to be applied to achieve an acceptable level of

control (typically 2 – 3 kg $ha^{-1}$ of dried mycelia or conidia ($10^{13}$ per ha)). This application rate goes up tenfold for cryptic insects such as beetle larvae in soil. In addition, formulations are bulky and preservation of fungal viability beyond a few months is low due to the fragile nature of the conidia (Federici 1999).

Large-scale production of entomopathogenic fungi concentrates mainly on three types of propagules: vegetative cells (blastospores), vegetative, multicellular mycelium (Andersch 1992) and conidia (Jenkins & Prior 1993; Jenkins & Lomer 1994). While blastospores and conidia can infect the host directly, the mycelium needs to first grow and form infectious propagules. Conidia can be produced easily and are more stable in challenging environmental conditions than blastospores. Spore germination on artificial media can differ greatly from germination on an insect cuticle. The insect cuticle is covered by a waxy layer containing fatty acids, lipids and sterols (Hackman 1987), some of which contain fungistatic compounds that retard spore germination (Latgé *et al.* 1987).

To overcome the myriad of field application challenges, strategies have been devised to improve fungal performance under such stresses. Commercial mycoinsecticides are formulated to ease field application, enhance shelf-life and to increase environmental persistence after application (Feng *et al.* 1994). Formulated microbials are typically prepared as technical concentrates, wettable powders or oil dispersions (de Faria & Wraight 2007) and usually have a combination of an active ingredient (typically conidia), a thinner and/or disperser, a wetting agent and an adherent (Latgé & Moletta 1988).

Conidia can also be suspended in aqueous liquid or mixed with a powder carrier and sprayed as a mist or a dust with conventional equipment used for the application of synthetic chemical insecticides. Dry formulations (in which the active ingredient is formulated and stored until used (Soper & Ward 1981), are the forms which have been used to date commercially for *B. bassiana* conidia, although oil and water-based formulations are also used (Feng *et al.* 1994).

Under field conditions, sub-optimum temperature, humidity and exposure to UV light can render the conidia ineffective. Some adjuvants and other ingredients can improve the persistence of microbials in the environment by protecting them from inactivation (Reddy *et al.* 2008; Shapiro 1992). Rangel *et al.* (2015) suggested that more environmentally robust strains can also be attained through the selection of more tolerant and virulent phenotypes (isolates).

Leger & Wang (2010) outlined the efforts that have been made to create superior *Beauveria* strains by direct genetic manipulation. The approach has been to use protoplast

fusion, combined with selection for improved virulence, aimed at a faster speed of kill and a reduction in the lethal dose required for effective control. *Beauveria* has also been enhanced by genetically modifying the fungus to express the *Bacillus thuringiensis* Vip3Aa insecticidal protein (Ren *et al.* 2011). The characterization of the complete genome of *B. bassiana* has opened new insights into the complex mechanisms involved in its different life-histories and enabled the manipulation of its genes for various industrial purposes, including biocontrol (Xiao *et al.* 2012).

Careful selection of substrates can also enhance fungal viability, virulence, disease transmission and field persistence. Most common substrates for fungal entomopathogens include primary products from agriculture (Alves & Pereira 1989; Moore & Prior 1993; Zimmermann 1993). For example, work done by Ummidi & Vadlamani (2014) revealed that vegetable oil can be used as a substrate because it promotes adherence of spores to the insect cuticle, facilitating spore germination and infection. Simple and low-cost substrates have been developed using agricultural waste products such as cracked maize, maize bran, and rice grains (Jenkins & Goettel 1997). Essentially, any substrate can be used that supports a high yield of conidia, is cheap and readily available, easy to culture, and is effective in the environment (Magara *et al.* 2004).

Novel techniques for increasing field efficacy are being developed. For example, one such technique is encapsulation of the bioactive ingredient. Encapsulation of microorganisms by enveloping spores, mycelia or blastospores in a matrix with adjuvants and phagostimulants, can increase virulence and prolong the viability of the microorganism over an extended period (Rodrigues *et al.* 2017). Hanafi *et al.* (2000) also reported that encapsulated bioinsecticides can minimize the effects of environmental factors, such as inactivation due to light and heat. Potential biocontrol agents can be encapsulated in polymeric matrices or in hybrid materials such as alginate and carrageen to preserve the intrinsic properties of the fungus and increase its effectiveness. Some of the inoculum carriers that are being used in encapsulation technology include cellulose and cassava starch, cyclodextrins, sodium alginate, aliphatic polyesters, such as homo- and co-polymers of lactate and glycolate (including polylactic acid, PLA; poly-gamma-glutamic acid, PGA; and poly-lactic-*co*-glycolic acid, PLGA), polylactone and caprolactone (PCL) and the polyhydroxyalkanoates, known as PHAs (Kumari *et al.* 2010). In biological systems, these polymers are biodegraded through the relaxation of the polymer chain, the breaking of the monomeric unit located at the end of the chain (erosion), or even by the random split of a link at any point along the polymer chain (Ré & Rodrigues 2006). When

hydrated or degraded, the polymer relaxation process releases the infective conidia. Degradation processes occur when the polymers react with oxygen, light, or ambient temperature, and when acted upon by microorganisms present in the environment (Mohan 2011).

Microencapsulation has great potential for use in EPF formulations as it has been used successfully to protect bioactive ingredients that are sensitive to temperature, photodegradation, oxidization, moisture and other undesirable reactions (Gonsalves *et al.* 2009).

## Conclusions

The need to adhere to MRLs for export avocado production, coupled with the need for insecticide resistance management, suggest that evaluation of entomopathogenic fungi for control of thrips in avocado could be worthwhile. Fungal biopesticides such as *B. bassiana* are suitable for incorporating into IPM programs because they can play a key role in resistance mitigation, avoidance of resistance by target pests to synthetic pesticides, avoidance of damage to non-target organisms, especially honeybees, and a reduction in the reliance of agriculture on agrochemicals. Several mycoinsecticides have been developed, registered and are in use worldwide against a wide range of insect pests of economic importance. However, little work has been done on the biological control of thrips on avocado using entomopathogens. Whilst acknowledging the challenges of using *B. bassiana* as a control agent, this review supports its evaluation for the sustainable control of thrips in an integrated pest management program on avocado. Indeed, other entomopathogens may also have potential to control thrips on avocado, as indicated in this review. However, research will be required to optimize their performance under the challenging environmental conditions that exist in commercial orchards, and with the cryptic life cycle of the target thrips.

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# Chapter 2: Determination of the natural host status of avocado fruit to pestiferous thrips (Thysanoptera: Thripidae) in KwaZulu-Natal, South Africa


**[2]Abstract**

The South African avocado industry is export-orientated, with approximately 2.1 % of international market share and with a five-year mean annual production of 118,000 t from 17,500 ha. Feeding by thrips results in fruit scarring and corky tissue development, making the fruit unsuitable for export. The study aimed to identify the spectrum of thrips species in avocado flowers and more importantly, to confirm the identity of the thrips species responsible for damaging fruit. Thrips were collected from flowering panicles and were identified using taxonomic keys. *Frankliniella occidentalis* (Pergande), *Scirtothrips aurantii* Faure, *Thrips gowdeyi* (Bagnall), *Thrips pusillus* Bagnall, *Thrips tenellus* Trybom, *Haplothrips gowdeyi* (Franklin), *Haplothrips bedfordi* Jacot-Guillarmod and *Megalurothrips sjostedti* (Trybom) were consistently collected from May to September 2018. The minute size of thrips warranted a pre-season trial to determine the best netting material to contain thrips. Insect screen (149 μm pore size), nylon netting (250 μm pore size), chiffon (210 μm pore size), voile (250 μm pore size), organza (500 μm pore size), tea filter paper (74 μm pore size) and coffee filter paper (53 μm pore size) were evaluated as thrips exclusion screens. The experiment was laid out as a randomized complete block design with 6 replications and the trials repeated twice. Only coffee filter paper and tea filter paper contained at least 85 % of the thrips and were therefore chosen for thrips exclusion trials. Surveillance by fruit sampling was undertaken to determine the natural host status of avocado to thrips. Avocado fruitlets were randomly sampled and incubated under laboratory conditions. *Scirtothrips aurantii* Faure (the South African citrus thrips) emerged from fruitlets and was sustained on that fruit to adulthood. This is the first study to demonstrate that avocado is a natural host to this pest in South Africa.



**Keywords:** Thrips spectrum, exclusion, South Africa, avocado, fruit scarring.


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[2] *This chapter has been slightly modified and published as*:


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## 2.1 Introduction

South Africa is Africa's second largest avocado exporter (also in the top ten globally) behind Kenya, and is followed by several African countries, among them Rwanda, Democratic Republic of Congo, Cameroon and Zimbabwe (FAOSTAT 2020). The South African avocado industry is export oriented, with approximately 40 % of the total production volume exported (Blakey & Wolstenholme 2014). Being an export-oriented industry, there is a commercial impetus to optimise the exportable percentage of avocado fruit. However, thrips pose a significant threat to the industry by scarring fruit, resulting in fruits being downgraded from export quality. Damage in the U.S.A. by *Scirtothrips perseae* (Nakahara) was estimated to cost the industry US$8.65 million annually (Hoddle *et al.* 2003).

South Africa currently has a five-year mean annual production of 118,000 tonnes, from 17,500 ha of commercial avocado orchards, concentrated mainly in the subtropical areas of Limpopo (60 %), Mpumalanga (29 %), KwaZulu-Natal (9 %) and parts of the Cape provinces (2 %). Three-hundred and forty commercial growers and 78 emerging growers anchor production, with the industry employing 8,200 permanent and 7,300 seasonal workers (Donkin 2018). Approximately 70 % of the trees produced by South African avocado nurseries are 'Hass' and the remaining 30 % are mostly 'Fuerte', 'Ryan' and 'Pinkerton' cultivars (Blakey & Wolstenholme 2014).

Thrips are fringe-winged insects in the order Thysanoptera, with most pestiferous thrips belonging to the sub-order Terebrantia. Thrips lay extremely small eggs, about 0.2 mm long, by cutting slits in plant tissue with their ovipositors, and inserting their eggs, one per slit (Gullan & Cranston 2010). Being hemimetabolous, thrips gradually metamorphose to the adult stage. The first two instars are wingless nymphs, feed on plant tissue, and are devoid of functional genitalia. The third and fourth instars are non-feeding, resting stages and are respectively referred to as prepupa and pupa. Individuals of this sub-order pupate in soil or in the tree canopy (Lewis 1997) and these resting stages can last three to five days before adults emerge (Chin & Brown 2008). During this pupal stage, the insects' body organs are reshaped, wing-buds are developed and genitalia formed (Gullan & Cranston 2010). In warm weather, the adult stage can be attained in three weeks (Mound & Walker 1982) and last about 45 days, with several generations in a single year being possible (Chin & Brown 2008).

A study in Mexico by Johansen *et al.* (1999) revealed 38 thrips species on avocado, but only six of them were primary pests. Worldwide, some of the thrips recorded on avocadoes include *Liothrips persea* Watson, *Scirtothrips aceri* Moulton, *Frankliniella cephalica*

(Crawford), *Heliothrips haemorrhoidalis* (Bouché) (Dorantes *et al.* 2004), *Selenothrips rubrocinctus* (Giard) (Denmark & Wolfenbarger 2010) and *Scirtothrips perseae* Nakahara (Hoddle *et al.* 2012). These cosmopolitan, polyphagous species survive on foliage by scraping and sucking the contents of epidermal cells, in the process leaving silver-white discoloured spots, which later darken. Silvering is common, due to air entering cells from which the contents have been removed, and on fruits, this leads to scarring and corky tissue development. Very large populations of thrips can induce premature flower loss, and can reduce available pollen to below critical levels (Childers 1997). Damage is mostly observed on leaves and fruits, but thrips can also be found on tender shoots, buds, and flowers. These insects may cause malformation of fruit, premature fruit drop, and lesions that become entry points for microorganisms such as the fungus *Sphaceloma perseae* Jenkins (González-Hernández *et al.* 1999). While minor thrips damage can be tolerated, any damage covering an area of more than 2 $cm^2$ will result in the fruit being unacceptable for premium export grade (Stevens *et al.* 1999). In 1997, heavily infested orchards in Ventura County, U.S.A, recorded between 50 % and 80 % avocado crop damage due to *S. perseae* (Hoddle *et al.* 2002).

The climates of southern African countries range from semi-arid and sub-humid in the east, to hyper-arid in the west, while the central part of southern African is classified as semi-arid (Daron 2014). Global warming is expected to make southern Africa warmer and drier (James & Washington 2013). The projected changes in climate will provide favourable conditions for the proliferation of insect pests such as thrips in the region (Mafongoya *et al.* 2019).

Therefore, thrips are economically important pests on avocado fruit, and important candidates for pest risk analysis. Surveillance by fruit sampling is the most reliable method of determining the natural host status, and is preferred because it does not interfere with the natural behaviour of thrips. It also has the advantage of taking into account high levels of variability in the fruit, insect behaviour and periods of activity. According to the International Standard for Phytosanitary Measures 37, the status of a host can be determined based on confirmation of natural infestation and development of the pest to adult stages by sampling fruit from the field (FAO 2016). For this reason, fruit sampling was chosen as the method to determine the host status of avocado to thrips.

The objectives of this study were to determine the spectrum of thrips species found in avocado flowering panicles, to establish the effectiveness of different fruit bagging material as

thrips exclusion screens and to identify the thrips species responsible for the scarring damage observed in avocado fruit in KwaZulu-Natal Province, South Africa.

## 2.2 Materials and methods

### Determination of the thrips species spectrum in avocado flowers of KwaZulu-Natal, South Africa

#### 2.2.1 Study site

This study was carried out in two avocado orchards ('Pinkerton' cultivar), at Conlink Trust (-29.453415 30.683398) and Baynesfield Estate (-29.756873 30.314269), in KwaZulu-Natal Province, South Africa. Every fortnight from May to September 2018, 10 avocado trees at early flowering were randomly selected, from which 10 panicles/tree were gently shaken 10 times using the beat cup method, modified from Reisig *et al.* (2010). A 2-l water jug was fitted with a funnel, a 2 mm metal sieve, and at the base, ice cubes, to immobilise the thrips that were dislodged from the foliage using the apparatus shown in **Figure 2.1Error! Reference source not found.**. Thrips were collected and aspirated into vials. Thereafter, the thrips were euthanised and preserved in 70 % alcohol before being sent to Plant Health and Protection (Pretoria, South Africa) for taxonomic identification. After the first identification, subsequent identifications were done at the University of KwaZulu-Natal laboratory using morphological keys provided by Hoddle *et al.* (2012).

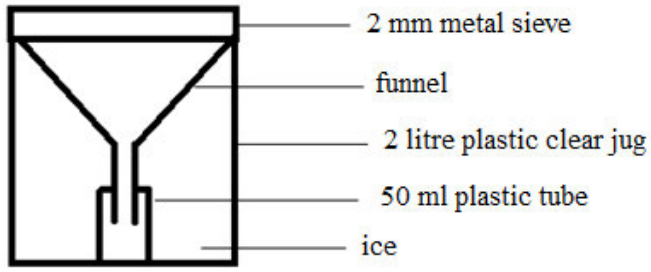


**Figure 2.1.** Apparatus used to collect thrips from flowering panicles.

#### 2.2.2 Pre-season determination of the effectiveness of different fruit bagging material as thrips exclusion screens

The minute size of thrips warranted a pre-season trial to determine the best material that could be used as a thrips exclusion screen. The pore opening sizes (µm) were determined under a stereo microscope (Leica M216 and image processing done using Leica application suite (LAS) 4.2). Thrips netting (149 µm pore size), nylon netting (250 µm pore size), chiffon (210 µm pore size), voile (250 µm pore size), organza (500 µm pore size), tea filter paper (74 µm pore

size) and coffee filter paper (53 µm pore size) were then evaluated against two thrips species, *Scirtothrips aurantii* Faure and *Haplothrips bedfordi* Jacot-Guillarmod, for their ability to contain the thrips. Ten thrips per species were introduced into each of 7 x 50 ml tubes. The tubes were then sealed off with the test material using tightly strung rubber bands. From one tree, six flowering panicles were selected and from these flowering panicles, experimental tubes were suspended 5 cm below the flowering panicles. The tubes were incubated in the field for 72 h, after which the tubes were assessed to see whether they successfully retained the thrips. The trial was repeated twice. The trial was laid out as a randomised complete block design with six replications and 42 sampling units. The results were analysed using the Bartlett test for homogeneity, Kruskal-Wallis non-parametric test and pairwise Wilcoxon test (for *post hoc* treatment separation).

### 2.2.3 Host status determination by means of fruit sampling

'Pinkerton' avocado fruitlets were collected from Conlink Trust and Baynesfield Estate from July to October 2018 and brought to the University of KwaZulu-Natal laboratory for incubation or 'rearing'. The actual selection of fruits was done using random sampling (probability based unbiased surveying technique) where the only basis of fruit selection was size (length ≤ 40 mm). Fruitlets were acquired for incubation trials every two weeks, with the target sample size for each sampling occasion being 80 fruitlets per sampling activity per site.

In the laboratory, individual fruits were washed in 0.035 % sodium hypochlorite solution and rinsed several times with tap water before being air-dried, fruitlet diameter measured (mm) and placed in clearly labelled incubation units (one fruit/unit) following the method modified from Ekesi & Billah (2006). An incubation unit consisted of a 25-ml transparent glass tube. The incubation tube was then sealed with coffee filter paper using tightly strung rubber bands. Incubation units were then held at 25 – 28°C, 75 ± 5 % relative humidity and 12L: 12D photoperiod.

Eggs of thrips are laid just below the skin of fruit, and larvae emerge from the eggs. The tubes were inspected and monitored daily for larval emergence (Ekesi & Billah 2006). After a few days, the larvae pupate to adults. All the larvae and pupae were held for two weeks to ensure maximum adult eclosure. After two weeks of incubation, the fruitlets were inspected once more before being discarded (by this time the fruits had dried).

The adults were kept alive for four days after adult eclosion to enable them to develop their full body colour and normal shape because morphological features were the main

identification tools. After four days, when adult body features had fully developed, the adults were identified using morphological keys provided by Hoddle *et al.* (2012). Adults were identified to species level and preserved in 70 % alcohol. The identity of the thrips as *Scirtothrips aurantii* Faure was also confirmed by ITS gene amplification and sequencing using the primers ITS1-F: TCGTAACAAGGTTTCCG and ITS1-R: GCTGCGTTCTTCATCGATGC at Inqaba Biotechnology Industries (Pretoria, South Africa). The fruit infestation index was calculated as the ratio of the number of larvae that emerged per number of fruits collected (Cowley *et al.* 1992).

### 2.3 Results

The thrips species *Frankliniella occidentalis* (Pergande), *Scirtothrips aurantii* Faure, *Thrips gowdeyi* (Bagnall), *Thrips pusillus* Bagnall, *Thrips tenellus* Trybom, *Haplothrips gowdeyi* (Franklin), *Haplothrips bedfordi* Jacot-Guillarmod and *Megalurothrips sjostedti* (Trybom) were consistently collected from avocado flower panicles during the May–September 2018 survey period from the two avocado farms in Pietermaritzburg (**Figure 2.2**; **Figure 2.3**).

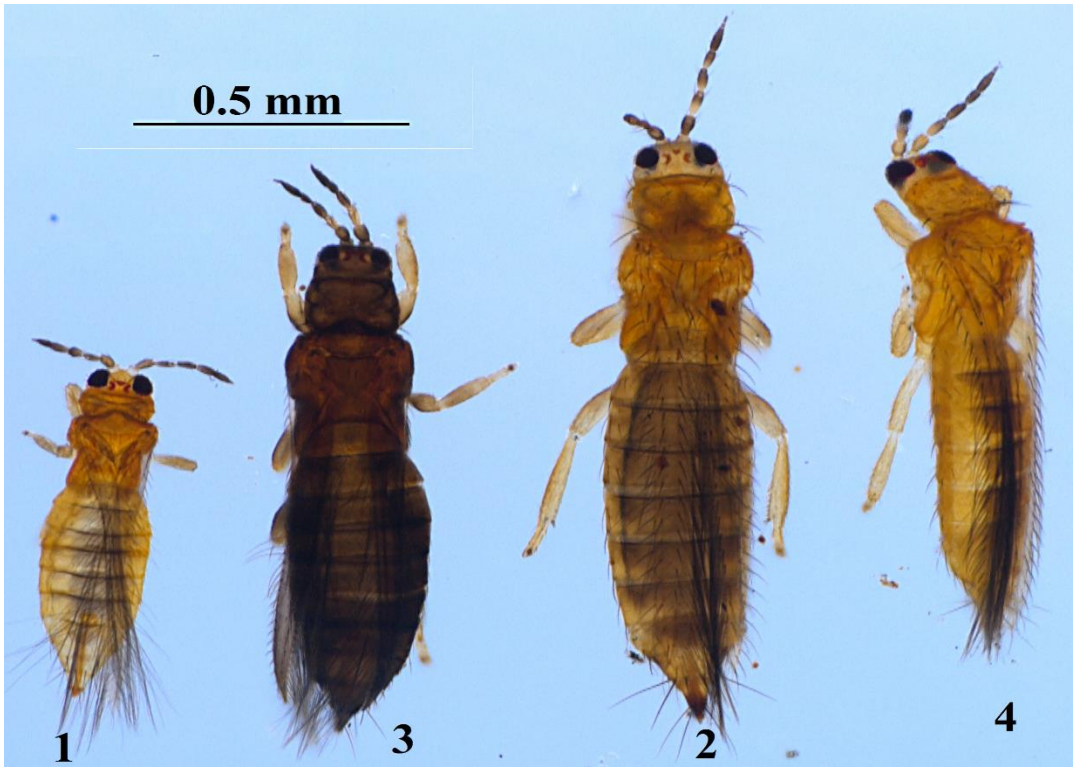


**Figure 2.2**. Thrips recovered from flower panicles: 1 = *Scirtothrips aurantii*; 2 = *Frankliniella occidentalis*; 3 = *Thrips gowdeyi*; 4 = *Thrips pusillus*.


**Figure 2.3.** Thrips recovered from flower panicles: 1 = *Megalurothrips sjostedti*; 2 = *M. sjostedti*; 3 = *Haplothrips gowdeyi*; 4 = *Thrips gowdeyi*; 5 = *H. bedfordi.*

*Scirtothrips aurantii* and *H. bedfordi* recovered from flower panicles at the end of winter/beginning of spring were used to test the effectiveness of various thrips screening material. The fruit bagging material were scrutinised under a stereomicroscope (X 57.5) (**Figure 2.4**) and their effective pore size was determined (**Table 2.1**).

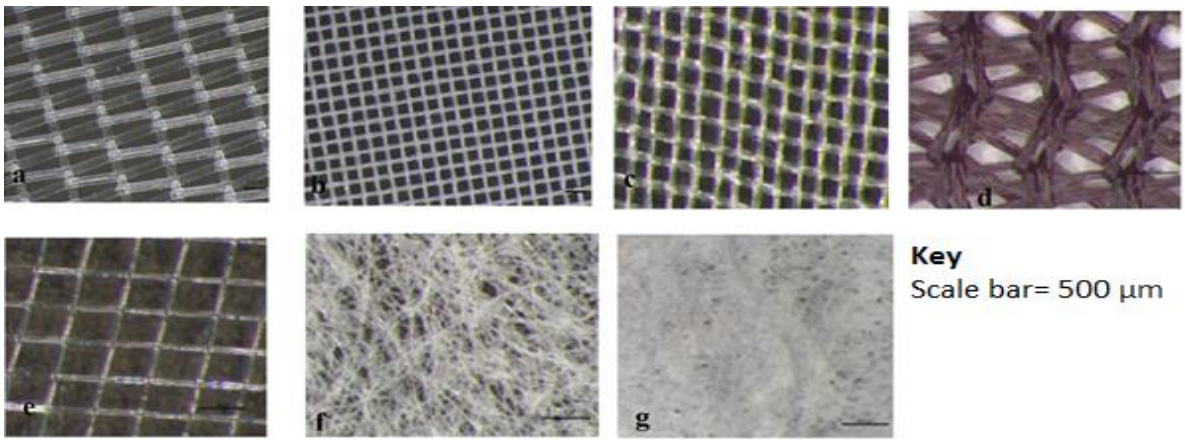


**Figure 2.4.** Test screening material: a – Thrips netting; b – Voile; c – Chiffon; d – Nylon netting; e – Organza; f – Tea filter paper; g – Coffee filter paper.

**Table 2.1.** Properties of bagging material used in the experiments.

| Material | Mesh No. | Pore size (μm) | Hole size | | Thread diameter, mm |
|---|---|---|---|---|---|
| | | | Length, mm | Width, mm | |
| Coffee filter | 270 | 53 | 0.052 | 0.005 | 0.02 |
| Tea filter | 200 | 74 | 0.074 | 0.0108 | 0.02 |
| Chiffon | 70 | 210 | 0.179 | 0.023 | 0.0334 |
| Thrips netting | 100 | 149 | 0.0524 | 0.1446 | 0.1476 |
| Nylon netting | 60 | 250 | 0.0468 | 0.2134 | 0.1196 |
| Voile | 60 | 250 | 0.2186 | 0.007 | 0.11 |
| Organza | 35 | 500 | 0.4722 | 0.022 | 0.0484 |

The mesh number represents the number of openings per linear 25 mm of material. The bigger the mesh number, the greater the number of pores, and the smaller the pore size. However, the opening pore size can vary slightly due to wear and distortion. The pore sizes determine the maximum size of particles and/or insects that can pass through. The mean length-width (l;w) of *S. aurantii* and *H. bedfordi* were measured to be 0.57 mm and 0.18 mm; and 1.29 mm and 0.21 mm, respectively. Containment of thrips after the 72-h evaluation period was statistically analysed using R Version 3.5.1 and the results are summarised in **Table 2.2**. Thrips netting, nylon netting, chiffon, organza and voile were ineffective at containing the thrips. Tea filter paper and coffee filter paper retained both thrips species (>85 %).

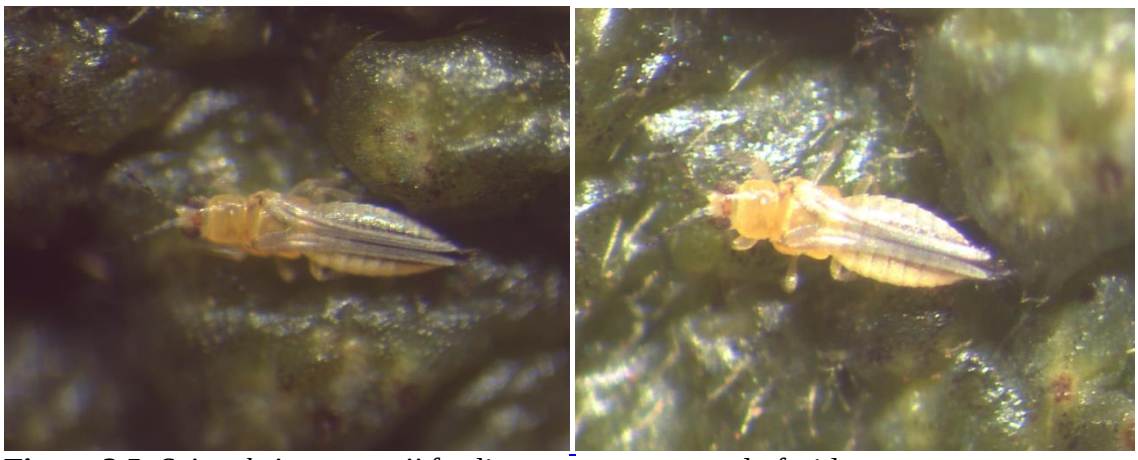

**Figure 2.5.** *Scirtothrips aurantii* feeding on young avocado fruitlets.

*Scirtothrips aurantii* larvae emerged from young avocado fruit and were sustained on the fruitlets to adulthood (**Figure 2.5**). During feeding, the thrips punch a hole by extruding a solid needle-like mandible. This is then withdrawn, and the maxillary stylets are inserted into the food source through the hole, with saliva being pumped into the tissues and the resultant fluid is then pumped back into the thrips' crop. Larvae and adults use a similar punch and suck feeding technique. Individuals tend to feed in localised patches on foliage, flowers and fruit, gradually moving out over undamaged areas of the fruit (Mound 2009).

From random sampling of 643 young avocado fruitlets, 70 *S. aurantii* larvae emerged from 31 fruitlets. Of the 70 larvae, 51.43 % successfully eclosed (36 adults) (**Table 2.3**). Thrips emerged from fruitlets of length 12 mm to 34 mm, with a mean of 20.74 mm. No thrips emerged from smaller fruitlets (**Table 2.4**). Of the 643 fruitlets surveyed, 31 fruitlets (4.82 %) were infested, and up to five larvae emerged from an infested fruit. The thrips larvae were observed feeding on the epidermal layer, leaving behind characteristic necrotic damage and were sustained on the fruitlets until adult thrips eclosed from pupae. Adult thrips survived on the drying fruitlet for several days until the fruit had either completely dried out or saprophytic fungi had completely covered the fruitlet.

**Table 2.2.** Containment of thrips by different bagging materials after 72 hours.

| | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | *S. aurantii* | *H. bedfordi* | *S. aurantii* | *H. bedfordi* |
| Bartlett's test for homogeneity | Bartlett's $K^2$ = 3.5681, df = 6, *P*-value = 0.7349 | Bartlett's $K^2$ = 5.983, df = 6, *P*-value = 0.4251 | Bartlett's $K^2$ = 10.708, df = 6, *P*-value = 0.09782 | Bartlett's $K^2$ = 1.7088, df = 6, *P*-value = 0.9444 |
| Kruskal-Wallis rank sum test | Kruskal-Wallis $\chi^2$ = 31.085, df = 6, *P*-value = 0.00002442 | Kruskal-Wallis $\chi^2$ = 31.63, df = 6, *P*-value = 0.00001921 | Kruskal-Wallis $\chi^2$ = 28.536, df = 6, *P*-value = 0.00007446 | Kruskal-Wallis $\chi^2$ = 34.328, df = 6, *P*-value = 0.000005813 |
| Pairwise comparisons of treatments using Wilcoxon rank sum test | Thrips netting $^{d,e,f}$<br>Nylon netting $^{d,e,f}$<br>Organza $^{b,d,f}$<br>Chiffon $^{b,c,d,}$<br>Voile $^{b,c,d}$<br>Tea filter $^{a}$<br>Coffee filter $^{a}$ | Thrips netting $^{d}$<br>Nylon netting $^{d,e,f}$<br>Organza $^{c,e,f}$<br>Chiffon $^{b,d,f}$<br>Voile $^{c,d,e,}$<br>Tea filter $^{a}$<br>Coffee filter $^{a}$ | Thrips netting $^{b}$<br>Nylon netting $^{b}$<br>Organza $^{b}$<br>Chiffon $^{b}$<br>Voile $^{b}$<br>Tea filter $^{a}$<br>Coffee filter $^{a}$ | Thrips netting $^{g,c}$<br>Nylon netting $^{b,c,e,f}$<br>Organza $^{c,e,f}$<br>Chiffon $^{d,e,f}$<br>Voile $^{b,c,d,e,f}$<br>Tea filter $^{a}$<br>Coffee filter $^{a}$ |
| Treatments with at least 85% thrips containment | Coffee filter (96.67 %); Tea filter (91.67 %) | Coffee filter (93.33 %); Tea filter (91.67 %) | Coffee filter (96.67 %); Tea filter (93.33 %) | Coffee filter (88.33 %); Tea filter (85.00 %) |

**Note**. Treatments sharing a letter in their superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$.

**Table 2.3.** Summary of *Scirtothrips aurantii* emergence from avocado fruitlets.

| Site | Total No. of fruitlets | No. of infested fruitlets | No. of emerging larvae | No. of adults eclosed | % Adult eclosure |
|---|---|---|---|---|---|
| Baynesfield | 500 | 20 | 47 | 24 | 51.06 |
| Conlink | 143 | 11 | 23 | 12 | 52.17 |
| Pooled | 643 | 31 | 70 | 36 | 51.43 |

**Table 2.4.** *Scirtothrips aurantii* fruit infestation indices (mean ± *SE*).

| Site | Total No. of fruitlets | No. of infested fruitlets | % fruit infestation | Mean No. of emerging larvae/fruit | Mean No. of adults/fruit | Mean length of fruitlet |
|---|---|---|---|---|---|---|
| Baynesfield | 500 | 20 | 4.00 | 2.35±0.27 | 1.20±0.22 | 23.50±1.08 |
| Conlink | 143 | 11 | 7.69 | 2.09±0.34 | 1.09±0.70 | 15.73±1.02 |
| Pooled | 643 | 31 | 4.82 | 2.26±0.21 | 1.16±0.16 | 20.74±1.03 |

## 2.4 Discussion

During spring, various species of thrips are attracted to avocado blossoms and this is reflected in the catches of various thrips species. *Frankliniella occidentalis*, *S. aurantii*, *T. gowdeyi*, *T. pusillus*, *T. tenellus*, *H. gowdeyi*, *H. bedfordi* and *M. sjostedti* were consistently collected during the survey. Of these species, only *S. aurantii* is known as a pest in citrus, and is responsible for much of the scarring damage caused on South African citrus (Gilbert & Samways 2018).

Of the possible thrips screening material tested, coffee filter paper out-performed the other test materials (greater than 85% thrips containment) (Kruskal-Wallis $\chi^2 = 31.63$; df = 6; $P < 0.001$); however, it is not very malleable and would be difficult to fit around avocado fruitlets. Tea filter paper is flexible, and was not significantly less effective than coffee filter paper (Kruskal-Wallis $\chi^2 = 31.09$; df = 6; $P < 0.001$) and can thus be used to contain the thrips. Thrips netting did not perform as well as expected, and thrips used in the test were observed to breach the screen within minutes of being introduced into the test tubes. This could possibly

be because thrips netting was designed to exclude much larger thrips species such as *F. occidentalis.* In this study, *F. occidentalis* was measured to be 1.04 mm x 0.27 mm, compared to 0.57 mm x 0.18 mm (l x w) of *S. aurantii.*

Fruit sampling revealed that the South African citrus thrips, *S. aurantii*, is a pest of concern in avocado. Studies in the 1990s by Steyn *et al.* (1993) revealed that two thrips species, the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouche), and the redbanded thrips, *Selenothrips rubrocinctus* (Giard), were the only thrips species attacking avocado fruit, accounting for a loss of 2.1 % of the fruits (Dennill 1992). Just a few years prior to this, De Villiers & Van den Berg (1987), reported that avocado orchards were relatively free from serious insect pests owing to good control by natural enemies. However, as a consequence of growth in monoculture production, there is an increase in the number and severity of insect pests and their impact on the avocado industry.

Milne (1973) noted that over 300 pests had been recorded on avocado worldwide and, of these, 76 occurred in southern Africa. Erichsen & Schoeman (1992) forecasted an increasing abundance and diversity of avocado insect pests. Prior to this study, *S. aurantii* was not considered as an economic pest of avocado in South Africa (Van den Berg *et al.* 2001), however, this study showed a thrips infestation index of 4.82 %, which is comparable to the 4.5 % infestation index found in another study conducted in Mexico, where the thrips infestation index of several other thrips species on avocado fruit were investigated (Morales *et al.* 2000). *Scirtothrips aurantii*, though polyphagous, is an established pest of citrus (Gilbert & Samways 2018), where it is known to cause quality losses of up to 50 % in export fruit. It is also a pest of concern in mangoes (Grové *et al.* 2000) and macadamia (Rafter & Walter 2012). In this study, *S. aurantii* was observed to emerge and feed on young avocado fruit < 4 cm long. Similarly, in the U.S.A., oranges were reported to be most susceptible to scarring by a related citrus thrips, *Scirtothrips citri* (Moulton), from petal fall until they are 4 cm in diameter. Fruit larger than 4 cm are rarely scarred (Flint 1991). Hoddle *et al.* (2002) also noted the preference of young, tender fruit by another related thrips species, the avocado thrips, (*Scirtothrips perseae* Nakahara), in the USA.

It was observed in this study that avocado fruit are particularly vulnerable to attack by *S. aurantii* for a short period between spring flush (new growth) and early fruiting. The fruit are most susceptible to infestation when the young fruit are the only new growth available to the thrips. Yee *et al.* (2001) reported that as the foliage matures, it becomes less attractive to the thrips and the insects begin to feed on immature fruit. The sporadic nature of thrips attack on avocado fruit, coupled with the complex interaction with the avocado tree phenology, makes it

difficult to accurately predict and forecast infestations. As thrips dwell in many microhabitats, they are consequently subject to host plant phenological changes. These changes are driven by the environment, since weather imposes conditions that determine when a plant will produce new flowers, fruits and leaves (Ananthakrishnan 1993). Weather influences host plant phenology by directly affecting production of flowers and leaves, which in turn attracts and maintain thrips on a given host.

## 2.5 Conclusion

The study found that *F. occidentalis*, *S. aurantii*, *T. gowdeyi*, *T. pusillus*, *T. tenellus*, *H. gowdeyi*, *H. bedfordi* and *M. sjostedti* constitute the current spectrum of thrips associated with avocado flowers in South Africa. Due to the minute size of *S. aurantii*, fabrics with tiny pores such as coffee and tea filter papers need to be used be used in thrips exclusion experiments. Thrips netting proved to be ineffective as a shield against penetration by this species.

*Scirtothrips aurantii* emerged from avocado fruitlets, and were observed to be feeding on young fruit, causing visible scarring damage. The fruitlets sustained the thrips to adulthood, confirming the host status of avocado as a natural host to *S. aurantii*. This is the first study to confirm that avocado fruit are a natural host to the South African citrus thrips.

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# Chapter 3: Attractiveness of different coloured sticky traps to the South African citrus thrips (*Scirtothrips aurantii* Faure) in avocado, KwaZulu-Natal, South Africa


## [3]Abstract

The South African citrus thrips *Scirtothrips aurantii* is a recently confirmed economic pest in South African avocado. Scarring damage by thrips results in corky tissue development, making the fruit unsuitable for export and potentially costing the farmers and country in lost export revenue. Thrips management is complicated by the multiple and protracted flowering patterns of susceptible cultivars. In addition, widespread pesticide resistance of thrips and the negative environmental effects, along with challenging minimum residue levels for export fruit, limit the application of pesticides for thrips control. Monitoring an insect pest's presence and abundance is the first step in thrips management. This study aimed to investigate *S. aurantii*'s colour preferences, establish the distribution of thrips in the orchard, and to capture its population dynamics from flowering to fruit establishment. Attractiveness followed the order yellow > blue, white, clear > green, red, black, purple and orange. Thrips were randomly distributed throughout the orchard, with the highest populations occurring during flowering (August), declining sharply, then picking up from mid-summer, starting in December. Yellow sticky cards are recommended as a monitoring tool.



**Keywords:** South Africa, avocado, *Scirtothrips aurantii*, sticky traps, population dynamics.


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## 3.1 Introduction

There are 71 avocado-producing countries in the world and, in 2017, world production reached 5.9 million tonnes, increasing by about 65 % from 2007 (FAOSTAT 2020). The industry has benefited from advances in post-harvest technologies, reductions in trade barriers, strong marketing and increased areas under cultivation (Duarte *et al.* 2016). South Africa is Africa's second largest avocado exporter, producing an average of 118,000 tonnes annually from 17,500 ha (Donkin 2018). The South African avocado industry is export-orientated, with approximately 40 % of the total production volume exported (Blakey & Wolstenholme 2014). In 2014, approximately 65,845 t (51 % of total production) were exported, 21 % was sold through the National Fresh Produce Markets (NFPMs), 15 % was sold to the informal markets (hawkers), 4 % was processed, while the remaining 8 % was delivered directly to retailers to a gross value of ZAR 1.1 billion (US$ 77.2 million) (DAFF 2015).

Being an export-oriented industry, there is a commercial impetus to optimise the exportable percentage of avocado fruit. However, thrips pose a significant threat to the industry by causing scarring damage to fruit, resulting in their being downgraded. Feeding by both adults and larvae causes permanent superficial scarring of the fruit epidermis. Minor thrips damage can be tolerated but any damage covering an area of more than 2 $cm^2$ will result in the fruit being unacceptable for premium export grade (Stevens *et al.* 1999). A study carried out by Bara & Laing (2019) established that *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) (South African citrus thrips) is an economic pest of avocado causing scarring of fruit. Damage to avocado fruit in the USA by a related thrips species, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), was estimated to cost that industry US$8.65 million annually 17 years ago (Hoddle *et al.* 2003). In South Africa, *S. aurantii* is an established pest of citrus (Gilbert & Samways 2018) and mangoes (Grové *et al.* 2000). Eggs are minute (less than 0.2 mm), bean-shaped, and are laid separately in slits cut into the soft tissue of green fruit, tender leaves and shoots (Bedford 1943). The two feeding larval stages that follow the egg stage are yellow to orange, and cigar-shaped. These are followed by two non-feeding pupal stages. The average duration of the larval stages, though temperature dependant, is about 7.6 days, whilst that of the pupal stages is 4.0 days. Adult thrips are yellowish-orange, and all stages are less than 1 mm in length and are barely visible to the unaided eye. In citrus, the duration of the life cycle varies from 44 days in winter to 18.4 days in summer, with 9.4 generations being possible every year (Bedford 1943). The species is not known to undergo diapause owing to the mild climatic conditions of the main citrus-producing areas in South Africa (Gilbert & Bedford

1998). This therefore means that, subject to the availability of suitable feeding conditions, larvae and adults can be found throughout the year.

Thrips control is complicated by their dispersal and spatial distribution potential. Thrips dwell in many microhabitats and are consequently subject to host plant phenological changes. These changes are driven by the environment, since climate imposes conditions that determine when a plant will produce flowers, fruits and leaves (Ananthakrishnan 1993). Weather influences thrips host plant phenology by directly affecting production of flowers and leaves, which in turn attracts and maintains thrips on a given host. Thrips on leaves and fruits are typically herbivorous species that inflict damage on cultivated plants and increase their populations in response to the availability of food types (Silva & Del-Claro 2010).

Although thrips may feed and reproduce on vegetative plant structures, adults preferentially orientate towards, and land on flowering plants (Kumar *et al.* 1995) and are relatively more abundant on plants with numerous, large flowers (Pearsall 2000). A large avocado tree typically produces very large numbers of flowers, often in excess of one million (Blanke & Lovatt 1993), attracting several thrips species, some of which are of economic importance (Bara & Laing 2019).

Dispersal in Thysanoptera is a function of active flight and air currents. Mound & Marullo (1996) pointed out that the frequency and duration of flight varies with the species, gender, climate, the suitability of food, and possibly crowding. Lewis (1997) further pointed out that in addition to the distribution of thrips population being strongly influenced by climatic conditions, these individuals have more or less no control over their flight path and their destination. The important factors are those that influence flight take-off. Wind-aided dispersal occurs when thrips (winged and wingless) are picked up and transferred long distances by air currents. During warm and humid weather, adults may climb to the tips of plants to leap off and catch air currents. Wingless species on the mountains of south eastern Australia were able to disperse to the northern part of South Island, New Zealand, a distance of more than 1600 km across the Tasman Sea. Long-distance migration flights have also been demonstrated with *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae) (Mound 2003).

Thrips, like most phytophagous insects, locate hosts by responding to a range of stimuli, including visual, mechanical, gustatory and olfactory characteristics (Prokopy & Owens 1983). Colour, shape and olfactory cues are usually involved in an insect's initial orientation to a plant, whereas once an insect has alighted on the plant, acceptance or rejection, and the initiation of feeding are determined by texture as well as the presence or absence of specific chemical

stimulants or deterrents (Renwick 1983). Herrin & Warnok (2002) demonstrated host preference by vector thrips at the cultivar level. Many factors influence host choice, such as plant chemistry, plant morphology and colour (Terry 1997). Other factors, such as protection from predators and adverse environmental conditions, and the nutritive qualities of each plant host may also be important (Fry 1996).

Nault *et al.* (2003) highlighted the importance of trapping tools in monitoring an insect pest's presence and abundance as the first step in Integrated Pest Management (IPM) of thrips. Population monitoring for pestiferous thrips in tree crops is hampered by the tree size (Hoddle *et al.* 2002) with some avocado trees growing between 9 and 18 m tall (DAFF 2012). Monitoring of thrips populations in tree crops such as citrus and mangoes can be achieved using suitably coloured sticky traps and dispersal/emergence traps (Grové *et al.* 2000; Gilbert & Samways 2018). Coloured sticky traps are a rapid and cost-effective tool for monitoring that is used for monitoring various insect pests (Bashir *et al.* 2014). Trap attractiveness and trap catches are known to vary with species (Kirk 1984) and trap colour (Cho *et al.* 1995). Yellow sticky traps are currently used to trap whiteflies (Hemiptera), aphids (Hemiptera), leafhoppers (Hemiptera), thrips (Thysanoptera), leafminers (Lepidoptera) and tephritid fruit flies (Tephritidae*)* (Hazir & Ulusoy 2012). Thrips such as the western flower thrips, *Frankliniella occidentalis* (Pergande), plague thrips (*Thrips imaginis* Bagnall), and onion thrips (*Thrips tabaci* Lindeman) (Thysanoptera: Thripidae) are reported to be attracted to blue, yellow and white, as opposed to clear, red, green or black (Broughton & Harrison 2012). Samways (1986) found that the citrus pest, *S. aurantii*, shows a positive phototactic response to yellow surfaces in citrus, particularly fluorescent yellow, with peak reflectance being at about 525 nm. On mango crops, Grové *et al.* (2003) demonstrated the effectiveness of yellow sticky traps for monitoring *S. aurantii* in Mpumalanga, South Africa.

The objectives of this study were to determine the colour of sticky traps that would be most suitable for monitoring *S. aurantii*, to establish the distribution of thrips and to monitor the populations of thrips during the critical infestation period in avocado orchards in KwaZulu-Natal Province, South Africa.

## 3.2 Materials and methods

### 3.2.1 Study site

All the trials were conducted from August to December 2018 (spring to early summer) in two commercial, pesticide-free avocado orchards ('Pinkerton' cultivar) at Conlink Trust, Wartburg (-29.453415 30.683398) and Baynesfield Estate, Richmond (-29.756873 30.314269), in

KwaZulu-Natal Province, South Africa. Richmond receives between 800 and 1500 mm of rainfall annually, mainly in spring and summer. The prevailing wind direction for Richmond is predominantly from east-north-east and north-east directions, with an average speed of 5 kph, with occasional south to north winds (CSIR 2017). The 10-year-old avocado orchard used for the study ('Pinkerton' on 'Dusa' rootstock) had trees of 3 m height, spaced at 7 m × 4 m, with a tree population of 357 trees/ha.

Vegetation in and around the Baynesfield avocado orchards included commercial *Eucalyptus* spp. (gum tree), *Pinus* spp. (pine tree), *Acacia mearnsii* De Wild (black wattle), *Aristida junciformis* Trin. & Rupr. (ngongoni three awn grass), *Cymbopogon plurinodis* (Stapf) Stapf ex Burtt Davy (narrow-leaved turpentine grass), *Cynodon dactylon* (L.) Pers. (couch grass), *Cyperus textilis* Thunb. (sedge), *Digitaria eriantha* Steud. (finger grass), *Hyparrhenia hirta* (L.) Stapf (common thatching grass), *Lantana camara* L. (lantana), *Senna didymobotyra* (Fresen.) Irwin & Barneby (peanut-butter bush), *Zantedeschia aethiopica* (L.) Spreng. (white arum lily), *Stenotaphrum secundatum* (Walter) Kuntze (buffalo grass), *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu grass), *Ipomoea purpurea* (L.) Roth (morning glory) and *Bidens pilosa* L. (blackjack).

The Wartburg climate is generally mild, warm and temperate with an annual mean precipitation of 905 mm. Precipitation is lowest in June, with an average of 14 mm and highest in January, averaging 142 mm. At 20.4 °C, February is the hottest month and June is the coldest at 12.3°C (https://en.climate-data.org/africa/south-africa/kwazulu-natal/wartburg-189711/). The traps were set up on 3 m tall, 11-year-old 'Pinkerton' on 'Duke 7' trees, spaced at 6 m × 6 m with a plant population of 278 trees/ha.

The Conlink avocado orchards are surrounded by commercial plantations of *Eucalyptus* spp. (gum tree), *Pinus* spp. (pine tree), *Saccharum officinarum* L. (sugarcane), *Citrus unshiu* Yu.Tanaka ex Swingle (satsuma), *Ipomoea purpurea* (morning glory) and *Bidens pilosa* (blackjack).

### 3.2.2 Preparation of coloured sticky traps

The traps consisted of nine different colours as treatments - red, orange, yellow, green, blue, purple, white and black, with clear (transparent) as the control. The sticky cards were prepared by laminating different colour Manila paper boards. Gladwrap® (shrinkwrap plastic) was wrapped around the laminated cards and FlyTac® (Insect Science®, Tzaneen, South Africa) adhesive glue was uniformly applied over the entire card surface. The FlyTac® on the card was replaced once every 2 weeks. Each trap consisted of a double-sided 8 × 12 cm card.

### 3.2.3 Experimental procedure

At each trial site, ten experimental trap units/trees were randomly subjected to the treatments (five at the border and five in the middle of the orchard). The treatment blocks were the border and interior regions of the orchard. Each tree served as a trap unit on which the nine different coloured sticky traps were deployed. Five trees were randomly selected per block, each with nine coloured sticky traps, giving 45 traps per block per sampling period. The different coloured traps were randomly placed 1 m apart around the tree canopy, suspended from flowering/early fruiting panicles. The trees were at least 15 m apart and traps were deployed at a height of 1.5 m - 2.0 m. The traps were suspended under the panicles because the panicles presented active growing tissue known to be attractive to *S. aurantii*. The colour trap order was random and trap positions were rotated at each collection period to minimise bias. Traps were deployed in 2018 during bloom right through to fruit set when the fruit were lemon size (70 mm diameter).

Sticky traps were collected after 14 days, wrapped in Gladwrap®, placed in clear plastic containers, transported to the University of KwaZulu-Natal laboratory and kept in a refrigerator (3.0 ± 0.2°C). The thrips species were identified under a stereo-microscope, using morphological keys (Hoddle *et al.* 2012). The numbers of each thrips species on each sampling date were recorded for each trap. The experimental design was a randomised complete block design with five replications, over two sites (Baynesfield and Conlink), with blocks (border and interior) and treatments (colour and period). The results were analysed using the Bartlett test for homogeneity, Kruskal-Wallis non-parametric test and pairwise Wilcoxon test (for *post hoc* treatment separation) in R Version 3.5.2.

## 3.3 Results

A total of 1689 *S. aurantii* individuals were caught on a total of 900 traps, comprising nine different colours during the study period. Yellow had the highest pooled mean number catch (3.93 ± 0.57) per trap whilst orange had the least (0.70 ± 0.10) (**Table 3.1**).

The trap catches were not normally distributed and the non-parametric Kruskal-Wallis chi-squared test was used for analysis. The count of thrips caught on yellow sticky traps was significantly greater than the other colours (Kruskal-Wallis $\chi^2 = 127.36$, df = 8, $P < 0.001$), followed by blue, white and clear. Whilst blue and white caught more thrips than the other remaining colour traps, this was not statistically different to the control (clear). Green, red, black, purple and orange traps caught less than the clear control and orange had the least catches (0.70 ± 0.11). At site level, yellow was the most attractive colour at both Baynesfield and

Conlink. However, at Conlink, although yellow was the most attractive colour, it was not statistically different to white and blue but different to the other colours (Kruskal-Wallis $\chi^2$ = 86.645, df = 8, $P < 0.001$).

**Table 3.1.** Pooled *S. aurantii* catches for the different coloured sticky traps.

| **Colour** | **No. of thrips** | ***N*** | **Mean ± *SE*** |
|---|---|---|---|
| White | 254 | 100 | $2.54 \pm 0.28^{b}$ |
| Orange | 70 | 100 | $0.70 \pm 0.11^{d,e}$ |
| Clear | 225 | 100 | $2.25 \pm 0.23^{b,c}$ |
| Black | 104 | 100 | $1.04 \pm 0.12^{d}$ |
| Green | 183 | 100 | $1.83 \pm 0.22^{c}$ |
| Purple | 98 | 100 | $0.98 \pm 0.14^{d,e}$ |
| Red | 108 | 100 | $1.08 \pm 0.15^{d,e}$ |
| Yellow | 393 | 100 | $3.93 \pm 0.40^{a}$ |
| Blue | 254 | 100 | $2.54 \pm 0.24^{b}$ |

**Note**. Treatments sharing a letter in superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$.

Significant differences were observed in trap catches between Baynesfield and Conlink (Kruskal-Wallis $\chi^2$ = 6.7948, df = 1, $P$ = 0.009142). Conlink Trust farm had significantly higher thrips catches (4.74 ± 0.60) than Baynesfield (3.12 ± 0.52) on yellow sticky cards (**Table 3.2**).

**Table 3.2.** Baynesfield and Conlink *S. aurantii* trap catches.

| **Site** | **No. of thrips** | ***N*** | **Mean ± *SE*** |
|---|---|---|---|
| Baynesfield | 156 | 50 | 3.12 ± 0.52 |
| Conlink | 237 | 50 | 4.74 ± 0.60 |
| Pooled | 393 | 100 | 3.93 ±0.57 |

Kruskal-Wallis $\chi^2$ = 6.7948, df = 1, $P$ = 0.009142

When data from both localities were pooled, border rows had slightly more *S. aurantii* thrips catches (4.00 ± 0.55) per yellow trap than the 3.86 ± 0.59 recorded for interior rows (**Table 3.3**). However, this difference was non-significant (Kruskal-Wallis $\chi^2$ = 0.18673, df = 1, $P$ = 0.6657).

At site level, with respect to border and interior areas, again no significant differences in *S. aurantii* catches were observed at Baynesfield (Kruskal-Wallis $\chi^2 = 0.061149$, df = 1, $P = 0.8047$) and Conlink (Kruskal-Wallis $\chi^2 = 0.34386$, df = 1, $P = 0.5576$).

**Table 3.3.** *Scirtothrips aurantii* trap catches (mean ± *SE*) using yellow sticky traps.

| Site | Block | No. of thrips | *N* | Mean ± *SE* |
|---|---|---|---|---|
| Pooled | Border | 200 | 50 | 4.00 ± 0.55 |
| | Interior | 193 | 50 | 3.86 ± 0.59 |
| Baynesfield | Border | 76 | 25 | 3.46 ± 0.69 |
| | Interior | 80 | 25 | 3.97 ± 0.79 |
| Conlink | Border | 124 | 25 | 4.96 ± 0.83 |
| | Interior | 113 | 25 | 4.52 ± 0.87 |

Kruskal-Wallis $\chi^2 = 0.18673$, df = 1, $P = 0.6657$

Pooled *S. aurantii* thrips catches declined significantly from 10.70 (± 0.70) at flowering and early fruit set in August 2018 to 2.30 (± 0.54) in early December 2018 (Kruskal-Wallis $\chi^2 = 65.831$, df = 4, $P < 0.001$). Thereafter, the number of thrips caught in yellow sticky traps began to increase in December. When pooled, this increase was not significant. However, there were site differences. At Baynesfield, the number of thrips caught continued to decline (Kruskal-Wallis $\chi^2 = 41.327$, df = 4, $P < 0.001$), whilst at Conlink (Kruskal-Wallis $\chi^2 = 35.762$, df = 4, $P < 0.001$), the number of thrips began to increase significantly (**Table 3.4**).

**Table 3.4.** Effect of period on *S. aurantii* trap catches for the period August to December 2018.

| Period | Baynesfield | | | Conlink | | | Pooled | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of thrips | *N* | Mean | No. of thrips | *N* | Mean | No. of thrips | *N* | Mean |
| August | 96 | 10 | $9.60 \pm 0.86^{a}$ | 118 | 10 | $11.80 \pm 1.04^{a}$ | 214 | 20 | $10.70 \pm 0.70^{a}$ |
| September | 35 | 10 | $3.50 \pm 0.27^{b}$ | 44 | 10 | $4.4 \pm 0.31^{b}$ | 79 | 20 | $3.95 \pm 0.22^{b}$ |
| October | 13 | 10 | $1.3 \pm 0.21^{c}$ | 16 | 10 | $1.6 \pm 0.52^{c}$ | 29 | 20 | $1.45 \pm 0.28^{c}$ |
| November | 8 | 10 | $0.8 \pm 0.20^{c,d}$ | 17 | 10 | $1.7 \pm 0.40^{c}$ | 25 | 20 | $1.25 \pm 0.24^{c}$ |
| December | 4 | 10 | $0.4 \pm 0.16^{d}$ | 42 | 10 | $4.2 \pm 0.65^{b}$ | 46 | 20 | $2.30 \pm 0.54^{c}$ |

**Note**. Periods sharing a letter in superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$.

### 3.3.1 Non-target insect captures

A total of 609 non-target arthropods were caught in the traps including *Frankliniella occidentalis* (Pergande), *Thrips gowdeyi* (Bagnall), *Thrips pusillus* Bagnall, *Haplothrips gowdeyi* (Franklin), *Megalurothrips sjostedti* (Trybom) and *Haplothrips bedfordi* Jacot-Guillarmod. Other arthropods captured on sticky cards included *Orius* spp. (pirate bugs), *Oxytate* sp. (Arachnida) and *Apis mellifera* (bees) (**Table 3.5**).

**Table 3.5.** Non-target arthropods trapped using yellow sticky traps.

| **Arthropod species** | **No.** |
|---|---|
| *Frankliniella occidentalis* | 209 |
| *Thrips gowdeyi* | 91 |
| *Haplothrips bedfordi* | 54 |
| *Thrips pusillus* | 154 |
| *Megalurothrips sjostedti* | 25 |
| *Haplothrips gowdeyi* | 54 |
| *Oxytate* spp.(spider) | 11 |
| *Apis mellifera* | 8 |
| *Orius* spp. | 3 |

## 3.4 Discussion

The South African citrus thrips, *S. aurantii*, was the only thrips species of economic importance in fruit tree crops that was captured in the traps. This is noteworthy, considering that in the 1990s Steyn *et al.* (1993) reported two thrips species, the greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouche), and the redbanded thrips, *Selenothrips rubrocinctus* (Giard), as the only pest thrips species that were attacking avocado, and causing a loss of 2.1 % of the fruits (Dennill 1992). A few years prior to this, De Villiers & Van den Berg (1987) reported that avocado orchards were relatively free from serious insect pests owing to good control by natural enemies. Thirty years later, *S. aurantii* was the only thrips species of economic importance captured in traps. The sporadic nature of *H. haemorrhoidalis* and *S. rubrocinctus* could possibly explain the absence of these two species in traps, i.e., that they were not present in the study areas, when the study was conducted. Dennill & Erasmus (1992) studied *H. haemorrhoidalis* and *S. rubrocinctus* thrips in mature avocado fruit (close to harvest) in Hazyview, Mpumalanga Province, South Africa. The present study looked at young fruits in

KwaZulu-Natal Province, South Africa. The different geographical areas and maturity stages of the fruits may act to modify and regulate the thrips species present during the study period. However, one of the problems with this opinion is that both *H. haemorrhoidalis* and *S. rubrocinctus* are known to be found throughout the season, attacking both young and mature fruits, and laying eggs in fruit or leaf surfaces (Steyn *et al.* 1993). From fruit incubation of field infested avocado fruits, these two species were also not collected in an earlier study conducted in KwaZulu-Natal by Bara & Laing (2019).

Another possibility that may need to be further tested is that *S. aurantii* may have displaced these two thrips species, and has established itself as the dominant thrips species of avocado in South Africa. If this is the case, then this would be a definite change in the thrips pest species composition. It is probable that *S. aurantii* is highly competitive and this, coupled with its polyphagous nature, may have enabled it to outcompete other species and to colonise new host plants. Several studies have shown that interspecific competition is high among insects and that the most severe outcome of this interspecific competition is competitive displacement (Reitz & Trumble 2002). The competitive ability of thrips species is well demonstrated in *Frankliniella occidentalis* (Pergande), a thrips species native to western North America but which has become a worldwide invasive pest (Reitz 2009). van Rijn *et al.* (1995) reported that *F. occidentalis* had replaced *Thrips tabaci* Lindeman in European greenhouses, as the major thrips pest, and Northfield *et al.* (2011) demonstrated the competitive superiority of *F. occidentalis* to *Frankliniella bispinosa* (Morgan), a common thrips species of southern Florida, USA. However, whilst interspecific competition has been reported to be a major factor in displacements, Zhao *et al.* (2017) noted that other ecological mechanisms may also contribute to displacements. *Scirtothrips aurantii* is already a major pest in other fruit tree crops such citrus (Gilbert & Samways 2018) and mango (Grové *et al.* 2000), crops that are often grown in proximity with avocado in South Africa.

The study determined yellow to be the most attractive colour to *S. aurantii* in avocado orchards. Yellow coloured sticky traps have also been reported to be effective in South Africa for monitoring *S. aurantii* in citrus (Samways 1986) and in mango (Grové *et al.* 2000). In California, a related species, *Scirtothrips perseae* Nakahara, is also known to be attracted to yellow sticky traps in avocado (Hoddle *et al.* 2002).

Conlink Trust farm had more thrips catches than Baynesfield during the study period. This also supports an earlier study, which found that Conlink had a higher thrips infestation than Baynesfield (Bara & Laing 2019). It is possible than Conlink provides greater food and

refuge sites on several plant species (including weed species) for *S. aurantii*, which is a polyphagous species.

Border rows did not have a significant effect on trap captures of *S. aurantii*. This is in spite of the winds blowing predominantly from the northwesterly direction at the trial sites during the course of this study. Whilst wind-aided dispersal has been reported in some Thysanoptera such as *Frankliniella schultzei* (Mound 2003), it was not demonstrated in this study. Lewis (1997) noted that thrips flying activity and directionality of flight is strongly influenced by atmospheric conditions and that some species fly only when conditions permit controlled flight. However, in citrus, the orchard is the main reservoir for the insect in comparison to the surrounding natural vegetation (Gilbert 1990). The new vegetative growth in citrus is used as a food source by citrus thrips that would have stayed in the orchards. (Bedford 1943; Gilbert & Samways 2018).

Overall, *S. aurantii* numbers were high during flowering, declined post-bloom and then increased in summer. The critical flowering/fruit set period overlaps with the spring vegetative flush where young leaves are actively growing (Wolstenholme & Sheard 2012). During spring, *S. aurantii* individuals are attracted to the young foliage. Feeding and reproducing then occurred on the developing fruit. Studies in cotton also revealed that thrips catches are highest during the vegetative stage of the crop (Prema *et al.* 2018). In avocado, the spring flush in South Africa typically ends in August (Bekker *et al.* 2014), at about the same time that high levels of thrips infestation in fruitlets were reported (Bara & Laing 2019). As the fruitlets harden, the thrips disperse in search of young, actively growing vegetation, hence the decline in the numbers of thrips captured in the sticky traps. The increase in thrips numbers in December at Conlink, coincided with the onset of the summer leaf flush and may explain the sudden increase in thrips populations.

Dispersal is of particular adaptive significance for insects that exploit ephemeral resources with a low carrying capacity (Travis & Dytham 1999) such as reproductive structures of plants (Thompson 1983). Thrips are known to increase their populations in relation to the availability of food (Silva & Del-Claro 2010) and disperse when the abundance of food is low, as is the case with many other insects such as butterflies (Matter & Roland 2002).

To an untrained eye, *Thrips pusillus* is morphologically similar to *S. aurantii* and as such, it is most likely to be confused with *S. aurantii*. *Thrips pusillus* was readily captured on sticky traps during flowering but it is a flower thrips and is not considered to be of economic importance to avocado. Avocado growers wishing to monitor thrips populations using sticky

traps would have to take care to avoid confusing these two species as incorrectly scoring of *S. pusillus* as *S. aurantii* would erroneously increase the apparent importance of non-avocado pests. A study by Gilbert (1990) in citrus also showed the high likelihood of confusing *T. pusillus* with *S. aurantii*. *Scirtothrips aurantii* is a minute insect and magnifying equipment will be required, together with training in identification, but thereafter, it should be possible for on-site, grower-led monitoring. Where challenges are encountered, extension support services could provide confirmatory identification.

In this study, yellow sticky traps were found to be the most attractive. To estimate the severity of thrips infestation, yellow sticky traps can be set up in early spring, within the avocado orchard prior to, and during, blossoming, to give advance warning of the level of infestation that can be expected, as is the case with citrus (Gilbert 1990). It is also important to note that the critical period for thrips damage is very short, typically only a few weeks after fruit set and that yellow traps only capture adults (the larval stages cause more damage due to their greater numbers) (Bara & Laing 2019), therefore control measures should be implementation-ready at the beginning of spring.

## 3.5 Conclusion

The study confirmed that yellow sticky traps are attractive to *S. aurantii* in avocado and that the incidence of this species is not restricted to border margins or the interior but is randomly distributed in avocado orchards. *Scirtothrips aurantii* numbers are high during the spring flush and in particular, during the economically and ecologically sensitive fruit-set period. This therefore represents the period when control measures should be implemented if necessary, to prevent scarring damage on the young developing fruit.

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# Chapter 4: An investigation into the role played by endophytic *Cladosporium* spp., wind abrasion and thrips (Thysanoptera: Thripidae) in scarring avocado fruit

**[4]Abstract**

Avocado fruit are an important subtropical fruit grown primarily for export in South Africa, earning the country ZAR 1.75 billion annually in export revenue. The need to produce export quality fruit is therefore commercially important. The export market demands that fruit be aesthetically attractive. However, several factors act to scar avocado fruit and to reduce export capacity. The study aimed to confirm the causal agents responsible for the economic scarring by investigating the damage and currently ascribed causal agents, and to provide growers with recommendations on how to reduce economic scarring. Fruits were subjected to abrasion and fungal inoculation treatments to determine fruit response and the results compared to typical field damaged fruit signs. *Sphaceloma perseae* Jenkins was suspected but it was not found in this study. Endophytic *Cladosporium* spp. were isolated from scarred avocado fruit; however, their pathogenicity was not proven when inoculated directly on bruised and intact fruit. Wind induced abrasion and thrips (Thysanoptera: Thripidae) were confirmed to be the main agents responsible for scarring avocado fruit in South Africa. Wind induced abrasion and damage by thrips, through their feeding on avocado fruit result in corky tissue development (scarring), making the fruit unsuitable for export.

**Keywords:** avocado, endophytic *Cladosporium*, fruit scarring, thrips, wind abrasion.

[4] *This chapter has been submitted for publication and is currently under review*

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## 4.1 Introduction

Avocado (*Persea americana* Miller) is an economically important fruit in South Africa, contributing ZAR 1.75 billion in export revenue to the gross domestic product of the country in 2019 (http://www.worldstopexports.com/avocados-exports-by-country/). FAOSTAT (2020) reported that in 2018, 6.4 million tonnes of avocado fruit were produced globally, with Mexico being the largest producer in the world, followed by the Dominican Republic, Indonesia, and Peru. In that year, Mexico produced over 2 million tonnes of avocado fruits, approximately 34 % of the world's production. On average, 73 % of the world's avocado fruit are produced in the Americas, 13 % in Africa, 12 % in Asia and 2 % in Europe and in the South Pacific. South Africa is Africa's second largest avocado exporter after Kenya, and is currently the 12$^{th}$ largest producer of avocado fruit in the world. Other African countries that produce avocado fruit include Zimbabwe, Cameroon, Rwanda, Tanzania, and the Democratic Republic of Congo. South Africa is among the top ten exporters of avocado fruit globally (FAOSTAT 2020) and has an export-oriented industry, primarily targeting the European market (Netherlands, UK, France and Spain). In 2016, South Africa exported 57,867 tonnes of avocado fruit (1.7 % of international market share) with a total value of ZAR 1,061 million (DAFF 2017).

According to Donkin (2019), South Africa produced 170,000 tonnes of avocado fruits in 2018, of which 51 % (86,000 tonnes), were exported, 10-15 % were processed into oil and purée with the rest being sold at fresh produce markets. The value of the export fruit was estimated at US$116.7 million (ZAR 1.75 billion), approximately 2.1 % of total income from exported avocado fruit globally (http://www.worldstopexports.com/avocados-exports-by-country/).

To remain competitive in the global market, the South African avocado industry needs to consistently produce fruits of high quality. Nelson (2014) identified poor fruit quality as the biggest challenge to export. Avocado production costs are high, with several pests and diseases acting as major limiting factors to production and export. Globally, diseases such as phytophthora root rot caused by *Phytophthora cinnamomi* Rands (Hardham & Blackman 2018), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Sacc.), avocado scab (*Sphaceloma perseae* Jenkins) and cercospora spot (*Pseudocercospora purpurea* (Cooke) Deighton) (Darvas & Kotze 1987) cause major economic losses. Insect pests such as fruit flies (Diptera: Tephritidae), thrips (Thysanoptera: Thripidae), false codling moth (*Thaumatotibia leucotreta* Meyrick) (Erichsen & Schoeman 1992), and the heart shaped scale (*Protopulvinaria pyriformis* (Cockerell)) (Du Toit *et al.* 1991) are among some of the pests which commonly reduce the quality of avocado fruit in South Africa.

With a continuously expanding market and changes in the production and market systems, thrips pose a constant threat to avocado production. Bara & Laing (2019) established that *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) (South African citrus thrips) is a significant threat to the production of avocado fruit in South Africa. *Scirtothrips aurantii* is an established pest of citrus (Gilbert & Samways 2018), macadamia (Rafter & Walter 2012) and mango (Grové *et al.* 2000). Female *S. aurantii* individuals make an incision in soft green fruit tissue, shoots and tender leaves using their serrated ovipositor and lay minute (less than 0.2 mm), bean-shaped eggs (Bedford 1943). The egg stage lasts about 6.0 days and hatch into yellow to orange, cigar-shaped larvae. Two non-feeding pupal stages, the prepupa and the pupa follow the two actively feeding larval stages (I & II). The average duration of the larval stages is about 7.6 days whilst that of the pupal stages is 4.0 days, depending on the temperature (Bedford 1943). Adult *S. aurantii* thrips are yellowish-orange and reproduce mainly through haplodiploidy where diploid females result from fertilized eggs, and haploid males develop parthenogenetically from unfertilized eggs (Gilbert 1992). All stages are less than 1 mm in length and are barely visible to the unaided eye. In citrus, where the thrips species has been extensively studied, the duration of the life cycle is 18.4 and 44.0 days in summer and winter respectively, with 9.4 generations being possible every year (Bedford 1943). As Gilbert & Bedford (1998) noted, owing to the mild climatic conditions of South Africa's citrus-growing areas, *S. aurantii* does not undergo diapause therefore, adults and larvae may be present throughout the year.

Bara & Laing (2019) determined that young avocado fruit were especially vulnerable to *S. aurantii* feeding damage. Hardening of young foliage from vegetative growth flushes during early fruit development results in the movement of adult female thrips from the foliage to feed and oviposit into young fruit. Feeding by the emerged larvae and adults results in damage to the skin of developing fruit as noted in avocado by *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) (Hoddle *et al.* 2002a). As Stevens *et al.* (1999) noted, minimal thrips scarring can be tolerated but damage in excess of 2 $cm^2$ area will result in the fruit being unacceptable for premium export grade (downgraded to local market or processing grade).

Damage to avocado in the USA by a related thrips species *S. perseae* has been estimated to cost that industry US$8.65 million annually (Hoddle *et al.* 2003). Feeding by low densities of *S. perseae*, approximately three larvae on young avocado fruit (< 5 cm in length), was determined to scar the entire fruit surface, with the localized feeding starting as discrete brown scars that elongate as fruit matures (Hoddle *et al.* 2002b).

Avocado scab (*Sphaceloma perseae* Jenkins) is a widespread, fungal disease of avocado in the humid tropics and subtropics that causes severe losses from premature abscission of infected fruit and downgrading of cosmetically scarred fruit. In Michoacán State, Mexico, crop losses of up to 53 % have been reported in susceptible cultivars (Vidales 1996). Fruit scarring due to *S. perseae* initially appears as corky, raised, oval or irregular shaped brown to purplish-brown spots. As the disease progresses, spots enlarge and coalesce to form large rough areas over the fruit surface. Avocado fruits are most susceptible to the disease during the early growth phases with cool moist weather exacerbating the disease progression. Disease expression is most severe under heavy rains and foggy conditions (Manicom 2001).

*Sphaceloma perseae* is a necrotrophic fungus that produces host-specific phytotoxic metabolites (Stergiopoulos *et al.* 2013) that trigger a hypersensitive response in the host, and which allow the fungus to feed on the damaged tissue. Marroquín-Pimentel (1999) demonstrated that there is a strong positive correlation between thrips feeding and avocado scab; the greater the thrips pressure, the greater the scab damage. Jenkins (1934) noted that avocado scab on a month-old avocado fruitlet manifested itself as the rupturing of the epidermis and production of hyaline conidia and conidiophores that form a dense purplish-brown velvety covering. The velvety layer is gradually lost due to weathering, leaving behind corky tissue. Pernezny & Marlatt (2007) reported that fruit usually become resistant to infection 1-month after fruit set.

Russet fruit damage is a type of fruit blemish typically associated with mechanical abrasion of young, tender fruit by foliage and insects. Carapace skin, a fruit disorder first described by Horne (1929), occurs when fruits mechanically rub against an abrasive object such as leaves branches and other fruits. Chronic wind damage early in the growth and development of young tender fruit causes the formation of rigid scars or calluses that repeatedly rip and heal as the fruit matures. The scars may grow to cover significant portions of the fruits, resulting in the ‘alligator skin’. As Dreistadt (2007) noted, the damage is cosmetic with the flesh underneath the scar undamaged but the external appearance of the fruit may result in downgrading of the fruit at packhouses.

The objectives of this study were to confirm damage signs exhibited by the abrasion damage (simulated thrips and wind) and to identify pathogenic agents associated with scarring on avocado fruits in KwaZulu-Natal Province of South Africa.

## 4.2 Materials and methods

In June 2019, fungal isolates obtained from scar damaged 'Carmen®-Hass' and 'Pinkerton' fruit samples were collected from Everdon Estate (-29.452322, 30.266425), Howick; and at Baynesfield Estate (- 29.756873, 30.314269), Richmond respectively. The field pathogenicity studies were carried out from August to November 2019 at Conlink Trust Farm (-29.453415, 30.683398), Wartburg and Baynesfield Estate (-29.756873, 30.314269), Richmond.

Conlink Farm has generally mild, warm and temperate climate and is situated 27 km northeast of Pietermaritzburg, in Wartburg, KwaZulu-Natal Province (KZN), South Africa. It has an annual mean precipitation of 905 mm, with June recording the least precipitation with an average of 14 mm, and January recording the highest precipitation, averaging 142 mm. February is the hottest month and June is the coldest at 20.4 and 12.3°C, respectively (https://en.climate-data.org/africa/south-africa/kwazulu-natal/wartburg-189711/).

Conlink Farm is surrounded by commercial plantations of *Saccharum officinarum* L. (sugarcane), *Pinus* spp. (pine tree) and *Eucalyptus* spp. (gum tree) and grows several different avocado cultivars such as 'Pinkerton', 'Hass', 'Fuerte', 'Lamb Hass', and 'Rinton'. *Citrus unshiu* Yu.Tanaka ex Swingle (satsuma), *Bidens pilosa* L. (blackjack) and *Ipomoea purpurea* (L.) Roth (morning glory) are also present on the farm.

Richmond is about 20 km southwest of Pietermaritzburg and receives on average between 800 and 1500 mm of rainfall annually, mainly in spring and summer. The prevailing wind direction is predominantly from east-north-east and north-east directions, with an average speed of 1.39 $ms^{-1}$, with occasional south to north winds (CSIR 2017). Surrounding Baynesfield Estate avocado orchards are commercial plantations of *Eucalyptus* spp. (gum tree) and *Pinus* spp. (pine tree). Other plant species in the area include *Aristida junciformis* Trin. & Rupr. (ngongoni three awn grass), *Acacia mearnsii* De Wild (black wattle), *Cymbopogon plurinodis* (Stapf) Stapf ex Burtt Davy (narrow-leaved turpentine grass), *Cyperus textilis* Thunb. (sedge), *Cynodon dactylon* (L.) Pers. (couch grass), *Digitaria eriantha* Steud. (finger grass), *Hyparrhenia hirta* (L.) Stapf (common thatching grass), *Stenotaphrum secundatum* (Walter) Kuntze (buffalo grass), *Senna didymobotyra* (Fresen.) Irwin & Barneby (peanut-butter bush), *Zantedeschia aethiopica* (L.) Spreng. (white arum lily), *Lantana camara* L. (lantana), *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu grass), *Bidens pilosa* L. (blackjack) and *Ipomoea purpurea* (L.) Roth (morning glory).

Everdon Estate is located in the cool, subtropical KwaZulu-Natal midlands and experiences cool, mesic conditions. Annually, it receives an average of 1052 mm and has an

average temperature of 16.4°C (https://en.climate-data.org/africa/south-africa/kwazulu-natal/howick-27052/). Several avocado cultivars are grown on the Estate, including 'Carmen®-Hass', 'Hass', and 'Fuerte', and the avocado orchards are surrounded by commercial *Eucalyptus* spp. (gum tree) and *Pinus* spp. (pine tree). The estate overlooks the Karkloof Valley, a nature reserve that is host to an exceptionally wide range of indigenous flora.

### 4.2.1 Isolations and cultures

Young fruitlets of 'Carmen®-Hass' and mature (ready to harvest) fruit of 'Pinkerton' showing scars "typical" of avocado scab were collected from Everdon Estate and Baynesfield Estate. Ten "symptomatic" fruit samples were surface sterilized using 70 % ethanol for 1 min, rinsed in 1 % sodium hypochlorite solution for 4 min and rinsed in sterile distilled water five times (Sun *et al.* 2017). Small pieces (5 to 10 $mm^2$) of disinfected tissue were cut from the samples using a sterile scalpel and then transferred onto potato dextrose agar (PDA; Neogen®, USA) and incubated upside down at 28°C, in the dark until fungal hyphae emerged from the plant tissue (Schuck *et al.* 2014; Timmer *et al.* 1996). The form, morphology of conidiophores and conidia, as well as the colour of the colonies on PDA were used as the initial criteria for identifying the colonies. The cultures were grown on PDA plates and transferred to freshly prepared PDA media every three weeks.

### 4.2.2 DNA barcoding and sequencing

The cultured specimens were morphologically identified according to the macroscopic and microscopic criteria (Bensch *et al.* 2012). However, because of species diversities and similarities, molecular techniques were employed to positively confirm the identity of the species. Three fruit samples showing "typical" avocado scab signs (**Figure 4.1**) collected from Everdon Estate and Baynesfield Estate were DNA fingerprinted using ITS sequencing to confirm the identity of the fungal isolates at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa, using methods described by White *et al.* (1990). A Quick-DNA™ Fungal/Bacterial miniprep kit (Zymo Research, catalogue No. D6005) was used to collect genomic DNA from pure cultures isolated from the avocado fruit samples. The primers listed in **Table 4.1** were used to amplify the ITS region using OneTaq® QuickLoad® 2X Master Mix (NEB, catalogue No. M0486). The PCR products were run on a gel and gel extracted using Zymocleam™ Gel DNA Recovery Kit (Zymo Research, catalogue no. D4001). Forward and reverse direction sequencing was done on the PCR products using Nimagen, Brilliant Dye™ Terminator Cycle sequencing kit V3.1 BRD3-100/1000 and purified using ZR-96 DNA Sequencing Clean-up Kit™ (Zymo Research, catalogue no. D4050). The purified fragments

were analysed on the ABI 3500xl Genetic Analyser (Applied Biosystems, ThermoFisher Scientific) and the CLC Bio Main Workbench v7.6 was used to analyse the .ab1 files. The sequences were then compared to databased sequences in NCBI's GenBank using the BLASTn search tool (http://www.ncbi.nlm.nih.gov/BLAST/).


**Figure 4.1**. Fruit showing scar tissue damage; a) and b) 'Carmen®-Hass' 2 week old fruitlets; c) 'Pinkerton' mature, ready-to-harvest fruit.

**Table 4.1.** ITS primer sequences (White *et al.* 1990).

| Name of Primer | Target | Sequence (5' to 3') |
|---|---|---|
| ITS1 | Small Sub-Unit | TCCGTAGGTGAACCTGCGG |
| ITS4 | Large Sub-Unit | TCCTCCGCTTATTGATATGC |

### 4.2.3 Pathogenicity tests

A conidial spore suspension for inoculation was obtained from the 14-day-old cultures by adding 10 mL of distilled water to each plate, scraping off the fungal mycelium using a flame sterilized scalpel blade and filtering the suspension through a double layer of nylon gauze. The desired spore concentration of $1 \times 10^5$ spores $mL^{-1}$ (Nam *et al.* 2015) was determined using a Neubauer chamber.

### 4.2.4 Field evaluation of scar tissue incidence and severity

On the 20th of August 2019, field evaluation trials were set up where a total of 1400 marble sized (10-20 mm long), 2 - 3 week old 'Pinkerton' fruitlets were randomly selected and marked using 2 mm cotton twine at Baynesfield Estate and Conlink Farm. At each of these sites, 14

fruiting trees were randomly selected, from which 50 marble sized fruitlets were randomly selected per tree. However, to guard against loss of data due to fruit abscission, 20 fruitlets per treatment down from the original 50 fruitlets were assessed per assessment effort.

To determine the role played by wind, thrips and the isolated pathogen, 7 treatments were applied to 2-3 week old randomly selected fruitlets. Abrasion damage by thrips and wind damage was simulated by lightly sanding fruitlets using a fine sandpaper (150 – 180 grit). The fruitlets were subjected to the following treatments:1) Inoculated fruitlets (sprayed with conidial suspension); 2) Sandpapered fruitlets (inoculated); 3) Sandpapered fruitlets (uninoculated); 4) Uninoculated control (plastic covered); 5) Filter bag covered fruitlets; 6) No filter bag (Filter bag control) and 7) Sandpapered fruitlets in filter bags (**Figure 4.2**).


**Figure 4.2.** Application of field treatments: a) plastic bag covered treatments; b) tea filter bag covered treatments c) treatments without filter or plastic bags.

For field inoculation, a conidial suspension of $1 \times 10^5$ spores $mL^{-1}$ (Nam *et al.* 2015) was applied to run-off on 2-3 week old 'Pinkerton' fruitlets using a hand held atomizer. At 72 hours after inoculation, scar tissue incidence was evaluated using the presence or absence of visible scars and severity was visually rated from 0-100 %. This was done for all the treatments for a further 10 weeks. Economic damage was measured as the percentage of fruit that had ≥10 % of their surfaces scarred. Percentages below 10 % were not considered economically damaging (Phillips *et al.* 1995). Scarring damage <10 % was given a rating of 1 and ≥10 % was rated 2. To eliminate biases in assessing scarring damage, a consistent team scored the fruit.

### 4.2.5 Statistical analysis

The trial was set out as a randomized complete block design with sites as blocks, 7 treatments and 700 experimental units. Scoring data were subjected to the Shapiro-Wilk normality test and analysed using non-parametric Kruskal-Wallis rank sum test and pairwise comparisons were done using Wilcoxon rank sum test in R (v. 3.6.1., R Foundation for Statistical Computing, Vienna, Austria).

## 4.3 Results

### 4.3.1 Isolation and identification

*In vitro* assessment of the colonies revealed *Cladosporium spp.,* which were olive green on PDA with diffuse aerial mycelia (**Figure 4.3**). However due to the complexities arising from variations in conidiophore and conidia size, molecular analysis was used to positively confirm the identity of the fungi. The ITS1 and ITS4 PCR sequence data was matched against Genbank reference library using NCBI's BLAST and tested positive for *Cladosporium* spp. (*C. cladosporioides* (Fresen.) G.A. de Vries, *C. westerdijkieae* Bensch & Samson and *C. perangustum* Bensch, Crous & U. Braun).

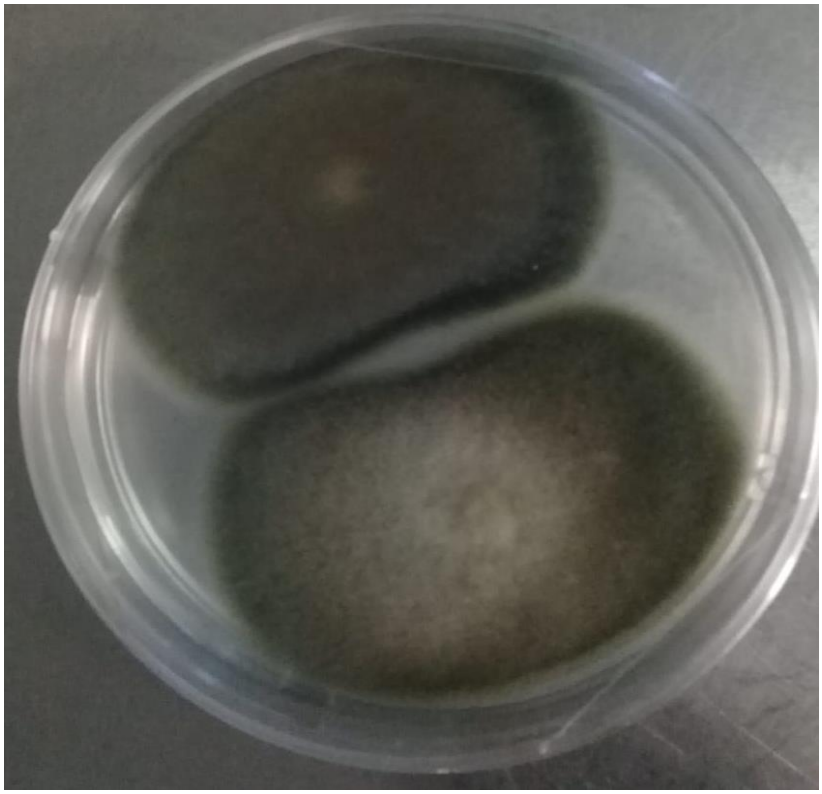

**Figure 4.3.** *Cladosporium* spp. on PDA, cultured from fruitlets.

After the 3rd and 8th day of field inoculations, none of the fruitlets treated with *Cladosporium* spp. showed any scar damage similar to that observed on naturally scarred avocado fruits. The experiment was set up on the 20th of August, with observation running to the 8th of November 2019 (11-weeks) at both sites. For the entire duration of the study, no fruit lesion, spot or necrotic symptoms were observed on any of the *Cladosporium* (only) inoculated fruitlets.

The damage incidence was noted as presence/absence of scar tissue on fruitlets directly attributable to the treatment, whereas the damage score was the rated appearance of scar

damage (1, No damage; 2 Damage ≥ 10 %). Damage between 0 and 10 % is not of economic significance. The damage data were subjected to the Shapiro-Wilk normality test and found not to be normal ($P < 0.05$). The damage scores were subsequently analysed using the non-parametric Kruskal-Wallis rank sum test. The damage scores for the two sites, Baynesfield Estate and Conlink Farm were not significantly different (Kruskal-Wallis $\chi^2 = 0.014$, df = 1, $P > 0.05$) and therefore the damage scores were pooled. Fruitlets sprayed with the conidial suspension (Treatment 1) and the uninoculated control (Treatment 4) did not exhibit scar damage. Filter bag (Treatment 5) and the no-filter bag covered control (Treatment 6) showed more fruit scarring. However, the damage scores of these treatments were not statistically different from each other. The sandpapered fruitlets inoculated and uninoculated (Treatment 2 and 3) as well as the sandpapered fruitlets in the filter bags (Treatment 7) showed similar levels of scar damage (**Figure 4.4**).

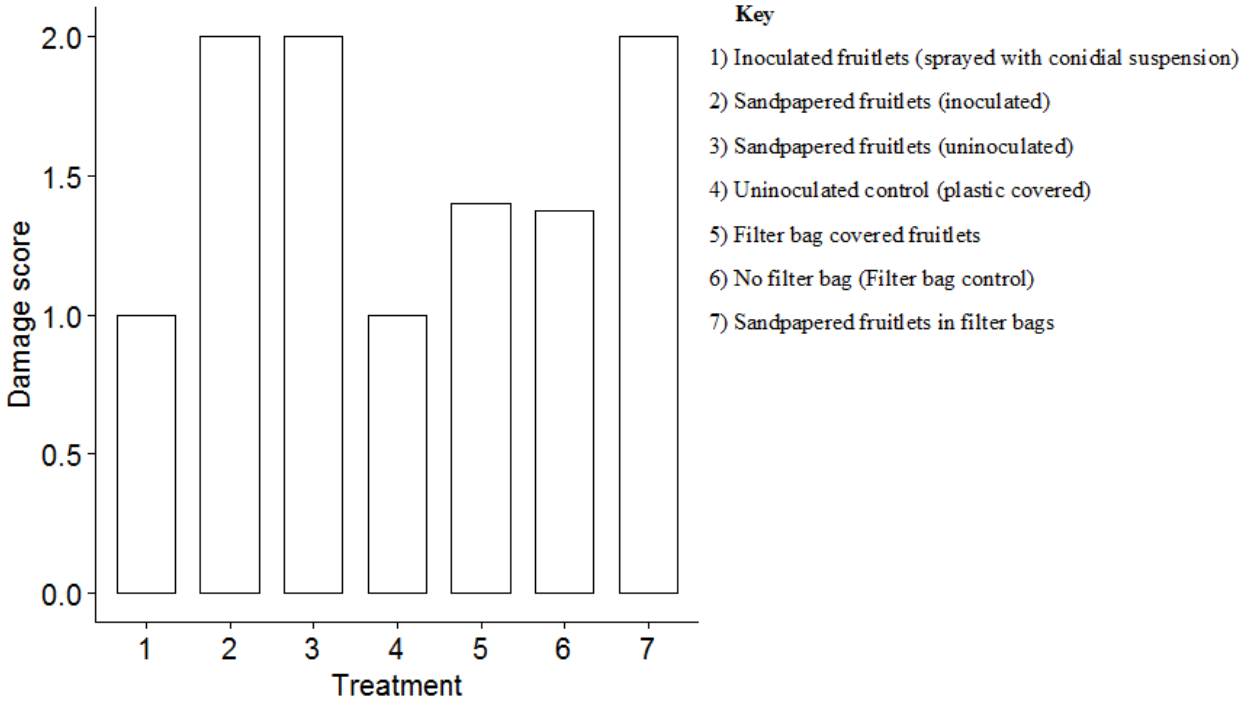


**Figure 4.4.** Barplot of the pooled damage scores: 1; No damage 2; Scarred.

The progress of scar development for up-to 11 weeks after the fruits were physically scarred is depicted in **Figure 4.5**. Avocado scar tissue development induced by sanding the avocado fruitlets using a fine sandpaper produced scar tissue comparable to that which occurred naturally, under field conditions (**Figure 4.6**). The damage score of the tea filter bag

covered fruitlets was not significantly different from that of the fruitlets in the filter bag control ($P > 0.05$).

**Figure 4.5.** Progress of scar tissue development from abrasion treatment: a) at the beginning of trial; b) 1 week; c) 3 weeks; and d) 11 weeks after physical abrasion (sanding).

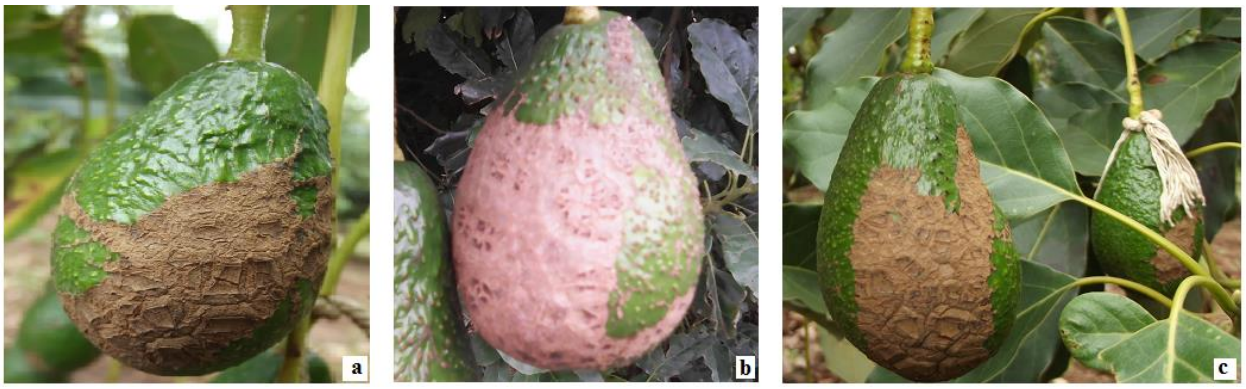

**Figure 4.6.** Comparison between scar tissue induced by artificial scarring using sandpaper, 11-weeks after sanding (a) and c); and that produced naturally under field conditions on mature ready to harvest fruit b).

## 4.4 Discussion

Molecular and morphological identification of 'diseased' samples revealed the presence of *C. cladosporioides* (Fresen.) G.A. de Vries, *C. westerdijkieae* Bensch & Samson and *C. perangustum* Bensch, Crous & U. Braun. However, no pathogenicity on avocado fruitlets was demonstrated. Previous studies carried out on 'Fuerte' in South Africa have found *Cladosporium* spp., *Alternaria* sp., *Fusarium oxysporum* Schlecht. Emend. Snyder & Hansen, *Pithomyces graminicola* R.Y. Roy & B. Ra and *Colletotrichum* sp. to be the main fungal genera isolated from avocado flowers (Thomas *et al.* 1994; Smith & Korsten 1996; Eicker *et al.* 1998). Riesen (1985) noted that *Cladosporium* spp. are good nutrient competitors and are well noted for their ability to utilize the host's resources.

The genus *Cladosporium* is a member of the largest group of dematiaceous hyphomycetes belonging to the order Capnodiales in the class Dothideomycetes (Yew *et al.* 2016), and is commonly encountered on all kinds of organic matter. *Cladosporium* spp. were

readily collected from surface sterilized avocado fruits in this study, which supported previous work that showed that *Cladosporium* spp. were fungal endophytes in avocado (Shetty *et al.* 2016; Hakizimana *et al.* 2011). Endophytic fungi live asymptomatically within intra- and intercellular spaces of plant tissues (Hyde & Soytong 2008) and their effect on the plants is wide ranging from temporary residents, mutualists, latent saprotrophs, and in some cases, latent pathogens (Hardoim *et al.* 2015; Zeilinger *et al.* 2015).

Wang *et al.* (2013) reported that *C. cladosporioides* is both a saprophyte and a pathogen on various plant hosts, and is known to cause raceme blight in macadamia nuts (Van den Berg *et al.* 2008), papaya scab (Chen *et al.* 2009), sooty mold in persimmon (Kwon & Park 2003), blossom blight in strawberry (Nam *et al.* 2015), grape (Briceño & Latorre 2008), mandarin (Tashiro *et al.* 2013), and wheat (Perelló *et al.* 2003). Thomas *et al.* (1994) showed that the application of *C. cladosporioides* spores on flowering avocado panicles resulted in greater abscission of flowers than in uninoculated controls. However, Smith & Korsten (1996) reported that although *Cladosporium* spp. were frequently isolated from avocado flowers, it is not known to be pathogenic on avocado fruit, a view supported by this study. In the present study, *Cladosporium* spp. were sprayed on healthy fruitlets and infection was not demonstrated. It is highly probable, that the spores were unable to cause infection in healthy avocado fruit tissue. It appears that the fungus acts as a saprophyte, feeding and living off damaged and decaying plant tissues.

Studies on grapevines by Nerva *et al.* (2019) revealed that, although *Cladosporium* spp. were isolated from asymptomatic plants, it occurred at a higher frequency in symptomatic grapevine plants, leading to the speculation that endophytic *Cladosporium* spp. can turn from saprophytes to being pathogens, depending on the host plant's response to different biotic or abiotic stresses, a view also shared by Alvarez-Loayza *et al.* (2011).

Bunchy and malformed top of *Carica papaya* L. (papaya cv. Eksotika) has been reported to be caused by the interaction of *Thrips parvispinus* (Karny) and *Cladosporium oxysporum* Berk. & M. A. Curtis. *Thrips parvispinus,* through their feeding (scraping and sucking) on young developing leaves, provided the infection site (the damaged tissues) for invasion by the normally saprophytic fungus *C. oxysporum* (Lim 1989). In this study, simulated abrasion followed by the application of *Cladosporium* conidial suspensions did not result in pathogenicity. Unfavourable environmental conditions could possibly account for this observation. One of the disadvantages of field pathogenicity evaluations is the reliance on favourable environmental conditions for pathogen development (Conner 2002; Walker *et al.* 2018). However, the absence of symptoms at both trial sites at the time the fruitlets were highly

susceptible to scarring damage makes it unlikely that *Cladosporium* is the primary cause of the typical scars found on damaged avocado fruits. The presence of *Cladosporium* spp. on scarred fruit tissue supports the view that on avocado fruit, the fungus is not pathogenic but acts as a saprophyte.

The scars that developed after sanding the fruitlets with sandpaper were similar to those observed in the field. Possible causal agents are thrips and wind induced abrasion. A study carried out by Bara & Laing (2019) established that *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) (South African citrus thrips) posed a significant threat to South African avocado production. Thrips have rasping mouthparts and a unique feeding apparatus (Heming 1993), which can physically scar fruit tissue. In this study, heavy thrips feeding action and chronic recurring wind action (bruising of fruits against other fruits, stems, or leaves) was simulated by lightly sanding 2-week-old 'Pinkerton' fruitlets, and the findings of this study suggest that thrips and wind could induce the scar tissue observed on avocado fruits.

The tea filter bags were not effective at reducing the abrasion damage and the damage observed was comparable to that observed from fruitlets in the filter bag control. This is probably because even inside the filter bags, the fruitlets were not sufficiently protected from bruising. The occurrence of scar tissue inside filter bags may also help to explain that it is not the wind itself that is bruising the fruits but the action of the fruits bruising against other physical objects.

Fruits and fruitlets bearing similar scar characteristics to those of avocado scab (*S. perseae*), which was suspected at the beginning of the study, was not confirmed. Jenkins (1934), citing Doidge & Bottomley (1931), reported the presence of the fungus in South Africa. It is interesting to note that since then, the fungus has never been isolated in South Africa, even though the pathogen is recorded as being present in the country. The fungus may be absent in South Africa. Secondly, *S. perseae* is notoriously difficult to isolate in pure culture (Fan *et al.* 2017). Further collections and pathogenicity studies will be required to confirm the status of avocado scab in the country. The use of real-time PCR with specific primers may discover the fungus where conventional isolation techniques based on artificial media do not succeed (https://www.frontiersin.org/articles/10.3389/fpls.2018.01086/full).

### 4.5 Conclusion

This study confirmed the presence of endophytic *Cladosporium cladosporioides, C. westerdijkieae* and *C. perangustum* in avocado fruits. Subsequent pathogenicity tests suggested that these endophytic species are not pathogenic to avocado fruits. The initial prime suspect

was *Sphaceloma perseae*. However, it was not isolated in this study. Further study may be required to confirm the role played by *S. perseae* in avocado scar tissue development in South Africa.

The damage observed from field scarred avocado fruits is similar to that observed when fruits were physically bruised, suggesting that physical abrasion either as a result of biotic agents (thrips, caterpillars) and abiotic factors (wind induced) is responsible for the development of wide areas of scar tissue on avocado fruit. Growers are advised to take precautions in wind prone areas, such as planting windbreaks to minimise wind abrasion, and to monitor thrips population for the successful implementation of IPM.

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# Chapter 5: Susceptibility of avocado fruit to *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and wind scarring damage in Limpopo and KwaZulu-Natal Provinces of South Africa


**[5]Abstract**

In South Africa, the avocado (*Persea americana*) is an important fruit, grown primarily for export and contributing ZAR 1.75 billion to the gross domestic product of the country. As an export driven industry, optimising exportable avocado fruit volume is a primary concern. Wind induced abrasion and damage by thrips (Thysanoptera: Thripidae), through their feeding on avocado fruit results in corky tissue development (scarring), making the fruit unsuitable for export. The study aimed to determine the economic losses caused by these agents as well as to assess different cultivar responses to scarring damage. Across cultivars, the industry loses 1.49 % revenue annually due to *Scirtothrips aurantii* downgrading (3.86 % loss factor), translating to ZAR 34.90 million (US$2.39 million). Packhouse study results showed that both thrips and wind abrasion damage accounted for 30 % scarring damage, a loss factor of 13.72 % and a combined revenue loss of 5.57 %. The cultivar 'Pinkerton' showed the greatest susceptibility to scarring damage by both wind and *S. aurantii* whilst the cultivar 'Carmen®-Hass' showed a natural predisposition to higher levels of thrips damage. The presence of macadamia trees near avocado trees increases the likelihood of *S. aurantii* damage occurring on avocado fruit.



**Keywords:** South Africa, avocado, fruit scarring, thrips, wind abrasion.


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## 5.1 Introduction

Grown mainly in the humid, subtropical areas of South Africa, avocado (*Persea americana* Miller, fam. Lauraceae) is an economically important tropical fruit, contributing ZAR 1.75 billion to the gross domestic product of the country (http://www.worldstopexports.com/avocados-exports-by-country/). In 2018, 6.4 million tonnes of avocado fruit were produced worldwide with the world's largest producer of avocado fruit, Mexico, contributing 34 % to the total volume of avocado fruits followed by the Dominican Republic, Indonesia, and Peru (FAOSTAT 2020). Approximately 73 % of the world's avocado fruit are produced in the Americas, 13 % in Africa, 12 % in Asia and 2 % in Europe and in the South Pacific (FAOSTAT 2020). South Africa is the 12$^{th}$ largest producer of avocado fruit and is among the top ten exporters of avocado fruit globally (FAOSTAT 2020). In Africa, Kenya is the largest avocado exporter, followed closely by South Africa. Other African countries that produce avocado fruit include Rwanda, Democratic Republic of Congo, Cameroon, Tanzania and Zimbabwe. As an export oriented industry, South Africa primarily targets the European market (the Netherlands, UK, France and Spain) and in 2016, exported 57,867 tonnes of avocado fruit with a total value of ZAR 1,061 million, accounting for approximately 1.7 % of international market share (DAFF 2017).

The worldwide demand for avocado fruit has seen the area under avocado trees in South Africa increase rapidly from 2,000 ha in the 1970's (DAFF 2017) to 17,500 ha in 2018, with forecasts of a further increase of 1,500 ha plantings to be planted annually for the next five years (Donkin 2019). Commercial avocado production is mainly concentrated in the subtropical areas of Limpopo (60 %), Mpumalanga (29 %), KwaZulu-Natal (9 %) and parts of the Cape provinces (2 %) (Donkin 2019). Approximately 70 % of the trees grown in South African avocado nurseries are 'Hass' and the remaining 30 % is comprised mostly of 'Fuerte', 'Ryan' and 'Pinkerton' (Blakey & Wolstenholme 2014).

Of the 170,000 tonnes produced in 2018, approximately 51 % (86,000 tonnes) were exported, mainly to Europe, approximately 10-15 % were processed (oil and purée) and the rest were sold at fresh produce markets (Donkin 2019). The value of 2018 South African avocado export fruit was estimated at US$116.7 million (ZAR 1.75 billion), approximately 2.1 % of total income from exported avocado fruit globally (http://www.worldstopexports.com/avocados-exports-by-country/).

As an export driven industry, optimising the volumes of exportable avocado fruit is a primary concern. The presence of poor quality fruit plagues the industry, and with several global competitors vying for the same market, the need to improve quality is paramount

(Nelson 2014). Several pests and diseases work to constrain production and export. Globally, diseases such as phytophthora root rot caused by *Phytophthora cinnamomi* Rands (Hardham & Blackman 2018), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Sacc.), avocado scab (*Sphaceloma perseae* Jenkins) and cercospora spot (*Pseudocercospora purpurea* (Cooke) Deighton) (Darvas & Kotze 1987) are believed to cause major economic losses. Thrips (Thysanoptera: Thripidae), false codling moth (*Cryptophlebia leucotreta* (Meyrick)), fruit flies (Diptera: Tephritidae), avocado scale (*Fiorinia fioriniae* (Targioni-Tozzetti)) (Erichsen & Schoeman 1992), and heart shaped scale (*Protopulvinaria pyriformis* (Cockerell)) (Du Toit *et al.* 1991) are among the insect pests which commonly reduce the quality of avocado fruit in South Africa.

Thrips pose a constant threat to avocado production and export. Feeding by both adults and larvae on the fruit epidermis causes permanent superficial scarring of the fruit that appears as corky 'alligator' tissue. As Stevens *et al.* (1999) noted, minimal thrips scarring can be tolerated but damage in excess of 2 $cm^2$ area will result in the fruit being unacceptable for premium export grade (downgraded to local market or processing grade).

Thrips are minute, fringe-winged insects in the order Thysanoptera, suborder Terebrantia or Tubulifera. With a few exceptions of thrips species belonging to the suborder Tubulifera (*Haplothrips*, *Liothrips*, *Gynaikothrips*), most pest species of thrips belong to the suborder Terebrantia. Important thrips pests belong to the family Thripidae that contains a number of widespread, polyphagous and multivoltine species (Lewis 1973).

A study carried out by Bara & Laing (2019) established that *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) (South African citrus thrips) was an economic pest of avocado fruit. Damage to avocado in the USA by a related thrips species *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) (avocado thrips) has been estimated to cost that industry US$8.65 million annually (Hoddle *et al.* 2003). Feeding by low densities of *S. perseae*, approximately three larvae per young avocado fruit (<5 cm in length), was determined to scar the entire fruit surface, with the localized feeding starting as discrete brown scars that elongate as fruit matures (Hoddle *et al.* 2002).

*Scirtothrips aurantii* is an established pest of citrus (Gilbert & Samways 2018), macadamia (Rafter & Walter 2012) and mango (Grové *et al.* 2000). Bean-shaped, minute eggs (less than 0.2 mm) are laid separately in soft green fruit tissue, tender leaves and shoots (Bedford 1943) by means of a serrated ovipositor. The two feeding larval stages that follow the egg stage are yellow to orange and cigar-shaped. These are followed by two non-feeding pupal stages, the prepupa and the pupa. The average duration of the larval stages is about 7.6 days

whilst that of the pupal stages is 4.0 days, depending on the temperature (Bedford 1943). Adult thrips are yellowish-orange. All stages are less than 1 mm in length and are barely visible to the unaided eye. In citrus, the duration of the life cycle varies from 44 days in winter to 18.4 days in summer, with 9.4 generations being possible every year (Bedford 1943). The species is not known to undergo diapause owing to the mild climatic conditions of the main citrus-producing areas in South Africa (Gilbert & Bedford 1998). This means that, subject to the availability of suitable feeding conditions, larvae and adults can be found throughout the year.

Young avocado fruit are especially vulnerable to *S. aurantii* feeding damage (Bara & Laing 2019). As young foliage from the spring growth hardens during the time of fruit set, adult female thrips move from the foliage to feed and oviposit into young fruit. Feeding by the emerged larvae and adults results in damage to the skin of developing fruit (Hoddle *et al.* 2002).

Carapace skin was first described by Horne (1929) as a type of abrasion (mechanical) injury on avocado fruit caused by wind induced rubbing of tender young fruit on leaves or stems. Chronic recurring wind damage on young fruit causes russet type blemishes (angular netting), which may grow to cover significant portions of the fruits as the fruit mature. The scar tissue is rigid and as the fruit grows, it fractures along lines of force, resulting in the scars showing angular netting. In mature fruit, the damage exhibits itself as external corky, cracked tissue with regular, angular divisions resembling a turtle's back. The flesh underneath the scar is undamaged but the external appearance of the fruit results in downgrading of the fruit at packhouses (Dreistadt 2007).

The objectives of this study were to quantify losses incurred, and to compare the relative susceptibility of various avocado cultivars to damage by wind abrasion and *Scirtothrips aurantii* in the Limpopo and KwaZulu-Natal Provinces of South Africa.

## 5.2 Materials and methods

### 5.2.1 Study sites

This study was carried out in South Africa, KwaZulu-Natal Province (KZN), at Conlink Trust Farm (-29.453415, 30.683398) Wartburg; Everdon Estate (-29.452322, 30.266425), Howick; and at Baynesfield Estate (-29.756873, 30.314269), Richmond. In the province of Limpopo, South Africa, the study was carried out at Lombard Avocado (-23.923729, 30.136581) and at three Westfalia farms – Macnoon (-23.719230, 30.131611); Waterval (-23.754705, 30.123056); and Fowey (-23.812237, 30.120610).

### 5.2.2 Fruit infestation

In May and June 2019, 515 ‘Carmen®-Hass’ fruitlets 15-20 mm long were randomly collected from fruiting trees at Macnoon (Limpopo) and Everdon (KZN) and brought to the University of KwaZulu-Natal laboratory for incubation or ‘rearing’, as described by Bara & Laing (2019). The fruit infestation index was calculated as the ratio of the number of *S. aurantii* larvae that emerge per fruit collected (Cowley *et al.* 1992).

### 5.2.3 Scarring damage assessment

From June 2018 to September 2019, 27,800 fruits from ‘Hass’, ‘Pinkerton’, ‘Fuerte’ and ‘Rinton’ were randomly sampled from in-field assessments from the two provinces (**Figure 0.1**). Each fruit was examined for thrips and wind scarring damage. Field assessments in Limpopo were conducted in May 2019, in which 60 trees per cultivar were randomly selected from blocks of ‘Hass’, ‘Fuerte’ and ‘Pinkerton’. From each of these 60 trees, 15 mature fruit per cardinal direction were selected, giving a sample size of 3,600 per block. In KwaZulu-Natal, 45 trees per cultivar (‘Hass’, ‘Fuerte’, ‘Rinton’ and ‘Pinkerton’) per block were randomly selected from June to July 2019 and from each of these trees, two mature (ready to harvest) fruit per cardinal direction were selected, giving a sample size of 360 per cultivar.


**Figure 0.1.** Scarring damage on avocado fruit: a) blemish free fruit; b) *Scirtothrips aurantii* damage; and c) wind induced abrasion damage.

The packhouse study was done at Conlink Trust farm, KZN during the August to September 2018 fruit packing period. Ten lugs (= crates) of freshly picked, unsorted avocado fruit were randomly selected from fruit being packed at the time. A total of 100 fruit were sampled per cultivar per sampling period and the sampling was done twice giving a sample size of 800 fruit.

The percentage fruit damage and damage severity was evaluated for each cultivar (Phillips *et al.* 1995), and expressed as Class 1 and Class 2 grade (for export), % local grade

(downgraded), % reject, to quantify damage and assess susceptibility of the cultivars to wind and thrips damage. Percent damage was calculated as:

$$\%\, damage = 100\, \left(\frac{a}{b}\right)$$

where:

a = the number of fruit with scarring damage; b = the total number of fruit assessed.

The amount of scarring covering the surface of fruit was visually rated using ranks modified from Phillips *et al.* (1995): 1, no scar; 2, < 2 $cm^2$ scarring damage; 3, 2 $cm^2$ – half of the fruit scarred; 4, greater than half of the fruit scarred; 5, completely scarred. To eliminate biases in assessing scarring damage, a fixed team of evaluators scored the fruit.

A fixed exchange rate of 1 EUR = ZAR 16.55 (https://www.exchange-rates.org/Rate/EUR/ZAR/10-10-2019) was assumed for all financial calculations to cater for fluctuations in the exchange rates. Due to currency fluctuations, seasons and the impact of competition from other exporting countries, sometimes the revenue earned from local sales exceeds that of exports. However, for purposes of this study, it was assumed that exports earn significantly more than local sales. Potential revenue export is the revenue that potentially accrues to the industry when avocado fruit are exported and this can be approximated using the equation:

$$Export\ revenue = a\ \times\ b$$

where:

a =% Class 1 and 2 (export) fruit; b = potential total revenue.

Potential revenue from local sales (downgraded fruit) is the revenue that growers gain per tonne when Class 3 and Class 4 fruits are sold on the local market. This is represented by the equation:

$$Revenue\ from\ downgraded\ fruit = \frac{a+b}{100}\ \times\ 1000\ \times\ c$$

where:

a =% Class 3; and b =% Class 4; c = market price.

Potential revenue loss is the estimated loss in revenue when fruit are downgraded, *i.e.*, the difference between potential revenue and revenue gained from downgraded fruit sales and estimated using the equation:

$$Potential\ revenue\ loss = a - (b + c)$$

where:

a = potential revenue; b = revenue gained from Class 1 and 2 export sales; and c = revenue from local, downgraded fruit sales.

The percent loss is the potential loss expressed as a percent of potential revenue and is calculated as:

$$\%\ loss = 100(\frac{a}{b})$$

where:

a = potential revenue loss; and b = potential revenue.

### 5.2.4 Statistical analysis

Scoring data was analysed using non-parametric Kruskal–Wallis rank sum test and pairwise comparisons done using Wilcoxon rank-sum test in R (v. 3.6.1., R Foundation for Statistical Computing, Vienna, Austria).

## 5.3 Results

The damage scores of 'Fuerte', 'Rinton', 'Hass' and 'Pinkerton' were determined by visual assessment. The damage scores were not normally distributed and the non-parametric Kruskal–Wallis rank sum test was therefore used for data analysis. Wind damage across all the cultivars investigated accounted for 25.33 % downgrading (loss factor %) in Pietermaritzburg (KwaZulu-Natal), which was significantly different from the 3.92 % recorded for Tzaneen (Limpopo) (Kruskal–Wallis $\chi^2$ = 3591.6, df = 1, $P < 0.001$). More fruits suffered from wind induced abrasion in KZN (55.06 %) than in Limpopo (8.27 %) (**Table 0.1**;

**Table *0.2***).

**Table 0.1.** Summary of wind induced damage on avocado fruits in Limpopo and KZN.

| Site | N | Export % | % Scarring | Loss factor % | Damage score (Mean ± *SE*) |
|---|---|---|---|---|---|
| Limpopo | 25200 | 96.08 | 8.27 | 3.92 | $1.13 \pm 0.003^a$ |
| KZN | 1800 | 74.67 | 55.06 | 25.33 | $1.81 \pm 0.02^b$ |
| Pooled | 27000 | 94.66 | 11.39 | 5.34 | 1.18 ± 0.003 |

**Note**: Sites sharing a letter in superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$. Kruskal–Wallis $\chi^2$ = 3591.6, df = 1, $P < 0.001$.

**Table 0.2.** Wind induced damage in Limpopo and KZN.

| Cardinal Direction | Limpopo | | KwaZulu-Natal | |
|---|---|---|---|---|
| | ***N*** | **Damage score (Mean ± *SE*)** | ***N*** | **Damage score (Mean ± *SE*)** |
| North | 6300 | $1.143 \pm 0.006^{b}$ | 450 | $1.742 \pm 0.038^{b}$ |
| East | 6300 | $1.117 \pm 0.001^{a}$ | 450 | $1.627 \pm 0.036^{a}$ |
| West | 6300 | $1.152 \pm 0.001^{b}$ | 450 | $1.947 \pm 0.041^{c}$ |
| South | 6300 | $1.110 \pm 0.005^{a}$ | 450 | $1.940 \pm 0.041^{c}$ |

**Note**: Directions sharing a letter in superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$. Kruskal–Wallis $\chi^2 = 3591.6$, df = 1, $P < 0.001$.

In Tzaneen, the greatest wind induced damage was recorded on fruits in the north-west direction and the least in the south-east direction (Kruskal–Wallis $\chi^2 = 27.749$, df = 3, $P < 0.001$). In Pietermaritzburg, the greatest wind damage was recorded in the south-west direction, whilst the least damage was recorded in the east direction (Kruskal–Wallis $\chi^2 = 27.749$, df = 3, $P < 0.001$).

The packhouse study of 800 mature fruit harvested from four cultivars at Conlink Farm in Wartburg, Pietermaritzburg, was done in August and September 2018. For this study, where scarring from thrips and wind damage were pooled, 30 % of the fruits showed some form of scarring damage, a loss factor of 13.72 %, and combined revenue loss of 5.57 % was recorded (**Table 0.3**). The data were not normally distributed and the Kruskal–Wallis non-parametric test was performed to analyse the data. 'Hass' showed the least scarring (1.16 ± 0.03), followed by 'Fuerte' (1.39 ± 0.04), 'Rinton' (1.49 ± 0.05) and 'Pinkerton' (1.80 ± 0.07). 'Rinton' and 'Fuerte' scarring was not significantly different from each other (Kruskal–Wallis $\chi^2 = 63.822$, df = 3, $P < 0.001$). In terms of revenue lost, a pooled 5.57 % was lost across the cultivars, with 'Fuerte' showing the least revenue lost (2.65 %), while 'Pinkerton' had the highest at 12.69 %. When pooled, thrips damage was highest on 'Pinkerton' with a mean damage score of 1.265, and the least thrips damage was recorded on 'Fuerte' (1.008 ± 0.001) (**Table 0.4**; **Figure 0.2**). The damage on the cultivars differed significantly from each other (Kruskal–Wallis $\chi^2 = 1172$, df = 3, $P < 0.001$). The mean damage score was between 1 and 2, implying that in most cases, the damage scar was < 2 $cm^2$. In Limpopo Province, 'Fuerte' was the least thrips scarred avocado cultivar (1.004 ± 0.001), followed by 'Hass' (1.121 ± 0.004) and then 'Pinkerton'

(1.272 ± 0.008). Very little 'Rinton' is grown in Limpopo Province, and no data for scarring damage was recorded. In KwaZulu-Natal, 'Rinton' had the least thrips scarring, with a damage score of 1.044 ± 0.016. 'Fuerte' (1.097 ± 0.018) scar damage was significantly different to 'Rinton' (1.044 ± 0.016), but was not significantly different to 'Hass' (1.122 ± 0.021). The highest damage score was recorded for 'Pinkerton' at 1.193 ± 0.019. However, this was not significantly different to 'Hass's (1.122 ± 0.021) (Kruskal–Wallis $\chi^2 = 37.733$, df = 3, $P < 0.001$).

**Table 0.3.** Summary of fruit scarring damage caused by wind induced abrasion and *S. aurantii*.

| Cultivar | *N* | Scarring % | Mean ± *SE* | Loss factor % | % Export | Revenue lost % |
|---|---|---|---|---|---|---|
| 'Fuerte' | 200 | 31.50 | $1.39 \pm 0.04^{b}$ | 7.00 | 93.00 | 2.65 |
| 'Hass' | 200 | 11.00 | $1.16 \pm 0.03^{a}$ | 4.50 | 95.50 | 2.77 |
| 'Pinkerton' | 200 | 44.00 | $1.80 \pm 0.07^{c}$ | 33.50 | 66.50 | 12.69 |
| 'Rinton' | 200 | 33.50 | $1.49 \pm 0.05^{b}$ | 15.50 | 84.50 | 5.87 |
| Pooled | 800 | 30.00 | 1.46 ± 0.03 | 13.72 | 86.28 | 5.57 |

**Note:** Treatments sharing a letter in their superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (***BH***) procedure, P < 0.05. Damage score: 1, no scar; 2, < 2 $cm^2$ scarring damage; 3, 2 $cm^2$ – half of the fruit scarred; 4, greater than half of the fruit scarred; 5, completely scarred.

A summary of the financial impact of damage caused by *S. aurantii* is presented in **Figure 0.2**. 'Pinkerton' consistently showed greater thrips scarring, downgraded percent and revenue loss. The revenue lost was higher in Limpopo (ZAR2,418.06 across the three cultivars) than in KZN (ZAR1,955.63 for the four cultivars).

**Table 0.4.** *Scirtothrips aurantii* scarring damage scores in Limpopo and KwaZulu-Natal Provinces.

| | Overall | | Limpopo | | KwaZulu-Natal | |
|---|---|---|---|---|---|---|
| Cultivar | *N* | Mean ± *SE* | *N* | Mean ± *SE* | *N* | Mean ± *SE* |
| 'Pinkerton' | 7920 | $1.265 \pm 0.008^{d}$ | 7200 | $1.272 \pm 0.008^{c}$ | 720 | $1.193 \pm 0.019^{c}$ |
| 'Hass' | 11160 | $1.121 \pm 0.004^{c}$ | 10800 | $1.121 \pm 0.004^{b}$ | 360 | $1.122 \pm 0.021^{b,c}$ |
| 'Fuerte' | 7560 | $1.008 \pm 0.001^{a}$ | 7200 | $1.004 \pm 0.001^{a}$ | 360 | $1.097 \pm 0.018^{b}$ |
| 'Rinton' | 360 | $1.044 \pm 0.016^{b}$ | | | 360 | $1.044 \pm 0.016^{a}$ |

**Note**: Treatments sharing a letter in superscript are not significantly different at the 0.05 level according to Benjamini-Hochberg (*BH*) procedure, $P < 0.05$.

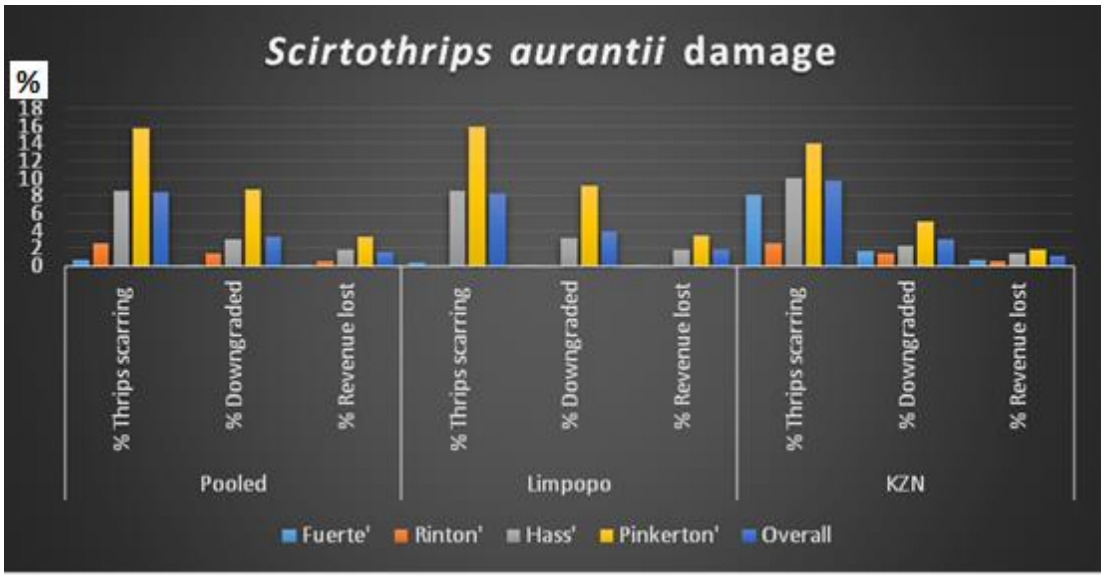


**Figure 0.2.** The impact of *Scirtothrips aurantii* damage on revenue.

Site differences were clearly illustrated at Westfalia, Tzaneen on blocks of 'Hass' and 'Carmen®-Hass'- a "Hass-type" cultivar. Thrips damage at Macnoon farm, where orchard blocks Macnoon 5C, Macnoon 4B and Macnoon 'Carmen®-Hass' were close to a macadamia (*Macadamia integrifolia* Maiden et Betche) orchard of the cultivar 695 ('Beaumont') were significantly different to block Fowey 'Hass' Z2A, which was about 10 km away from the macadamia orchard (Kruskal–Wallis $\chi^2 =$ 854.4, df = 3, $P < 0.001$). In particular, block Macnoon 4B recorded the highest level of thrips damage (1.272 ± 0.010) and was adjacent to the macadamia orchard, whereas the least scarred fruit was from the block at Fowey of 'Hass' cultivar at 1.0023 ± 0.001 (**Figure 0.3**). The higher damage scores recorded at Macnoon was further supported by thrips infestation studies carried out in May and June 2019, which revealed a fruit infestation index of 53.33 % compared to Everdon's 17.6 %. The 'Carmen®-Hass' at Macnoon is planted very close to a macadamia block while the 'Carmen®-Hass' at Everdon was not near macadamias. When pooled, *S. aurantii* fruit infestation was 38.45 % with a mean number of 2.60 ± 0.175 larvae emerging per fruitlet (

**Table *0.5***).

**Table 0.5.** *Scirtothrips aurantii* fruit infestation indices (mean ± *SE*) in Tzaneen and Pietermaritzburg.

| Site | Province | Cultivar | Total No. of fruitlets | No. of infested fruitlets | % fruit infestation | Mean No. of emerging larvae/fruit |
|---|---|---|---|---|---|---|
| Macnoon | Limpopo | 'Carmen®-Hass' | 330 | 176 | 53.33 | 2.60 ± 0.18 |
| Everdon | KZN | 'Carmen®-Hass' | 125 | 22 | 17.6 | 2.64 ± 0.19 |
| Pooled | | 'Carmen®-Hass' | 515 | 198 | 38.45 | 2.60 ± 0.175 |

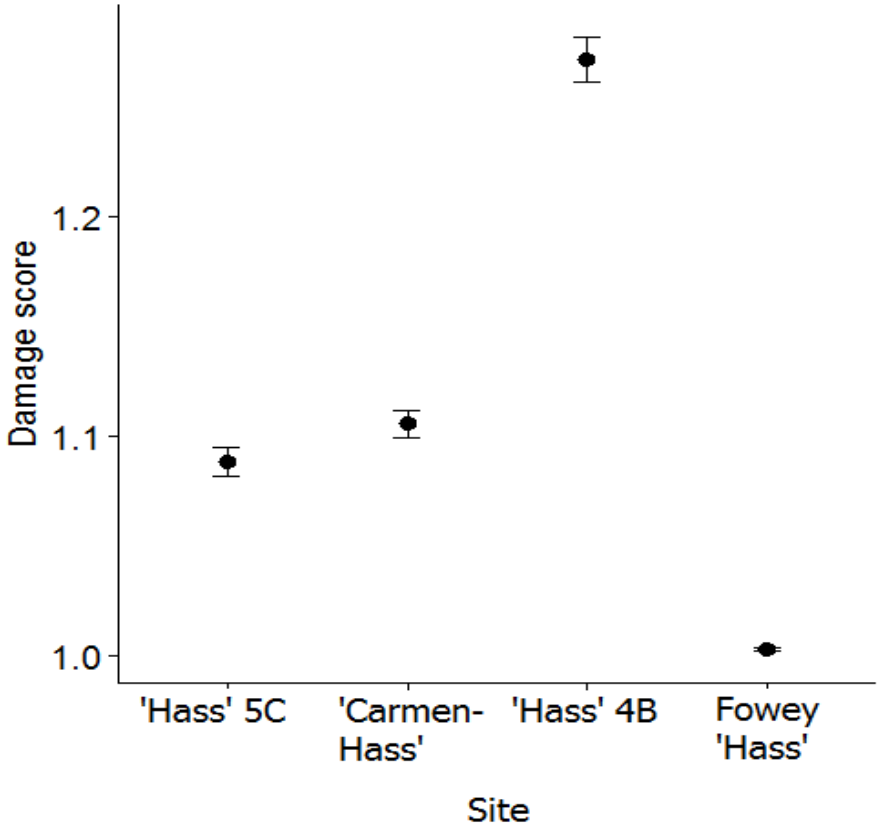


**Figure 0.3.** Errorplot (mean ± *SE*) of damage scores of "Hass-type" cultivars at Westfalia Estates, Tzaneen.

### 5.3.1 Economic impact of scarring damage

Due to pricing fluctuations, a fixed price range of EUR7.00-15.00 per 4 kg carton export price was assumed, resulting in gross revenue of ZAR37,237.50 to ZAR59,993.75 per tonne (price dependant on cultivar). 'Pinkerton', 'Fuerte', 'Hass' and 'Rinton' all have different yield potentials and different market prices and consequently different levels of revenue. Potential revenue from 'Hass' was highest at ZAR59,993.75 whilst the other cultivars peaked at

ZAR37,237.50 per tonne (**Table 0.6**). ‘Pinkerton’ had the highest potential revenue loss of ZAR1,227.28 per tonne, representing a 3.296 % revenue loss from *S. aurantii* scarring damage, followed by ‘Hass’ at 1.878 %. ‘Fuerte’ showed the least thrips scarring at 0.035 %. In ZAR terms, *S. aurantii* potentially costs the industry ZAR1,227.28 per tonne of ‘Pinkerton’ harvested, marginally followed by ‘Hass’ with a potential loss of ZAR1,126.39. ‘Rinton’ and ‘Fuerte’ showed the least thrips scarring losses at ZAR195.94 and ZAR13.06 per tonne harvested respectively.

**Table 0.6.** Potential revenue and losses per tonne after downgrading due to scarring caused by *S. aurantii*

| | | South African Rand (ZAR) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **EUR/ 4 kg** | **Mean ZAR/kg** | **Potential revenue/t** | **Export class/t** | **Local sales/t** | **Total revenue earned/t** | **Potential Revenue loss/t** | **Potential revenue lost %/t** |
| ‘Fuerte’ | 7-11 | 37.24 | 37237.50 | 37203.02 | 21.42 | 37224.44 | 13.06 | 0.035 |
| ‘Hass’ | 14-15 | 59.99 | 59993.75 | 58160.61 | 706.75 | 58867.36 | 1126.39 | 1.878 |
| ‘Pinkerton’ | 7-11 | 37.24 | 37237.50 | 33998.03 | 2012.19 | 36010.22 | 1227.28 | 3.296 |
| ‘Rinton’ | 7-11 | 37.24 | 37237.50 | 36720.31 | 321.25 | 37041.56 | 195.94 | 0.526 |

The percentage of potential revenue lost was lowest for ‘Fuerte’ at 0.005 % and highest for ‘Pinkerton’ at 3.44 %. No ‘Rinton’ fruits were assessed in Limpopo (

**Table *0.7***).

**Table 0.7.** Effect of *S. aurantii* scarring on revenue accrued per tonne harvested in Tzaneen, Limpopo.

| | | South African Rand (ZAR) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **EUR/ 4 kg** | **Mean ZAR/kg** | **Potential revenue/t** | **Export class/t** | **Local sales/t** | **Total revenue earned/t** | **Potential Revenue lost/t** | **Potential revenue lost %/t** |
| 'Fuerte' | 7-11 | 37.24 | 37237.50 | 37232.33 | 3.21 | 37235.54 | 1.96 | 0.005 |
| 'Hass' | 14-15 | 59.99 | 59993.75 | 58143.94 | 713.18 | 58857.12 | 1136.63 | 1.89 |
| 'Pinkerton' | 7-11 | 37.24 | 37237.50 | 33860.27 | 2097.76 | 35958.03 | 1279.47 | 3.44 |

In KwaZulu-Natal, the lowest *S. aurantii* scarring induced revenue loss was recorded on 'Rinton' at 0.53 %, whilst the highest potential revenue loss was for 'Pinkerton' at 1.89 %. However, the potential revenue loss per tonne of harvested fruit was highest for 'Hass' at ZAR819.19, followed by 'Pinkerton's ZAR705.38, 'Fuerte's ZAR235.13 and 'Rinton's ZAR195.94 (**Table 0.8**).

**Table 0.8**. Effect of *S. aurantii* scarring on revenue accrued per tonne harvested in Pietermaritzburg, KwaZulu-Natal.

| | | South African Rand (ZAR) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **EUR/ 4 kg** | **Mean ZAR/kg** | **Potential revenue/t** | **Export class/t** | **Local sales/t** | **Total revenue earned/t** | **Potential Revenue loss/t** | **Potential revenue lost %/t** |
| 'Fuerte' | 7-11 | 37.24 | 37237.50 | 36616.88 | 385.50 | 37002.38 | 235.13 | 0.63 |
| 'Hass' | 14-15 | 59.99 | 59993.75 | 58660.56 | 514.00 | 59174.56 | 819.19 | 1.37 |
| 'Pinkerton' | 7-11 | 37.24 | 37237.50 | 35375.63 | 1156.50 | 36532.13 | 705.38 | 1.89 |
| 'Rinton' | 7-11 | 37.24 | 37237.50 | 36720.31 | 321.25 | 37041.56 | 195.94 | 0.53 |

## 5.4 Discussion

Adverse biotic (pests and diseases) and abiotic factors (wind, temperature, humidity) act to limit the exportable percent of harvested avocado. In this study, wind damage caused the downgrading of avocado fruit of 25.33, 3.92 and 5.34 % in Pietermaritzburg (KZN), Tzaneen (Limpopo) and pooled, respectively. Significant differences in wind damage were observed between these sites, with fruit in KZN showing a greater mean damage score due to greater

wind damage of 1.81 ± 0.02 compared to Tzaneen's 1.13 ± 0.003. Traditionally, wind induced abrasion damage has been managed by using wind breaks. However, given the "hilly" terrain in which avocadoes are often planted, they are not always effective. A further issue is that tree species such as *Casuarina cunninghamiana* Miq. (beefwood), which is reported to reduce wind damage by 26 % in 'Hass', also competes with the avocado trees for water, sunlight and nutrients (Holmes & Farrell 1993). This presents a production challenge, especially in water-scarce environments such as South Africa. Other tree species commonly used as windbreak in citrus such as *Grevillea robusta* A. Cunn. ex R. Br. have been found to also harbour *S. aurantii* (Grout & Richard 1990), making the selection of tree species to be used as a windbreak important.

From July 2018 to October 2018, predominately north-easterly winds of an average speed of 1.54 m $s^{-1}$ were recorded in Tzaneen (https://www.windfinder.com/windstatistics/tzaneen-grenshoek). This period was chosen because, the early fruiting stage is the most susceptible to scarring damage, and assessments of fruit in May 2019 was of fruit exposed to these winds during development. The greatest damage on fruit per tree was observed on fruits facing the west and north cardinal directions (1.152 ± 0.001 and 1.143 ± 0.006 respectively). In KZN, the highest wind damage was recorded on west and south facing fruit (1.947 ± 0.041 and 1.940 ± 0.041, respectively). The apparent susceptibility of 'Pinkerton' to wind damage may have to do with its early fruiting, which coincides with periods when the weather is windy (August, September, October) (https://weatherspark.com/y/96783/Average-Weather-in-Durban-South-Africa-Year-Round).

In addition, 'Pinkerton' has short internodes and crowded branches against which young developing fruit can bruise in windy conditions. The predominant winds experienced in Pietermaritzburg from July to October 2019 were South easterlies of average speed 2.44 m $s^{-1}$ (https://www.windfinder.com/windstatistics/pietermaritzburg). There appears to be a relationship between wind direction and the damage observed.

When pooled, all the cultivars had significantly different scores for scar damage (Kruskal–Wallis $\chi^2 = 1172$, df = 3, $P < 0.001$). 'Pinkerton' had the highest level of scar damage (1.265 ± 0.008), followed by 'Hass' (1.121 ± 0.004), 'Rinton' (1.044 ± 0.016) and 'Fuerte' (1.008 ± 0.001). This trend was observed both in Limpopo (Kruskal–Wallis $\chi^2 = 1165.8$, df = 2, $P < 0.001$) and KZN. However in KZN, damage to 'Hass' was not significantly different to 'Pinkerton' (Kruskal–Wallis $\chi^2 = 37.733$, df = 3, $P < 0.001$). Dennill & Erasmus (1992) noted that in the 1990s' *Heliothrips haemorrhoidalis* (Bouché) and *Selenothrips rubrocinctus* (Giard) were the thrips of economic concern for South African avocado farmers, and that at the time,

farmers were complaining that ‘Hass’ was the most susceptible cultivar. In this study, these two thrips species were not collected, only *S. aurantii*, as found in Bara & Laing (2019). However, thrips damage to ’Hass’ featured prominently, with damage to the cultivar second only to ‘Pinkerton’, and in KZN this was not significantly different to ‘Pinkerton’. *Scirtothrips aurantii* damage in ‘Fuerte’ was consistently lower than that of ‘Pinkerton’ and ‘Hass’ in both provinces.

‘Pinkerton’ appears to be highly susceptible to scarring damage. Susceptibility of ‘Pinkerton’ to thrips, may perhaps be related to the degree of compatibility between the seasonal phenology of the trees and the environment. ‘Pinkerton’ may be susceptible to thrips feeding in spring due to its early development when the temperatures are low and when it develops young fruit that present fresh growing tissue favourable to thrips feeding. According to Scholefield *et al.* (1985), floral initiation occurs in autumn followed by flowering in late winter/ early spring, with fruit maturation occurring the following winter. A spring vegetative flush coincides with the end of flowering and early fruit development. This is the time when the greatest scarring damage occurs.

‘Pinkerton’ is a high yielding cultivar that does well under South African conditions. However, the cultivar is plagued by problems associated with its early flowering and extended flowering period (June to December) (Sippel *et al.* 1994). This extended flowering period causes the tree to have various sized fruit at differing maturity levels, and this predisposes fruits to thrips feeding over a longer period than other cultivars.

The level of damage caused by *S. aurantii* to avocado fruit of the four cultivars is a function of a range of biotic and abiotic factors. The presence of macadamia trees near avocado orchards appears to modify the avocado-thrips population dynamics. This was demonstrated when comparing the levels of damage of blocks of similar ‘Hass’ trees grown near and distant from macadamia orchards. Fruitlets collected from ‘Carmen®-Hass’ blocks near macadamias showed much higher thrips incidence (53.33 %) than the ‘Carmen®-Hass’ at Everdon Estate where there are no macadamia grown in the vicinity (17.60 %). The blocks near the macadamia orchard exhibited high levels of *S. aurantii* damage. This is supported by work down by Schoeman & Linda (2019), who also concurred that thrips damage was significantly greater in orchards near macadamia trees. Macadamia is a well known host of *S. aurantii* (Rafter *et al.* 2013). The proximity of macadamia trees to avocadoes allows for host switching when conditions are unfavourable in one crop, for example, after chemical spray application, thrips may disperse to untreated crops/blocks, and return when the environment is favourable. This

means that within these farms, there will usually always be a resident population on one of the tree crops that forms the base population for subsequent outbreaks.

In South Africa, 'Carmen®-Hass' is unique in its ability to flush continuously (both fruit and foliage) through-out the year, predisposing it to *S. aurantii* damage because there is a constant supply of young tissue in the trees that can support *S. aurantii* populations throughout the year. This was probably why there was a high thrips incidence in 3 week old 'Carmen®-Hass' fruitlets at both Macnoon (53.33 % (situated near macadamia orchards) and at Everdon, Pietermaritzburg (17.60 %)). These infestation levels differ from those obtained on 'Pinkerton' in Pietermaritzburg (4.82 %), as determined in an earlier study (Bara & Laing 2019).

'Hass' earns more money per unit volume exported to the EU (potentially up-to ZAR59,993.75 per tonne), due to the European market's preference for the cultivar (DAFF 2017). However, it is susceptible to *S. aurantii* feeding damage and was second to 'Pinkerton' in potential revenue loss. The potentially losses of 'Pinkerton' of ZAR1,227.281 per tonne of fruit harvested was closely followed by 'Hass' at ZAR1,126.392. The 2018 season was an 'on' year for avocado fruit, producing 170,000 tonnes of avocado fruit. Of this, 86,000 tonnes were exported (Donkin 2019), with an estimated value of ZAR 1.75 billion (US$116.7 million) (http://www.worldstopexports.com/avocados-exports-by-country/). South Africa's five-year annual average production stands at 118,000 tonnes (Donkin 2018).

Across cultivars, the industry loses 1.49 % revenue annually due to *S. aurantii* downgrading (3.86 % loss factor), translating to ZAR34.90 million (US$2.39 million). In Ventura (USA), a related thrips species, *S. perseae*, was reported to cause an economic damage of at least 15 % through fruit being downgraded at packinghouses in 1997-98 (Phillips *et al.* 1999). Faber *et al.* (2000) noted that the losses could be higher, given that the reported economic loss did not include fruit that dropped off trees and never made it to the packhouses.

## 5.5 Conclusion

The South African avocado industry loses 1.49 % revenue annually due to *S. aurantii* downgrading (3.86 % loss factor), translating to ZAR34.90 million (US$2.39 million). In our study, *S. aurantii* caused scarring damage on 'Pinkerton' of 3.296 % per tonne of fruit harvested, followed by 'Hass' (1.878 %), 'Rinton' (0.526 %) and 'Fuerte' (0.035 %).

The presence of macadamia trees near avocado trees appeared to expose the avocado fruit to greater frequencies of *S. aurantii* feeding damage. 'Carmen®-Hass' in South Africa is naturally predisposed to higher levels of thrips damage owing to its continuous flush phenology.

The loss to russet netting (wind abrasion damage) was higher in Pietermaritzburg (25.33 %) than in Tzaneen (3.92 %). Growers are advised to consider site, locality and the presence/absence of macadamia orchards in the area when selecting cultivars to grow. In wind prone areas, growers can take cultural management measures such as planting windbreaks and planting on the leeward side of slopes to minimise wind induced abrasion damage.

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# Chapter 6: First report of tussock moths (*Bracharoa mixta* (Snellen) (Lepidoptera: Erebidae)), scarring avocado fruit in KwaZulu-Natal, South Africa


## [6]Abstract

Avocado (*Persea americana*) is an important export crop for South Africa, contributing ZAR 1.75 billion in export revenue to the gross domestic product. As an export-oriented industry, increasing the exportable percentage of avocado fruit is a major concern. A tussock moth, *Bracharoa mixta* (Snellen) is reported for the first time on avocado trees, scarring fruit and defoliating leaves. Feeding damage by the larvae results in corky tissue development, making the fruit unsuitable for export. The study aimed to determine the identity of the larvae (morphologically and using DNA barcoding) and to ascertain the levels of damage it causes naturally. Sequencing of the barcoding region of cytochrome oxidase subunit I (*COI*) gene was done. The taxonomic studies identified the pest as a tussock moth (*Bracharoa mixta* (Snellen)). There were no prior DNA barcodes for this pest. In-field fruit damage assessment of infested trees showed an 11 % scarring damage level, resulting in the downgrading of 3.67 % of fruit. This represents a potential revenue loss of up to ZAR 1,352.90 per tonne (2.26 % revenue loss). The defoliating caterpillars also caused an 18.22 % reduction in leaf area. This study documents the potential of *B. mixta* to cause significant economic losses in sporadic, isolated outbreaks.



**Keywords**: South Africa, avocado, fruit scarring, *Bracharoa mixta*, DNA barcoding.


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## 6.1 Introduction

Avocado (*Persea americana* Miller) is an economically important subtropical fruit tree grown in South Africa for its much sought-after nutritious pear-shaped berry. Globally, annual production is estimated to be in excess of 3.5 million tonnes, 20 % of which is traded amongst countries (Schaffer *et al.* 2013). South Africa is ranked 12th in the world in terms of production but is among the top 10 exporters of avocado fruit in the world (FAOSTAT 2020). South Africa is currently Africa's second largest avocado exporter, behind Kenya, followed by Rwanda, the Democratic Republic of Congo and Cameroon. Avocado production in South Africa is an export-oriented industry primarily targeting the European market. In 2016, SA exported 57 867 tonnes of avocado fruit, with a total value of R1,061 million, accounting for approximately 1.7 % of international market share (DAFF 2017).

The area under avocadoes has increased rapidly from 2,000 ha in the 1970s (DAFF 2017) to 17 500 ha in 2018 (Donkin 2019). Production in 2018 stood at 170,000 tonnes of which approximately 51 % (86,000 tonnes) was exported, mainly to Europe, while approximately 10 % was processed (oil and purée) and the rest was consumed domestically (Donkin 2019). The value of the 2018 export crop was estimated at US$116.7 million, which was 2.1% of total income from exported avocado fruit globally (https://community.cochrane.org/style-manual/tables-and-figures/tables).

Being an export-oriented industry, there is a commercial impetus to optimise the exportable percentage of avocado fruit. However, several pests constitute a major constraint to production. Among the insect pests are thrips (Thysanoptera: Thripidae), fruit flies (Diptera: Tephritidae), false codling moth (*Thaumatotibia leucotreta* Meyrick) (Prinsloo & Uys 2015) and the heart shaped scale (*Protopulvinaria pyriformis* (Cockerell)) (Du Toit *et al.* 1991). Diseases such as phytophthora root rot (*Phytophthora cinnamomi* Rands) (Reeksting *et al.* 2014), stem canker (*Phytophthora cinnamomi* Rands), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Sacc.) (Sanders & Korsten 2003) and cercospora spot (*Pseudocercospora purpurea* (Cooke) Deighton) (Darvas & Kotze 1987) are also known to cause significant losses.

Towards the end of the 2018/2019 avocado season, unidentified Lepidoptera larvae were observed feeding on avocado fruits and leaves (both young and mature) in Wartburg, KwaZulu-Natal, South Africa. Tree foliage through photosynthesis provides the nutrients necessary for the growth, development and health of the tree. Insect feeding has both immediate and long-term effects on tree growth and development, as well as seed/fruit production. In response, the

trees counter foliage loss by concentrating resources on re-growing these food-producing tissues at the expense of other tissues, including fruit (Shepherd 1994).

Lepidoptera (moths and butterflies) make up the second largest order of insects, comprising of more than 16 000 species globally (Dai *et al.* 2015). Accurate identification of agricultural pests is a crucial step in the implementation of a successful integrated pest management (IPM) programme as control options are tailor made against a specific target species. Traditionally, identification has been achieved using morphological traits (Jinbo *et al.* 2011). However, as Barrett & Hebert (2005) noted, identifications based on morphology are often problematic, time consuming and error prone. In addition, immature life stages are often difficult to identify using routine taxonomy because most of the morphotaxonomic keys are only appropriate for evaluating adult stages. Murugan *et al.* (2016) pointed out that the difficulties in morphological identification are compounded by phenotypic plasticity, where one genotype produces more than one phenotype under different environments. The pea aphid (*Acyrthosiphon pisum* Harris) has been shown to alternate between asexual and sexual reproduction, as well as growing wings between generations when plants become too populated (Eisen 2010). Ball & Armstrong (2006) noted the complexity of morphological identification of cryptic species when using taxonomic keys that requires a high level of training and experience.

Molecular techniques, such as DNA barcoding, provide solutions to some of the challenges associated with morphology-based identifications. DNA barcoding is a technique that allows for the rapid and accurate identification of biological specimens through sequencing of the mitochondrial cytochrome-c oxidase subunit 1 (COI) gene (Jalali *et al.* 2015). The use of DNA barcoding is increasingly becoming popular among entomologists (Jinbo *et al.* 2011), with more arthropods being subject to barcoding than any other taxonomic group (Taylor & Harris 2012).

The insect mitochondrial genome (mitogenome) is a closed, double-stranded, circular DNA molecule, 14-19 kb in length that encodes two ribosomal RNA genes (*12S rRNA* and *16S rRNA*), 22 tRNA genes, a highly conserved set of 37 genes, 13 protein-coding genes (PCGs): [ATPase subunits (*ATP*) *6* and *8*, cytochrome c oxidase subunits 1–3 (*COI–III*), cytochrome (*Cyt*) *B*, and NADH dehydrogenase subunits (*ND*) *1–6* and *4L*] (Wolstenholme 1992; Boore 1999). The mitogenome is preferred over nuclear genes as a molecular marker for insect species identification because it is compact, lacks genetic recombination, exhibits maternal inheritance and undergoes a relatively fast evolutionary rate of variation (Cameron *et al.* 2006; Timmermans *et al.* 2014). In DNA barcoding of insects, the short standardised gene

region of mitochondrial COI is used. Ball & Armstrong (2006) have previously confirmed the accuracy of barcoding as a tool to identify lymantriids (tussock moths).

In DNA barcoding, the short standardised gene region of mitochondrial COI (the DNA barcode) is used as a species tag for each species (Jinbo *et al.* 2011). DNA barcoding is a two-step process where a barcode library of known species is established and then the barcode sequence of the unknown specimen is matched to the barcode library for local alignment similarity. The first step requires taxonomic expertise in morphologically identifying the specimen that will serve as the reference sample in the barcode library (Kress & Erickson 2012). Ratnasingham & Hebert (2007) pointed out that a sequence alignment algorithm is used to match the unknown specimen to a known species by finding the closest database sequence to the sample sequence. The National Center for Biotechnology Information (NCBI) in the USA, operates the Basic Local Alignment Search Tool (BLAST) as one of its main matching tools designed to search for correspondence between a query sequence (sequence from unknown specimen) and a known sequence reference library (Kress & Erickson 2012).

This was the first time that this pest was reported feeding on avocado fruits and leaves in South Africa, potentially causing economic losses. The first step in any IPM programme is to correctly identify the problem pest and its biology. The absence of knowledge therefore creates a knowledge gap.

The objectives of this study were to identify the caterpillar using morphology and DNA barcoding and to estimate the levels of damage caused by the insect in avocado orchards in KwaZulu-Natal Province, South Africa.

## 6.2 Materials and methods

### 6.2.1 Study site

The caterpillars were first observed in a 'Hass' cultivar block in June 2019 at Conlink Trust, Wartburg (-29.457936, 30.675975) and then in a 'Rinton' orchard at St. Paul's farm (- 29.447031, 30.668446) in September 2019, about 1.37 km away (**Figure 0.1**). The two orchards are separated by sugarcane (*Saccharum officinarum* L.) plantations and blocks of 'Lamb-Hass' avocado trees. In August 2019, the caterpillars were reported in 'Lamb-Hass', in an adjacent block at Conlink. Wartburg is a small farming community, 27 km northeast of Pietermaritzburg, KwaZulu-Natal Province, South Africa. The climate is generally mild, warm and temperate with an annual mean precipitation of 905 mm. Precipitation is lowest in June, with an average of 14 mm and highest in January, averaging 142 mm. At 20.4°C, February is

the hottest month and June is the coldest at 12.3°C (https://en.climate-data.org/africa/south-africa/kwazulu-natal/wartburg-189711/).


**Figure 0.1.** Site locations, Wartburg, KwaZulu-Natal province, South Africa.

Commercial plantations of *Eucalyptus* spp. (gum tree), *Pinus* spp. (pine tree) and *Saccharum officinarum* L. (sugarcane), populate the landscape. Vegetation in and around the Conlink and St. Paul's avocado orchards included *Saccharum officinarum* L. (sugarcane), *Citrus unshiu* Yu.Tanaka ex Swingle (satsuma), *Ipomoea purpurea* (L.) Roth (morning glory) and *Bidens pilosa* L. (blackjack).

### 6.2.2 Insect specimen collection

Live caterpillars were hand-picked directly from avocado fruit and leaves, and transported to the University of KwaZulu-Natal laboratory in glass jars. The site of collection, collection date, collector's name and location were recorded at each sampling period. Sampling was conducted from July 2019 to September 2019. Collected caterpillars were placed in insect jars and provided with avocado leaves 'Hass' *ad libitum*.

### 6.2.3 Morphological identification

Lepidoptera larvae at different developmental stages were collected from the field and reared in 500 ml insect jars at 25-28°C, 75 ± 5 % relative humidity and 12L:12D photoperiod. The insect jars were sealed with nylon netting (250 µm pore size) using tightly strung rubber bands and the larvae were provided with fresh avocado leaves *ad libitum*. The adults did not feed and were kept alive for as long as possible to enable them to develop their full body colour and

normal shape, because morphological features were the main identification tools. As this was the first observation in avocado, in KwaZulu-Natal, there was no local expertise available for consultation. Specimens were sent to several experts in a quest to identify the species including entomologists at the Plant Health and Protection division of the Agricultural Research Council in Pretoria, South Africa.

### 6.2.4 DNA barcoding and sequencing

Two larval caterpillar specimens preserved in 80 % alcohol were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa for DNA barcoding. They were sequenced using methods modified from Folmer *et al.* (1994). Genomic DNA was extracted from the larval specimens using the ZR Quick-DNA miniprep plus kit™ (Zymo Research, catalogue No. D4068). The cytochrome COI region was amplified using One Taq® Quick-Load ®2X Master Mix (NEB, catalogue no. M0486) with the primers presented in **Table 0.1**. The PCR products were run on a gel and extracted with Zymoclean™ Gel DNA recovery kit (Zymo Research, catalogue no. D4001). The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator cycle sequencing kit V3.1 BRD3-100/1000) and purified (Zymo Research, ZR3500x sequencing clean-up kit™, Catalogue No. D4050). The purified fragments were analysed on the ABI 3500xl Genetic analyser (Applied Biosystems, ThermoFisher Scientific) for each reaction for the two samples. CLC Bio Main Workbench v. 7.6 was used to analyse the .ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by NCBI's BLAST search tool.

**Table 0.1.** Primers used in the sequence amplification (Folmer *et al.* 1994).

| Primer | Target gene | 5' to 3' sequence |
|---|---|---|
| LCO1490 | COI | ggtcaacaaatcataaagatattgg |
| HCO2198 | COI | taaacttcagggtgaccaaaaaatca |

### 6.2.5 Leaf loss estimation due to leaf eating caterpillars

To study the incidence and severity of the tussock moth caterpillar damage, a roving survey was conducted at the two study sites at the harvesting stage for avocado fruit at Conlink, and at the early fruiting stage at St. Paul's. As this was a survey to determine the presence of the pest and the damage levels it can cause, purposive sampling was conducted. In the affected

blocks, tree infestation was localized with about 10 % of the trees showing signs of tussock moth damage. Fifteen infested trees per site were selected, from which 30 leaves were randomly selected in the tree canopy from all the cardinal campus positions (north, east, west and south). Using methods modified from Kozlov *et al.* (2014), degrees of defoliation were determined by visual assessment using the following rating scale: 1) (no leaf lamina damage); 2) 0.01-25.00; 3) 25.01-50.00; 4) 50.01-75.00; 5) 75.01-100.00 %.

Two indices were used to quantify the incidence and severity of the insect feeding damage. The Leaf Damage Index 1 (LD1) is an index of the proportion of leaves damaged through larval feeding and is calculated as the proportion of affected leaves to the total number of leaves sampled, i.e:

$$\text{LD1}=\frac{\text{No. of affected leaves}}{\text{Total No. of leaves sampled}}$$

The Leaf Damage Index 2 (LD2) is the estimated area of leaf damaged expressed as a percent of the total leaf area (Alliende 1989). LD2 is the frequency of the damage classes (product of the number of leaves in each damage class and the respective median value of that class). The sum total of these frequencies will then be divided by the total number of leaves (*N*), to get leaf area damage (Kozlov *et al.* 2014).

### 6.2.6 Fruit scarring damage assessment

In July 2019, 300 fruits (20 fruits/infested avocado tree) were randomly sampled. Each fruit was examined for tussock moth caterpillar scarring damage. The percentage fruit damage and damage severity was evaluated and expressed as % export grade, % local grade, % reject. Percent damage was calculated as:

$$\%\, damage = 100\left(\frac{a}{b}\right)$$

Where:

*a* = the number of fruit with scarring damage; and *b* = the total number of fruit assessed.

The amount of scarring covering the surface of fruit was visually rated using ranks modified from Phillips *et al.* (1995): 1) no scar; 2) <2 $cm^2$ scarring damage; 3) 2 $cm^2$ – half of the fruit scarred; 4) greater than half of the fruit scarred; 5) completely scarred. To eliminate biases in assessing scarring damage, a consistent team visually scored fruit.

A fixed exchange rate of 1 EUR = ZAR 16.55 (https://www.exchange-rates.org/Rate/EUR/ZAR/10-10-2019) was assumed for all financial calculations to cater for

fluctuations in the exchange rates. Potential revenue export is the revenue that potentially accrues to the industry when class 1 and 2 fruit are exported and this can be approximated using the equation:

$$Export\ Revenue\ = a\ x\ b$$

Where:

*a* = % class 1 and 2 (export) fruit; and *b* = potential total revenue.

Potential revenue from local sales is the revenue that growers' gain/t when class 3 and class 4 fruits are sold on the local market and is represented by the equation:

$$Revenue\ from\ local\ sales = \frac{a+b}{100}\ x\ 1000\ \text{x}\ c$$

Where:

*a* = % class 3; and *b* = % class 4; *c* = market price.

Potential revenue loss is the estimated loss in revenue when fruit are downgraded, *i.e.* the difference between potential revenue and revenue gained from local sales and estimated using the equation:

$$Potential\ revenue\ loss = a - (\ b + c)$$

Where:

*a* = potential revenue; *b* = revenue export; and *c* = revenue from local sales.

The percent loss is the potential loss expressed as a percent of potential revenue and is calculated as:

$$\%\ loss = 100(\frac{a}{b})$$

Where:

*a* = potential revenue loss; and *b* = potential revenue.

### 6.2.7 Statistical analysis

Scoring data was analysed using non-parametric Kruskal-Wallis rank sum test and pairwise comparisons were done using Wilcoxon rank sum test in R (v. 3.6.1., R Foundation for Statistical Computing, Vienna, Austria).

## 6.3 Results

### 6.3.1 Morphological identification

Spherical, white eggs approximately 1 mm wide with a circular depression hatched into larvae showing typical tussock moth morphological characteristics - a pair of dark-grey pencil tufts on the prothorax and a shorter mid-dorsal one on the 8th segment growing up to a length of 22 mm. Each of the four abdominal segments were observed to have a white to creamy-buff, dense, short tuft, with the second abdominal segment projecting an additional pair of dark grey lateral tufts. The adult male moth was 10 mm long and 3-4 mm wide with greyish-brown forewings and plain grey hind wings of wingspan 20-25 mm. The wings also had an irregular patchy, white band and a prominent spot near the apex. The light brown, flightless adult female was roughly the same length as the male (10 mm), however, it is stouter (5-6 mm wide). The female pupa also differs from the male pupa in not having developed wing pads nor remnants of hair tufts. The insect was identified from its morphology as *Bracharoa mixta* (Snellen 1872) (Erebidae: Lymantriinae), and specimens (voucher I.D AcP 9636) were deposited with the Agricultural Research Council's Plant Health and Protection and Health, Biosystematics division, Roodeplaat, Pretoria, South Africa. The various life stages observed in South Africa are shown in **Figure 0.2**.


**Figure 0.2.** *Bracharoa mixta* life stages: a) eggs; b) larva; c) pupa; d) adult male moth; e) adult female; f) female with eggs.

### 6.3.2 DNA barcoding and sequencing

The COI sequence data was matched against the Genbank reference library using NCBI's BLAST, as well as other genomic databases such as BOLD. However, there were no conclusive matches obtained. The closest match in Genbank was a Lepidopteran sp. voucher K052, accession KC172813.1 at 90.58 %. The mitochondrial COI gene region for the tussock moth had not yet been deposited with any major database. The COI gene sequence was subsequently submitted to NCBI's Genbank for databasing (accession I.D: GenBank MN527963).

### 6.3.3 General observation on life history in South Africa

Thirty larvae were collected from the field and reared under laboratory conditions, as described under the morphological identification section. The larvae were collected at different growth stages but all the larvae had pupated within 50 days from the date of collection. At 25-28°C, 75 ± 5 % relative humidity and 12L:12D photoperiod, the pupal period was observed to take 4-7 days in females and 11-13 days in males. The flightless female was light brown in colour and 8-10 mm long, 3-5 mm wide at the thorax and had well developed blacklegs and a pointed abdomen. After emerging from her cocoon, the female clung to the edge of the exit hole of the cocoon, mated with a male moth and then laid eggs outside her empty pupal case. The female laid white, spherical eggs 1 mm long with a depression around the micropyle. The adults did not feed and lived for about 8 days.

### 6.3.4 Feeding damage

*Bracharoa mixta* was recorded for the first time causing damage to fruits and leaves on two avocado farms in Wartburg, a 'Hass' and 'Lamb-Hass' orchard at Conlink and a 'Rinton' orchard at St. Paul's farm (**Figure 0.3**).


**Figure 0.3.** *Bracharoa mixta* feeding damage: a) close-up of early and late feeding damage; b) mature 'Hass' fruit scarred showing characteristic 'woody' appearance; c) leaf defoliation.

The leaf damage scores were not normally distributed and the non-parametric Kruskal-Wallis chi-squared test was used for analysis. Conlink had more foliar damage (2.08 ± 0.05)

than St. Paul's (1.92 ± 0.05) but not statistically different to St. Paul's farm (Kruskal-Wallis $\chi^2$ = 3.72, df = 1, $P$ > 0.05) (**Table 0.2**).

**Table 0.2.** Summary of leaf feeding damage caused by *Bracharoa mixta*.

| Site | *N* | Damage score (Mean ± *SE*) | LD1 % | LD2 % |
|---|---|---|---|---|
| Conlink | 450 | 2.08 ± 0.05 | 55.56 | 19.93 |
| St. Paul's | 450 | 1.92 ± 0.05 | 52.00 | 16.50 |
| Pooled | 900 | 2.00 ± 0.04 | 53.78 | 18.22 |

**Note.** Feeding damage at site level are not significantly different at the 0.05 level (Kruskal-Wallis $\chi^2$ = 3.72, df = 1, $P$ > 0.05): Damage score: 1) 0 (No leaf lamina damage); 2) 0.01-25.00; 3) 25.01-50.00; 4) 50.01-75.00; 5) 75.01-100.00 percent.

Damage to leaves was evaluated using damage scores, LD1 and LD2 (**Table 0.2**). The damage score for both sites were close to 2.0 (0.01-25.00 %). Conlink had a slightly higher leaf damage index (LD1) (55.56 %) than St. Pauls' (52.00 %). Conlink also showed a higher leaf area damaged (19.93 %) compared to St. Paul's 16.50 %. However, the indices at the two sites were not statistically different to each other (Kruskal-Wallis $\chi^2$ = 3.72, df = 1, $P$ > 0.05). When pooled, the damage score, LD1 and LD2 were 2.00 ± 0.04, 53.78 % and 18.22 %. A damage score of 2 implies that the area damaged is between 0.01-25.00 %. This speaks to the LD2 damage estimated at 18.22 %.

**Table 0.3.** Summary of fruit scarring damage caused by *Bracharoa mixta* at Conlink.

| N | Percent scarred | Damage score | % Class 1 | % Class 2 | % Class 3 | % Class 4 |
|---|---|---|---|---|---|---|
| 300 | 11.00 | 1.15 ± 0.03 | 88.67 | 7.67 | 3.33 | 0.33 |

**Note.** Damage score: 1) no scar; 2) < 2 $cm^2$ scarring damage; 3) 2 $cm^2$ – half of the fruit scarred; 4) greater than half of the fruit scarred; 5) completely scarred.

Eleven percent of study fruit at Conlink showed typical "woody" scar tissue resulting from *B. mixta* feeding, with a mean damage score of 1.15 ± 0.03 (**Table 0.3**). Of the 300 fruit, 3.67 % fruit scarring led to a 2.26 % revenue loss (**Table 0.4**). (World avocado prices accessed from http://www.farmingportal.co.za/index.php/agri-index/68-crops/2668-overview-global-avocado-market-july-2019). At St. Paul's farm, 3-week old 'Rinton' fruitlets were also observed to have been scarred by the tussock moth larva (**Figure 0.4**).

**Table 0.4.** Summary of potential revenue gained from sales and lost through downgrading of scarred fruit.

| | | | South African Rand (ZAR) | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **EUR/ 4 kg** | **Average ZAR/kg** | **Potential revenue/t** | **Export class/t** | **Revenue local sales/t** | **Total revenue earned/t** | **Potential revenue lost/t** | **Potential revenue lost %/t** |
| 'Hass' | 14-15 | 59.99 | 59993.75 | 57791.98 | 848.87 | 58640.85 | 1352.90 | 2.26 |

(1 EUR = ZAR 16.55)


**Figure 0.4.** 'Rinton' tissues damaged by *B. mixta*: a) larva feeding on leaf; b) and c) fruitlets scarred by larvae.

## 6.4 Discussion

The voracious caterpillars were identified morphologically as *Bracharoa mixta* (Snellen 1872), syn. *Orgyia mixta:* (Lepidoptera: Lymantriinae). *Bracharoa mixta* feeding scars show distinct, deep, irregular shaped "woody" scar tissue and in-field damage assessment showed that 11 % scarring in 'Hass' fruit resulted in 3.67 % downgrading and a 2.26 % loss in revenue.

First described by Snellen in 1872, this tussock moth is widespread throughout tropical Africa (Austara & Migunda 1971). It has been recorded in Angola, Malawi, Mozambique, Zambia, Zimbabwe (Pinhey 1975), and South Africa (Vári *et al.* 2002). *Bracharoa mixta* is a lepidopteran insect in the super family Noctuoidea, family Erebidae; subfamily Lymantriinae. The Lymantriinae has approximately 350 genera and over 2,500 recognised species in every continent except Antarctica (Zahiri *et al.* 2012). Members of the Lymantriinae are well known defoliators of forest trees, among which are the Douglas-fir tussock moth *Orgyia pseudotsugata* (McDunnough), gypsy moth *Lymantria dispar* (Linnaeus), and the nun moth *Lymantria monacha* (Linnaeus).

*Bracharoa mixta* was first reported in Kenya in the 1950s as a pest defoliating softwoods, and subsequently caused a number of localised sporadic outbreaks notably in the Kenyan

Highlands (Austara & Migunda 1971). The tussock moth is highly polyphagous, feeding on several plant species including the fever tree (*Vachellia xanthophloea* (Benth.) P.J.H. Hurter) (Agassiz & Harper 2009), African acacias (*Acacia* spp.), the Monterey pine (*Pinus radiata* D. Don), the pepper tree (*Schinus molle* L.), the Siberian elm (*Ulmus pumila* L.), the mendocino cypress (*Cupressus pigmaea* (Lemmon) Sarg.) (Gardner 1957), citrus (*Citrus* spp.), maize, (*Zea mays* L.), geranium (*Geranium* sp.), coffee (*Coffea* spp.), cotton (*Gossypium* spp.) (Pinhey 1975), European plum (*Prunus domestica* L.) and jacaranda (*Jacaranda mimosifolia* D. Don) (Austara & Jones 1971).

In this study, spherical, white eggs approximately 1 mm wide with a circular depression around the micropyle were laid singly on foliage, usually outside the cocoon, by the gravid females. This is consistent with reports by Austara & Migunda (1971), who also noted that egg hatching is temperature dependent, with eggs hatching in 6 to 19 days with the optimum incubation temperature being 23-25°C. The eggs hatch into minute larvae with up to 5 instars for the males and 7 for the females. Austara & Migunda (1971) observed that at 17-19°C, the larval stage can take 43-106 days to develop whilst at 22-24°C, the larval period can be as short as 30-63 days. The first instar larvae are 3-5 mm long, and blackish-brown in colour. As the larvae mature, bright red, dorso-lateral verrucae become visible, one on each side of segments five to eight.

The young caterpillars' are voracious feeders, feeding gregariously before dispersing. As the females are flightless, dispersal is achieved mainly by the larvae, particularly the early instars. During dispersal, larvae attach themselves to foliage by exuding a thin silk thread from the labial spinneret and as they drop to the ground, they can be carried by air currents (ballooning) over considerable distance. Austara & Migunda (1971) noted that dispersal is a chance event and dependent on prevailing wind and air currents.

The larvae pupated by spinning brownish-yellow ovoid cocoons made of some of the caterpillar's body hair in the silk. The duration of the pupal period varied from 4-7 days in females and 11-13 days in males. Austara & Migunda (1971) reported that the pupal stage was 6-9 days and 13-18 days for females and males, respectively, and that the females could lay up to 200 eggs.

Both the males and females do not feed, and in our study, the adults survived for about 8 days. Austara & Migunda (1971) found that the adults could live for 8-13 days. In Kenya, the life cycle is 48-85 days, with the potential to produce up to six overlapping generations a year, allowing all stages to occur in one place at the same time. The duration and number of generations produced per year in South Africa were not determined in the present study.

As the adults do not feed, all the damage is caused by the larval stages that skeletonize leaves and feed on the fruit. As the larvae mature they tend to feed in a solitary manner, after becoming dispersed through the crown of the tree. Austara & Migunda (1971) estimated that 20-25 larvae per metre of branch can cause 100 % defoliation on the Monterey pine, *P. radiata*, with outbreaks possible any time of the year.

Under field conditions, *B. mixta* is susceptible to several natural enemies, among them parasitic flies, nematodes and wasps. The braconid *Apanteles africanus* Cam. and the ichneumonoids, *Pimpla mahalensis* Gribodo and *Charops* sp., are well known parasitoids (Migunda 1970). According to Migunda (1970), *Charops* sp. is probably the most important parasitoid of this tussock moth. This is also a motivation for avocado growers to continue with current regimen of using minimal amounts of pesticides because frequent pesticide application may affect the natural enemy complex, which in turn may lead to pest repercussion outbreaks.

Another possible cause for mortality emanates from the insect's dispersal mechanism. Wind dispersal to hosts is a chance event and a significant proportion of the dispersing larvae may not land on suitable hosts, thus resulting in considerable mortality.

A Leaf Damage Index (LD2) of 18.22 % was observed when both sites were pooled. This represents a loss in leaf area of about 18.22 %. It is not yet clear whether this level of leaf loss results in yield loss. However, defoliation is known to reduce yield as growth is reduced both radially and terminally. Mason & Wickman (1988) in their work on Douglas fir trees pointed out that most trees have a surplus of foliage to meet minor foliage losses, and as such, 30-50 % defoliation must occur before significant growth reduction can be detected. The focus of this current study was on avocado, and the levels of leaf damage observed were unlikely to have resulted in significant yield losses. However, it is possible that higher infestation levels could result in more extensive defoliation and crop losses.

A leaf damage score rating of 2.00 obtained in this study aligns with the LD2 finding, suggesting that both methods were consistent in estimating leaf damage. Avocado is an evergreen, subtropical fruit tree that can potentially support *B. mixta* populations through-out the year, provided other intrinsic (parasitism, disease) and extrinsic (temperature, humidity) factors are non-limiting. The polyphagous nature of *B. mixta,* coupled with its overlapping generations, make it difficult to predict with certainty during which period of the year heavy infestations might occur. It is also probable that under favourable environmental conditions, an outbreak may occur at any time during the year. This prediction is supported by work done by Austara & Migunda (1971).

It is interesting to note that the tussock moth was first noticed in a 'Hass' orchard at Conlink in June 2019. The caterpillars were then noted in an adjacent 'Lamb-Hass' block in August 2019. These two orchards were sprayed using *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) and the populations of tussock moth died down. However, some caterpillars survived and were routinely collected weeks after the spray. The tussock moth caterpillars were then noted on an adjacent farm (St. Paul's) in 'Rinton' early September 2019. The damage assessments were carried out early October 2019 at St. Paul's a month after the caterpillars were first reported. However, the leaf damage area at these two farms was not statistically different, with evidence of the voracious feeding nature of the caterpillars (3 week-old fruitlets and foliage showed typical signs of tussock moth larval feeding damage).

## 6.5 Conclusion

This is the first report of *Bracharoa mixta* causing scarring damage on avocado fruit and foliage in South Africa. This species was identified morphologically. A DNA barcode (GenBank MN527963) and voucher specimens (voucher I.D AcP 9636) were deposited for future referencing.

Scarring damage by *B mixta* can result in potential revenue losses of up to ZAR1,352.90 per tonne (2.26 % revenue loss) through downgrading. As several natural enemies keep *B. mixta* populations in check, avocado growers are encouraged to continue with current regimen of using minimal amounts of pesticides because frequent pesticide application may affect the natural enemy complex, which in turn may lead to pest repercussion outbreaks.

This study documents the potential of *B. mixta* to cause economic losses and cause sporadic, isolated outbreaks in avocado orchards.

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# Chapter 7: Biological and chemical control of the soil-dwelling stages of the South African citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae), on avocado, *Persea americana* Mill. (Lauraceae)


## [7]Abstract

*Scirtothrips aurantii* is a recently confirmed economic pest of avocado (*Persea americana* Mill.) causing 1.49 % annual revenue losses (US$2.39 million) due to downgrading (3.86 % loss factor) in South Africa. Damage by *S. aurantii*, through their feeding on avocado fruit results in corky tissue development (scarring), making the fruit unsuitable for export. The study aimed to monitor *S. aurantii* population dynamics during the critical early fruiting period by means of dispersal/emergence (D/E) traps and fruit infestation indices, to determine the field efficacy of biologicals (*Beauveria bassiana*, *Steinernema feltiae*), the pyrethroid beta-cyfluthrin, and the semi-synthetic spinosyn, spinetoram, against soil-dwelling stages of *S. aurantii* during early fruit development. Finally, the study aimed to establish the pupation sites of *S. aurantii* in avocado. Above ground, population dynamics fluctuated directly in response to the host plant's phenological changes and were modified by rainfall. Control of soil-dwelling stages by the insecticides and the effectiveness of D/E traps in monitoring *S. aurantii* populations were not demonstrated, probably because the populations trapped using the D/E traps were not representative of the above ground populations, as evidenced by fruit infestation indices. Under laboratory conditions, 81.60 % of mature larvae pupated away from the ground, demonstrating that *S. aurantii* does not passively drop to the ground in an avocado orchard, but actively seeks out dark, sheltered areas to pupate, mostly in the canopy.



**Keywords:** *Scirtothrips aurantii*, avocado, fruit scarring, pupation, control.


[7] *This chapter has been submitted for publication and is currently under review*

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## 7.1 Introduction

*Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) (South African citrus thrips) is a recently confirmed economic pest of avocado fruit in South Africa, causing 1.49 % annual revenue losses (US$2.39 million), due to downgrading (3.86 % loss factor) (Bara & Laing in-press). Permanent superficial scarring of the fruit epidermis caused by feeding *S. aurantii* adults and larvae on avocado fruit appears as corky 'alligator' tissue. Whilst thrips feeding damage is superficial and does not affect internal fruit quality, severe fruit scarring results in the downgrading of fruit from premium export to local market or processing grade (Stevens *et al.* 1999).

*Scirtothrips aurantii* feeding damage is most severe on young avocado fruit (Bara & Laing 2019). The preference for young fruit has been demonstrated for a closely related thrips species, *Scirtothrips perseae* Nakahara, in avocado (Hoddle *et al.* 2002) and *Scirtothrips citri* (Moulton) in oranges (Flint 1991) in the USA. Hoddle *et al.* (2002) noted that young actively growing tissue is particularly vulnerable to attack, because adult female thrips move from the hardening foliage to feed and oviposit into young fruit during early fruit development.

As Mound & Teulon (1995) noted, due to their evolutionary history, *Scirtothrips* spp. are polyphagous and are highly adapted to life as crop pests, dwelling in many microhabitats and being able to exploit host plant phenological changes. Environmental conditions modify and influence plant phenological changes by directly impacting on when plants produce leaves, flowers and fruits (Ananthakrishnan 1993). Silva & Del-Claro (2010) highlighted that thrips respond positively to the availability of suitable food resources and these attract and maintain thrips on host plants.

*Scirtothrips aurantii* has been studied in citrus (Gilbert & Samways 2018) and mango (Grové *et al.* 2000). The females use their serrated ovipositor to lay minute, bean-shaped eggs (less than 0.2 mm) separately into young, soft, tender fruit tissue, leaves and shoots (Bedford 1943). Yellow to orange, cigar-shaped actively moving and feeding larval stages (I & II) follow the egg stage and, depending on temperature, the larval stage lasts ± 7.6 days (Bedford 1943). Two non-feeding, quiescent pupal stages, the prepupa and the pupa lasting ± 4.0 days, possess developing wing buds and follow the larval stages (Bedford 1943). Adult thrips are highly mobile, yellow-orange winged insects. After a pre-oviposition period of 2.5 days, the females mate and lay on average 0.4 and 1.2 eggs per female daily in winter and summer, respectively (Gilbert & Bedford 1998). All the life stages are minute (usually less than 1 mm long). Bedford (1943) noted that the duration of the life cycle in citrus is temperature dependent, taking 18.4 and 44.0 days in summer and winter, respectively. In South Africa,

owing to its mild climatic conditions, *S. aurantii* does not undergo diapause and is reported to have 9.4 generations every year (Gilbert & Bedford 1998). This means that, subject to the availability of suitable feeding conditions, larvae and adults can be found throughout the year.

Bedford (1943) demonstrated that mature *S. aurantii* larvae drop to the soil to pupate. Gilbert & Samways (2018) suggested that dispersal/emergence (D/E) traps are effective in sampling *S. aurantii*, as evidenced in South African mango orchards (Grové *et al.* 2000) and orange orchards (Gilbert 1992). D/E traps have also been reported to be successful in monitoring a related thrips species, the Californian citrus thrips, *Scirtothrips citri* (Moulton), in oranges in the USA (Reed & Rich 1975; Tanigoshi & Moreno 1981). Dispersal/emergence traps capture falling mature larvae as well as adults when they emerge from the soil.

Sudo *et al.* (2019) noted that field evaluations of insecticide efficacy are problematic, primarily because the fate of the insects exposed to the insecticides cannot be directly observed in the field and that pest populations vary greatly, even within the same field. Consequently, estimates of control are taken from samples of an unknown parent population size (EPPO 2012; Yamamura & Suzuki 2006).

Control of *S. aurantii* is particularly challenging owing to the short generation time (18.4 days in summer), (Bedford 1943)), small size, polyphagy, high fecundity (1.2 eggs per female per day (Gilbert & Bedford 1998)). Generally, thrips are poorly controlled by foliar insecticide applications, because of their cryptic feeding behaviour (Thöming 2005). For this reason, soil drench applications may be helpful in disrupting the thrips life cycle and preventing populations from reaching economically damaging levels.

As no soil-applied insecticides are currently registered in South Africa for the control of *S. aurantii* in avocado, some biologicals and synthetic insecticides were evaluated for possible control efficacy. Spinetoram, a semi-synthetic spinosyn insecticide, has been shown to be effective against several insect pests including Thysanoptera, Lepidoptera, Diptera, Hymenoptera and Coleoptera (Dripps *et al.* 2008). Spinosyns are unique in that they do not target the same active sites that pyrethroids, neonicotinoids and avermectins target. Spinosyns act through a novel site in the nicotinic receptor, that is distinct from neonicotinoids or any other nicotinic actives (Crouse 2007). Orr *et al.* (2006) identified the spinosyn target site as an α7-like nicotinic acetylcholine receptor known as Dmα6-nAChR. Insect death results from the activation of this α6-nAChR.

Beta-cyfluthrin is a non-systemic, synthetic pyrethroid insecticide with contact and stomach action known to be effective against several insect orders including Coleoptera, Orthoptera, Lepidoptera, Hymenoptera and Blattodea (Leicht *et al.* 1996; Athanassiou *et al.*

2013). As a neurotoxic synthetic insecticide, beta-cyfluthrin disables the target insect's sodium channels of the neuronal membranes, resulting in death (Narahashi 1996).

*Steinernema feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding (Rhabditida: Steinernematidae) is a soil-dwelling, microscopic, entomopathogenic nematode (EPN). The efficacy of EPNs has been demonstrated on pupal and prepupal western flower thrips, *Frankliniella occidentalis* (Pergande) (Buitenhuis & Shipp 2005), and onion thrips, *Thrips tabaci* Lindeman (Azazy *et al.* 2018). Infective juvenile stages of EPNs respond to carbon dioxide, vibration and other chemical cues (Kaya & Gaugler 1993), as they actively seek out their hosts and enter through natural openings such as the mouth, spiracles and anus. Symbiotic bacteria released by the nematodes cause bacterial septicaemia resulting in insect death.

*Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) is an entomopathogenic fungus (EPF) that can infect insects through direct penetration of the host cuticle (Hajek & St. Leger 1994). After killing the insect host, the fungus produces conidia on the insect cadaver, releasing infective spores into the environment, facilitating long-term biological control. Soil-dwelling pupal phases of *F. occidentalis* have been reported to be susceptible to soil drenches of *B. bassiana* in eggplants (Zhang *et al.* 2019) and tomatoes (Lee *et al.* 2017).

These insecticides were chosen based on their modes of action and the possibility of their inclusion in an integrated management strategy.

The objectives of the study were to monitor *S. aurantii* population dynamics during the critical early fruiting period by means of (D/E) traps and fruit infestation indices, to determine the field efficacy of biologicals (*B. bassiana* and *S. feltiae*), the pyrethroid beta-cyfluthrin, and the semi-synthetic spinosyn, spinetoram, against soil dwelling stages of *S. aurantii*. Finally, the study aimed to establish the pupation sites of mature *S. aurantii* larvae in avocado orchards.

## 7.2 Materials and methods

Field evaluation of potential control agents - biologicals (*B. bassiana*, *S. feltiae*), the pyrethroid beta-cyfluthrin, and the semi-synthetic spinosyn, spinetoram against soil dwelling stages of *S. aurantii* were conducted over two seasons in KwaZulu-Natal Province, South Africa. The first season's study was conducted from 20.08.19 to 23.10.19 on early fruiting 'Pinkerton' cultivar at Baynesfield Estate (-29.756873, 30.314269), Richmond and Conlink Trust Farm (- 29.453415, 30.683398), Wartburg. The methodology was refined in Season Two and was conducted from 10.02.20 to 30.03.20 on 'Carmen®-Hass' avocado cultivar at Everdon Estate (-29.452322, 30.266425), Howick. Commercial formulations of *S. feltiae* (Entonem®, Koppert

SA (Pty) Ltd, Lanseria, South Africa), *B. bassiana* strain R444 (Eco-Bb®, Plant Health Products (Pty) Ltd, Nottingham Road, KwaZulu-Natal, South Africa), beta-cyfluthrin (Bulldock® Beta 125SC, Bayer (Pty) Ltd, Isando, South Africa) and spinetoram (Delegate™, Dow Agrosciences Southern Africa (Pty) Ltd, Bryanston, South Africa) were used in all trials (

**Table *7.1***). Each treatment was mixed in a 20l water bucket, and applied as a soil drench covering a radius of 1 m around the tree trunk using a watering can. No treatment was applied in the control plots. The top 3 cm of soil were kept moist for the entire duration of the study.

### 7.2.1 Evaluation method

Small 9-year-old avocado trees (2.5 m wide canopy) at Baynesfield ('Pinkerton'), Everdon ('Carmen®-Hass') and mature 20 year-old, recently-pruned, 3m wide canopy trees ('Pinkerton') at Conlink were selected for this study. From each of the three sites, 24 trees were randomly selected, with each tree serving as an experimental unit. D/E traps were made from rings cut from PVC cylindrical pipes (internal diameter 190 × 200 mm high), as described by Gilbert & Samways (2018). FlyTac® (Insect Science®, Tzaneen, South Africa) adhesive glue was uniformly applied on both sides of a cling film (Gladwrap®) covered clear glass 220 × 220 × 5 mm square. The coated glass square (trapping surface) was placed on top of the cylinder, catching falling larvae above and emerging adults below. A single trap was randomly placed in no particular cardinal direction, 30 cm from the tree trunk and its position was changed every 7 days (Gilbert & Samways 2018). For the duration of the study, every 14 days, four replications (trees) of six insecticide treatments were applied per study site, 1 m radius from the tree trunk in the drip zone (

**Table *7.1***). In 2019, beta-cyfluthrin and spinetoram were applied only twice, on the 4$^{th}$ and 27$^{th}$ of September. An additional 16 D/E traps (4 per tree) placed in the cardinal quadrants were also placed on four untreated trees to gather additional data on population dynamics in 2020. The glass squares containing trapped insects were collected after 7 days, wrapped in Gladwrap®, transported to the University of KwaZulu-Natal laboratory and the number of *S. aurantii* individuals counted under a stereomicroscope within 48 hours of collection to avoid distortion of specimens (shrinking, swelling and colour changes).

**Table 7.1.** Summary of treatments and application rates.

| Pesticide active ingredient | Trade name | Trial application rate | Treatment application interval |
|---|---|---|---|
| *Beauveria bassiana* | Eco-Bb® | 0.32 g/tree ($2x\ 10^9$ spores/g) | 14 days |
| *Beauveria bassiana* | Eco-Bb® | 0.64 g/tree ($2 \times 10^9$ spores/g) | 14 days |
| *Steinernema feltiae* | Entonem® | 500,000 juveniles/$m^2$) | 14 days |
| Control | (No treatment) | | |
| Beta-cyfluthrin | Bulldock® Beta 125 SC | 0.012 ml/tree | 14 days |
| Spinetoram | Delegate™ 250 WG | 0.03 g/tree | 14 days |

The methodology was refined in season two to collect additional information on weather parameters (rainfall, temperature), fruit infestation indices as well as establishing *S. aurantii* pupation sites. The second series of trials were conducted from 10.02.20 to 30.03.20 on 'Carmen®-Hass' avocado cultivar at Everdon Estate (-29.452322, 30.266425), Howick.

### 7.2.2 Fruit infestation

From 2 March to 30 March 2020, 72 'Carmen®-Hass' fruitlets 15-20 mm long were randomly collected every 7 days from the 24 experimental trees (3 fruitlets/tree) at Everdon Estate and incubated to allow *S. aurantii* larvae to emerge, at the University of KwaZulu-Natal laboratory, as described by Bara & Laing (2019). In total, 360 fruitlets were sampled over a period of 5 weeks. The fruit infestation index was calculated as the ratio of the number of larvae that emerge per fruit collected (Cowley *et al.* 1992).

### 7.2.3 Site of pupation

In a quest to understand the population dynamics of *S. aurantii* in the canopy and at the ground level at Everdon Estate, four untreated trees were randomly selected in a 1.8 Ha 'Carmen®-Hass' avocado block. Underneath the canopy, 4 D/E traps were randomly placed 30 cm from the tree trunk in the 4 cardinal directions: north, east, west and south (Gilbert & Samways 2018). The number of mature $2^{nd}$ instar larvae and emerging adults were monitored every 7 days from the week ending 9 March to 30 March 2020.

For the above ground population dynamics, the fruit infestation indices were also monitored during the same period. A simple model was used to generate the weekly-

hypothesised (expected) number of *S. aurantii* larvae and adults per tree for comparison to the weekly D/E trap catches. The hypothesised number of larvae was calculated as the product of the mean number of fruitlets (10-40 mm) per experimental tree and the mean number of *S. aurantii* larvae/fruitlet.

$$Hypothesised\ No.of\ S.aurantii\ larvae\ per\ tree = a\ \times b$$

Where:

a= mean number of fruitlets/tree; and b=mean number of *S. aurantii* larvae/fruitlet.

Bara & Laing (2019), in their work on *S. aurantii* on avocadoes, estimated that 51.43 % of larvae successfully pupated to adults in incubation units under laboratory conditions. Using this value, the mean (hypothesised) number of adults to emerge was calculated as the product of the number of larvae and the percent adult emergence.

$$Hypothesised\ No.of\ S.aurantii\ adults\ per\ tree = c\ \times d$$

Where:

c= mean number of larvae/tree; and d= percent adult emergence (51.43 %).

### 7.2.4 Laboratory determination of pupation sites

To verify the observations and to obtain an estimate of the proportion of larvae pupating above ground and in particular in the tree canopy, a pupation study was conducted in the laboratory with naturally infested avocado fruitlets. A total of 288 'Carmen®-Hass' fruitlets collected from the experimental trees at Everdon Estate were placed in incubation units. An incubation unit consisted of a 25-ml transparent glass tube. A single fruitlet (15-20 mm long) was placed in each incubation unit and a coffee filter paper (53 µm pore size) used to seal the unit using tightly strung rubber bands (**Figure 7.1**). Incubation units were then held at 25–28 °C, 75 ± 5 % relative humidity and 12L:12D photoperiod (Bara & Laing 2019). A total of 72 fruitlets were collected weekly, incubated on the day of collection on the 9th, 17th , 23rd and 30th of March 2020 and the individual pupation sites were recorded 12 days after incubation.

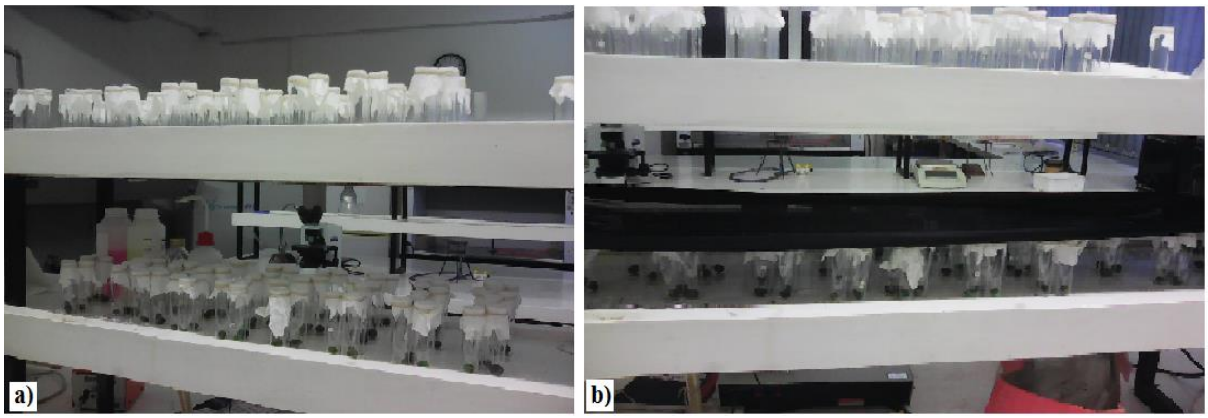


**Figure 7.1.** Fruit incubation units: a) standard with white coffee filter paper seals; b) nylon netting spread over incubation units.

In order to test the hypothesis that mature, 2nd instar larvae actively seek 'suitable' sheltered places to pupate (as opposed to passively dropping to the ground), the 144 fruitlets collected on 23 and 30 March 2020 were reared in the incubation units (**Figure 7.1**). On day 7 (after incubation) a black nylon cloth (250 µm pore size) was spread over the incubation chambers to create a dark environment at the roof of the units and on day 12 the number of *S. aurantii* individuals pupating on the fruit, floor and roof of the units were recorded.

**Statistical analysis**

The efficacy of the biological and chemical control agents was evaluated using a Poisson regression, Generalised linear model (GLM) and the coefficients separated using Wald's chi squared test. Over-dispersion was corrected using an over-dispersion term, the quasi-likelihood model (McCullagh & J.A 1989; Ver Hoef & Boveng 2007).

Fruit infestation indices were modelled using a logistic regression (GLM), while the larval emergence per fruit was modelled using Poisson regression GLM. The coefficients were separated using Wald's chi squared test. Pearson correlation coefficient ($r$) and the coefficient of determination ($R^2$) were calculated to explain the relationship between the number of larvae that emerge from the fruitlets and the fruit infestation indices.

Pupation site data were analysed using Poisson regression GLM and the coefficients separated using Wald's chi squared test. Over-dispersion was corrected using an over-dispersion term, the quasi-likelihood model (McCullagh & J.A 1989; Ver Hoef & Boveng 2007). All statistical analyses were done in R (v. 3.6.3., R Foundation for Statistical Computing, Vienna, Austria).

## 7.3 Results

### 7.3.1 Season 1, 2019 Field efficacy trials, 'Pinkerton', Conlink Farm and Baynesfield Estate

A similar characteristic trend was observed in the 2019 avocado season on the two farms, (Conlink and Baynesfield Estate) on all the treatments, where the highest adult *S. aurantii* populations were recorded at the start of the study in August, towards the end of the 'Pinkerton' flowering period, where after populations tailed off significantly. Very few dispersing larvae were collected in the D/E traps during the study period.

Results of the 2019 Conlink emergence data showed that the differences between the untreated control and all the other treatments were not significant ($\chi^2$ = 2.7, df = 5, $P(> \chi^2)$ =0.74). The control emergence data were also not significant ($P$ = 0.052). Although the control in dispersal traps was significantly different from zero ($P$ = 0.0069), the differences in the overall effect of the treatments were not significant ($\chi^2$ = 4.6, df = 5, $P(> \chi^2)$ = 0.47), and pairwise comparisons between the control and the other treatments were not significant (***Table 7.2***).

**Table 7.2.** Estimated parameter values for the degree of control based on GLM, Conlink 2019.

| | **Dispersal** | | **Emergence** | |
|---|---|---|---|---|
| **Parameter** | **Estimate** | ***P*(>\|t\|)** | **Estimate** | ***P*(>\|t\|)** |
| Control | -3.00 | 0.0069 | 0.79 | 0.052 |
| *B. bassiana* (std) | 1.95 | 0.098 | 0.087 | 0.88 |
| *B. bassiana* (x2) | 1.10 | 0.38 | 0.41 | 0.43 |
| Beta-cyfluthrin | -16.31 | 0.99 | 0.13 | 0.82 |
| *S. feltiae* | 0.69 | 0.60 | -0.38 | 0.54 |
| Spinetoram | 1.61 | 0.18 | -0.35 | 0.58 |

During the same period, dispersal trap data collected at Baynesfield also show that the overall effect of the treatments on dispersing *S. aurantii* larvae were not significant ($\chi^2$ = 1.9, df = 5, $P$ ($> \chi^2$) = 0.87). Differences between the dispersal coefficients of the control and the other treatments were also not significant. However, the control coefficient (-3.00) was significant ($P$ = 0.016). The differences between the emergence coefficients between the control and the other treatments were not significant. The overall effect of the treatments was

not significant on emergence ($\chi^2$ = 3.1, df = 5, $P(> \chi^2)$ = 0.68), although the control emergence coefficient (1.31) was significant ($P < 0.001$) (**Table 7.3**).

**Table 7.3.** Estimated parameter values for the degree of control based on GLM, Baynesfield 2019.

| | **Dispersal** | | **Emergence** | |
|---|---|---|---|---|
| **Parameter** | **Estimate** | ***P*(>\|t\|)** | **Estimate** | ***P*(>\|t\|)** |
| Control | -3.00 | 0.016 | 1.31 | 0.00023 |
| *B. bassiana* (std) | 1.49 | 0.28 | -0.41 | 0.46 |
| *B. bassiana* (x2) | 1.26 | 0.37 | -0.25 | 0.65 |
| Beta-cyfluthrin | -16.31 | 0.9954 | -0.31 | 0.57 |
| *S. feltiae* | 1.10 | 0.44 | -1.13 | 0.11 |
| Spinetoram | 0.16 | 0.93 | -0.65 | 0.30 |

### 7.3.2 Season 2, 2020 Field efficacy trials, 'Carmen®-Hass', Everdon Estate

The Poisson regression model estimates indicate that the overall effect of treatments were not significant ($\chi^2$= 7.4, df = 5, $P(> \chi^2)$ = 0.19). This is probably due to the low numbers of emerging *S. aurantii* adults trapped in the D/E traps. With respect to the emerging adults, beta-cyfluthrin was the only treatment significantly different to the control ($P$ = 0.0186) reducing *S. aurantii* populations by 34.48 % (**Table 7.4**).

**Table 7.4.** Estimated emerging adult parameter values for the degree of control based on GLM, Everdon 2020.

| **Parameter** | **Estimate** | ***SE*** | **t value** | **2.50%** | **97.50%** |
|---|---|---|---|---|---|
| Control | 0.035 | 0.23 | 0.16 | -0.45 | 0.45 |
| *B. bassiana* (std) | -0.59 | 0.38 | -1.56 | -1.38 | 0.13 |
| *B. bassiana* (x2) | -0.42 | 0.36 | -1.17 | -1.15 | 0.27 |
| Beta-cyfluthrin | -1.06 | 0.45 | -2.38 | -2.02 | -0.24 |
| *S. feltiae* | -0.11 | 0.33 | -0.33 | -0.77 | 0.54 |
| Spinetoram | -0.32 | 0.35 | -0.92 | -1.03 | 0.36 |

As there were no significant treatment effects ($\chi^2$= 7.4, df = 5, $P(> \chi^2)$ = 0.19), generalizations can be drawn across all the treatments. Generally, the *S. aurantii* larval and adult populations caught in the D/E traps during the critical period were low, as was observed in 2019 in the early 'Pinkerton' fruiting season. The *S. aurantii* adult emergence populations were highest at full bloom, gradually declining before slightly picking up during the week

ending 23 March to 30 March 2020. Though fluctuating, the number of dispersing larvae started low and began to increase steadily to the week ending 30 March 2020. As there were no significant differences between the treatments and control in all trials, the control D/E traps are shown in **Figure 7.2**, with the detailed trap data in Appendices 1, 2 and 3.

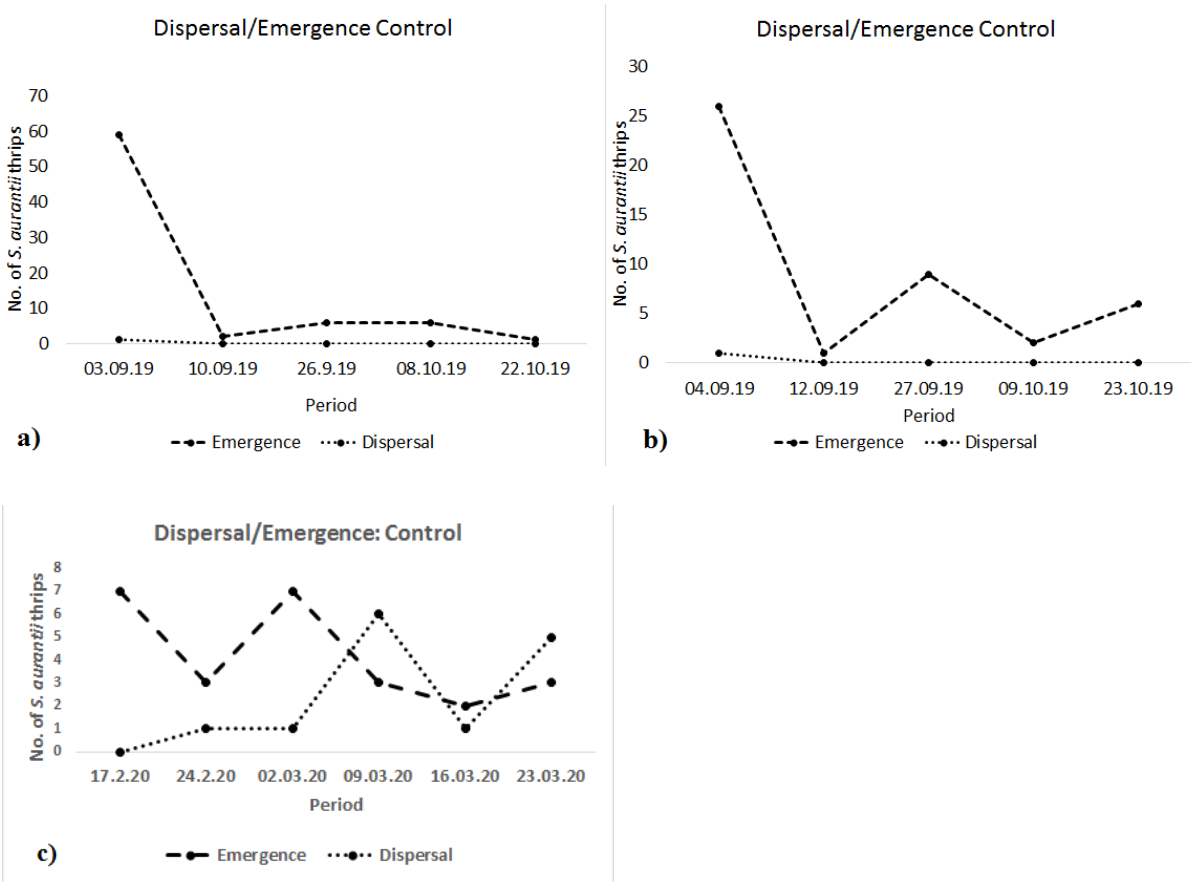


**Figure 7.2.** Summary of Control Dispersal/Emergence data at: a) Baynesfield ' Pinkerton' 2019; b) Conlink 'Pinkerton' 2019; and c) Everdon 'Carmen®-Hass' 2020.

### 7.3.3 Population dynamics

The trial was established on 10 February 2020, in a 'Carmen®-Hass' orchard under trees at 50 % bloom and early initiation of fruiting. The number of *S. aurantii* adults that emerged from under the trees were highest at the beginning of the trial (7 per 4 traps) and gradually declined to three adults on 30 March 2020. At the start of the trial, no *S. aurantii* larvae were collected in dispersal traps and the number increased in a series of erratic fluctuations until the week ending 30 March 2020 (five *S. aurantii* larvae per four traps).

Rainfall and temperature were recorded daily throughout the study period. The weekly average, minimum and maximum rainfall were recorded as 3.55 mm, 0.33 mm and 10.16 mm whilst that of temperature were 19.50°C, 17.51°C and 21.15°C respectively. Of particular note

is the apparent link between the highest weekly rainfall of 10.16 mm and a marked drop in fruit infestation index and in D/E trap catches. The highest number of adults caught in traps of 6 adults was recorded in the week ending 09 March which also recorded a relatively 'high' weekly mean temperature of 21.01°C and a fruit infestation index of 56.94 %.

Fruit infestation indices were collected once fruitlet reached at least 15 mm length (when detached from the parent tree, smaller sized fruits shrivelled and dried up before *S. aurantii* larvae emerged). Fruitlets collected on 2 March recorded a 50 % fruit infestation level that increased to 56.94 % the following week before dropping to 36.72 % in the third week and then increased to 59.72 %, where it remained to the end of the study on March 30, 2020 (**Figure 7.3**).

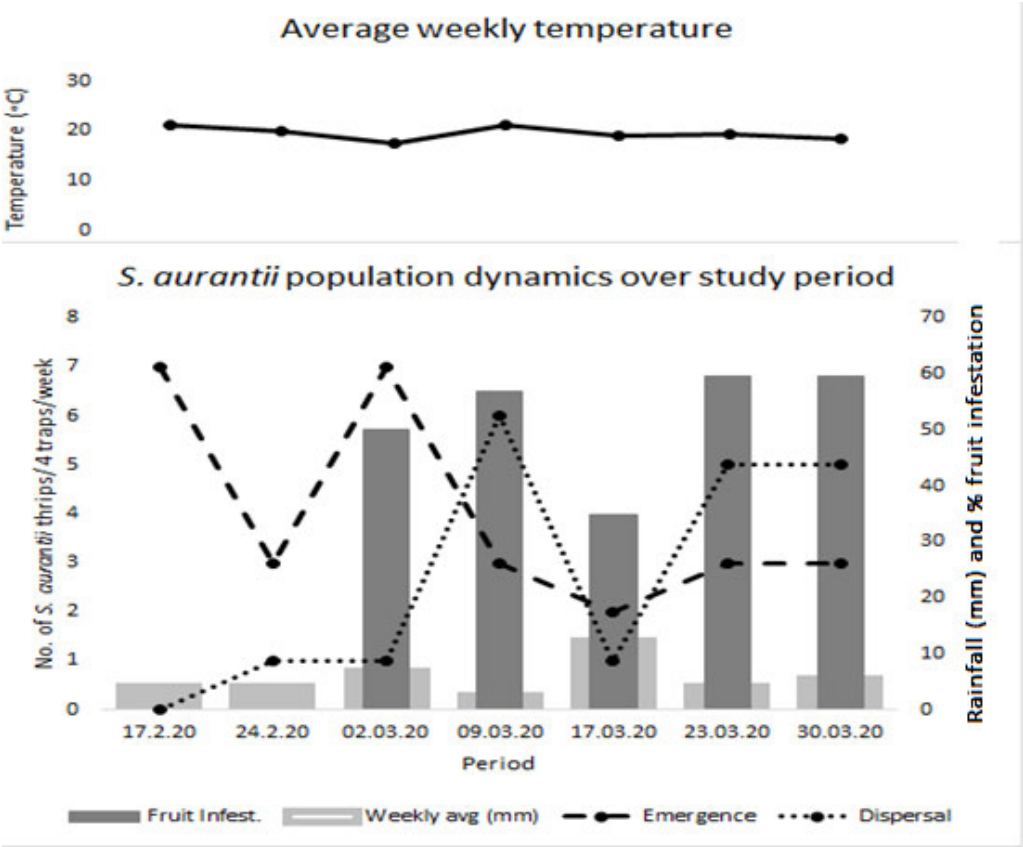


**Figure 7.3.** Population dynamics of *S. aurantii* in untreated control 'Carmen®-Hass'.

### 7.3.4 Fruit infestation index

To determine the dynamics of the larvae infesting fruits during the period from 2nd March to 30th March 2020, the number of larvae that emerged from 360 fruitlets were recorded. The baseline larvae per fruit densities in the field at the start of the assessment on the 2nd March, 2020 were estimated as the parameter intercept = 0.66 (95% CI: 0.32 to 0.96). The coefficient of the following week ending 09.03.20 was estimated as = 0.25 (95% CI: -0.17 to 0.69) representing an increase of 28.78 % in the number of larvae that emerged from the fruitlets. When period 17.03.20 was compared to the baseline (02.03.20), the coefficient of the 17th March was estimated at -0.52 (95 % CI: -1.06 to 1.4 x $10^{-3}$) which represents a decline of larvae emerging from the fruitlets by 40.29 %. The subsequent weeks ending 23rd March and 30th March showed a decline of 25.18 % and 28.06 %, respectively, when compared to the baseline population of the week ending 2nd March, 2020 (**Table 7.5**).

**Table 7.5.** Summary of the estimated fruit larval emergence parameter values of the GLM model.

| **Parameter** | **Estimate** | ***SE*** | **t value** | **2.5 %** | **97.5 %** |
|---|---|---|---|---|---|
| Baseline_02.03.20 | 0.66 | 0.16 | 4.01 | 0.32 | 0.96 |
| Period_09.03.20 | 0.25 | 0.22 | 1.16 | -0.17 | 0.69 |
| Period_17.03.20 | -0.52 | 0.27 | -1.92 | -1.06 | $-1.4x10^{-3}$ |
| Period_23.03.20 | -0.29 | 0.25 | -1.16 | -0.79 | 0.20 |
| Period_30.03.20 | -0.33 | 0.25 | -1.30 | -0.84 | -0.16 |

**Note**: The 95% confidence interval for each variable was calculated using 'Wald' function of the R lme4 package.

The differences in the estimated coefficients for the different periods (pairwise) were compared using the Wald $\chi^2$ test and the results summarised in Appendix 4. Larval emergence from fruit for the week ending 02.03.20 was not significantly different to that of the following week ($\chi^2 = 1.3$, df = 1, $P(> \chi^2) = 0.26$) but significantly different to the other periods. The results for the week ending 09.03.20 were not significantly different to all the periods, with the exception of the first week ending 02.03.20, whilst the results of the following week (17.03.20), were not significantly different from the results for the week ending 23.03.20 ($\chi^2 = 0.63$, df = 1, $P(>\chi^2) = 0.43$) and the last week 30.03.20 ($\chi^2 = 0.42$, df = 1, $P(>\chi^2) = 0.52$). The emergence of larvae per fruit for the week ending 23.03.20 were not significantly different to those

recorded for the 17th and the 30th of March 2020 ($\chi^2$ = 0.63, df = 1, $P(>\chi^2)$ = 0.43 and $\chi^2$ = .021, df = 1, $P(>\chi^2)$ = 0.88, respectively).

The chi-squared test statistic of 11.5 with 4 degrees of freedom associated with a $P$ = 0.022, suggests that the differences in fruit infestation index coefficients over the sampling periods are significant. The fruit infestation coefficient for the week ending 9th March 2020, a week after the study was initiated, showed an increase in fruit infestation of about 32.26 % from the baseline coefficient ($-1.2 \times 10^{-16}$), but was not different to that period ($\chi^2$ = 0.28, df = 1, $P(>\chi^2)$ = 0.6) or the other 3 periods (**Table 7.6**). The coefficient for the week ending 9th March (0.28) is significantly different to the coefficient of the following weeks ending 17th March ($\chi^2$ = 7.0, df = 1, $P(>\chi^2)$ = 0.008), but was significantly different to all the other periods. The coefficient of the week ending 17th March (-0.63) when compared to the coefficient of the baseline fruit infestation index ($-1.2 \times 10^{-16}$) represents a decline of 46.81 % but this difference is not statistically significant ($\chi^2$ = 1.4, df = 1, $P(>\chi^2)$ = 0.24). Seven of the 10 pairwise comparisons in the fruit infestation indices in the period coefficients are not significantly different from each other. The summary of all period coefficient comparisons are presented in Appendix 5.

**Table 7.6.** Summary of the estimated parameter values of the fruit infestation indices (GLM).

| **Parameter** | **Estimate** | ***SE*** | ***z value*** | **2.50 %** | **97.50 %** |
|---|---|---|---|---|---|
| Baseline_02.03.20 | $-1.2 \times 10^{-16}$ | 0.24 | 0.00 | -0.46 | 0.46 |
| Period_09.03.20 | 0.28 | 0.34 | 0.84 | -0.38 | 0.94 |
| Period_17.03.20 | -0.63 | 0.34 | -1.85 | -1.31 | 0.03 |
| Period_23.03.20 | 0.28 | 0.34 | 0.84 | -0.38 | 0.94 |
| Period_30.03.20 | 0.39 | 0.34 | 1.17 | -0.26 | 1.06 |

When the percent fruit infestation levels (ratio of fruits infested) and the fruit infestation indices (mean number of larvae that emerge per fruit) are considered together, there appears to be a positive association ($r$ = 0.65). The coefficient of determination, $R^2$ = 0.43, implying that 43 % of the variation in the number of larvae/fruitlet can be explained by the percent fruit infestation. At the beginning of the study, a fruit infestation level of 50.00 % and a mean larvae per fruit value of 1.93 ± 0.34 was recorded The following week ending 9th March 2020, the fruit infestation level increased to 56.94 % and the number of larvae per fruit increased to 2.49 ± 0.42. The week ending 17th March saw a marked decline in both the fruit infestation level and the mean number of larvae per fruit to 34.42 % and 1.15 ± 0.27, before increasing to 59.72 %

and 1.56 ± 0.23, respectively. For the week ending 30th March 2020, the percent fruit infestation level levelled off at 59.72 % and a slight decline in the mean larvae per fruit to 1.39 ± 0.20 was recorded (**Table 7.7**; **Figure 7.4**).

**Table 7.7.** Summary of mean larvae that emerged per fruit in March 2020.

| Period | No. of emerged larvae | No. of fruits infested | % Fruit infestation | Mean larvae/fruit ± *S.E* |
|---|---|---|---|---|
| 02.03.20 | 139 | 36 | 50 | $1.93 \pm 0.34^{a}$ |
| 09.03.20 | 179 | 41 | 56.94 | $2.49 \pm 0.42^{a}$ |
| 17.03.20 | 83 | 25 | 34.72 | $1.15 \pm 0.27^{b}$ |
| 23.03.20 | 112 | 43 | 59.72 | $1.56 \pm 0.23^{b}$ |
| 30.03.20 | 72 | 34 | 59.72 | $1.39 \pm 0.20^{b}$ |

**Note**. Periods sharing a letter in superscript are not significantly different at the 0.05 level according to *Wald* $\chi^2$ *test* statistics of the predictors, $P < 0.05$.

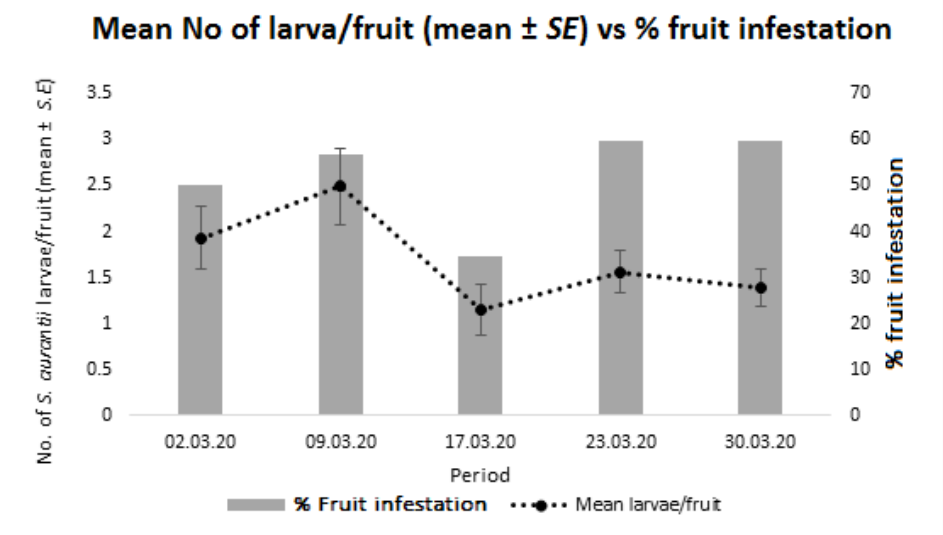


**Figure 7.4.** The mean number of *S. aurantii* larvae that emerged per fruit in relation to the corresponding fruit infestation indices.

### 7.3.5 Sites of pupation in the field

The mean number of larvae per fruit ranged from 1.4 to 2.5 whilst the estimated number of larvae per tree ranged from 46.8 to 164.4. The estimated number of adults emerging per tree ranged from 24.1 to 84.6 in March 2020. However, the actual number of *S. aurantii* caught (dispersing and emerging) were less than 10 per tree per week throughout the study period. The model results are presented in **Table 7.8**, with the graphical representation in **Figure 7.5**. It is important to note that the model presents a 'rough guide' to the number of thrips that can emerge from the fruits. The actual number of *S. aurantii* thrips in the canopy will likely be far greater than proposed in this model as other already existing life stages not captured in this

model (such as 1st and 2nd instar larvae, pupae and adults) will undoubtedly impact the population dynamics.

**Table 7.8.** Summary of actual and hypothesised number of larvae and adults per tree per week.

| Aspect | 09-Mar-20 | 17-Mar-20 | 23-Mar-20 | 30-Mar-20 |
|---|---|---|---|---|
| Mean No. of fruitlets/tree | 86 | 117 | 177 | 113.75 |
| Mean No. of *S. aurantii* larva/fruitlet | 2.49 | 1.153 | 1.56 | 1.39 |
| Percent fruit infestation | 56.94 | 34.72 | 59.72 | 59.72 |
| Estimate No. of infested fruit/tree | 48.97 | 40.63 | 105.71 | 67.93 |
| Estimate No. of larvae/tree | 121.75 | 46.83 | 164.44 | 94.35 |
| % adult emergence | 51.43 | 51.43 | 51.43 | 51.43 |
| Estimated No. of adults emerging/tree | 62.62 | 24.09 | 84.57 | 48.53 |

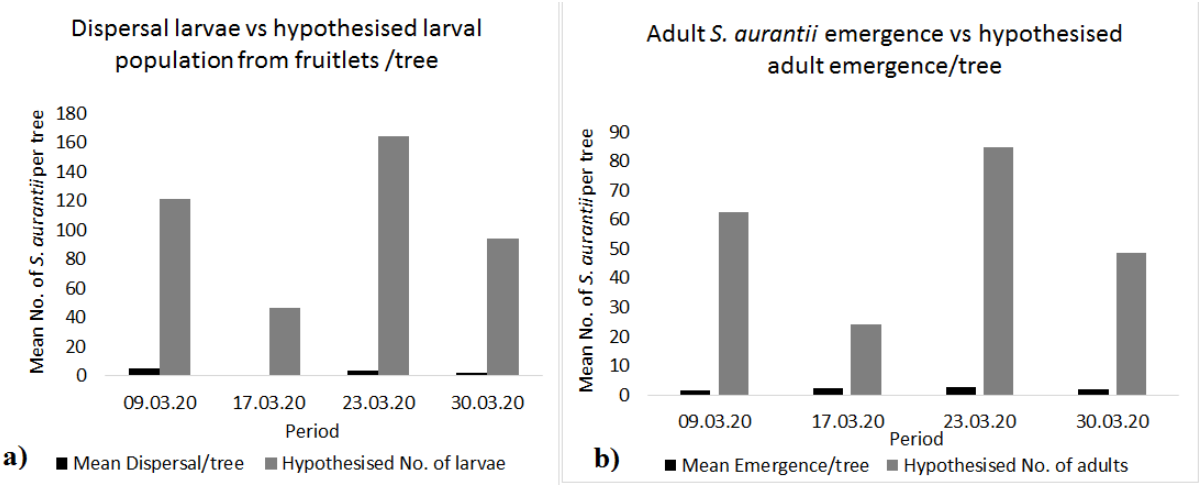


**Figure 7.5.** Model estimates a) Dispersal larvae vs hypothesised larval population b) Adult *S. aurantii* from D/E traps compared to hypothesised means estimated from fruit emergence.

### 7.3.6 Sites of pupation in the laboratory

When the pupation data (without netting) were pooled, 11.58 % of *S. aurantii* pupated on the fruitlet surface, whilst 44.21 % dropped to the floor of the incubation unit to pupate and an equal number (44.21 %) climbed to the top of the incubation unit and incubated in the crevices and folds on the roof. This represents 55.79 % of the pupae pupating above the floor of the incubation chamber. When the nylon netting was applied, 67.20 % of the thrips pupated on the

roof while 18.40 % and 14.40 % pupated on the floor and on the fruit respectively. This marks a 22.99 % increase in the number that pupated on the roof and an overall 81.60 % that pupated away from the floor (**Table 7.9**).

**Table 7.9.** Pupation site summary.

| Date | Fruit | Floor | Roof | Total |
|---|---|---|---|---|
| a) 09.03.20 | 16 (11.11) | 69 (47.92) | 59 (40.97) | 144 |
| b) 17.03.20 | 6 (13.04) | 15 (32.61) | 25 (54.35) | 46 |
| **Pooled (a & b)** | **22 (11.58)** | **84 (44.21)** | **84 (44.21)** | **190** |
| c) 23.03.20 | 6 (8.70) | 11 (15.94) | 52 (75.36) | 69 |
| d) 30.03.20 | 12 (21.43) | 12 (21.43) | 32 (57.14) | 56 |
| **Pooled (c & d)** | **18 (14.40)** | **23 (18.40)** | **84 (67.20)** | **125** |

**Note.** The value in parenthesis is the percent pupation: a) & b) No shade netting applied; c) & d) with shade netting.

The Poisson regression model estimates indicate that the overall effect of pupation sites was statistically significant ($\chi^2$ = 20.5, df = 5, $P(> \chi^2)$ = 0.001) (**Table 7.10**). Nine of the 15 pairwise comparisons were significantly different. Of particular note is the coefficient of the number of larvae that pupated on the roof with the nylon netting that was significantly different to the coefficient of the number that pupated on the floor of the incubation chamber ($\chi^2$ = 7.9, df = 1, $P(>\chi^2)$ = 0.005). In addition, the number which pupated on the incubation floor were not significantly different to those which pupated on the roof of the incubation units not covered by the nylon shade cloth ($\chi^2$ = 3.6, df = 1, $P$ $(>\chi^2)$ = 0.059). A summary of the pairwise coefficient comparisons for the pupation sites is reported in Appendix 6.

**Table 7.10.** Summary of the estimated parameter values of pupation sites using the generalized linear model (GLM)

| Parameter | Estimate | *SE* | t value | 2.50 % | 97.50 % |
|---|---|---|---|---|---|
| Baseline_Floor (without netting) | 0.65 | 0.10 | 6.76 | 0.45 | 0.83 |
| Floor (with netting) | -0.51 | 0.21 | -2.46 | -0.93 | -0.12 |
| Fruit (with netting) | -0.46 | 0.23 | -2.04 | -0.94 | -0.037 |
| Fruit (without netting) | 0.14 | 0.21 | 0.68 | -0.29 | 0.54 |
| Roof (with netting) | 0.047 | 0.14 | 0.34 | -0.22 | 0.31 |
| Roof (without netting) | 0.26 | 0.14 | 1.91 | -0.008 | 0.52 |

**Note**: The 95% confidence interval for each variable was calculated using 'Wald' function of the R lme4 package.

## 7.4 Discussion

Avocadoes grown in warm, subtropical climates exhibit one winter and three summer vegetative flushes (Salazar-García *et al.* 2006). 'Carmen®-Hass' is unique in having indeterminate bloom and vegetative flushes (Illsley-Granich *et al.* 2011). *Scirtothrips aurantii* thrips were recorded both in D/E traps and on infested fruitlets throughout the critical early fruit development period, demonstrating the sustained prevalence of the pest in avocado trees during this period. The critical early fruit development period coincided with the summer vegetative flush when young leaves were actively growing. During this period, *S. aurantii* individuals are sustained on the young foliage, some of them shifting to feed and reproduce on actively developing fruits. The preference for young actively growing tissue has also been shown in the closely related *S. perseae,* in avocado (Hoddle *et al.* 2002) and *S. citri* in citrus (Flint 1991). The highest number of larvae per fruit emerged during the first 2 weeks of March with early fruit set and then declined in subsequent weeks. This seems to suggest that as the season progressed and the fruits became larger, the number of *S. aurantii* emerging from the fruit declined significantly.

The percent fruit infestation levels were generally high during the early fruit set period (34.72-59.72 %). Apart from one exception, fruit infestation levels of more than 50% were recorded throughout the trial. This implies that *S. aurantii* larvae emerged from at least 50 % of the avocado fruitlets. Bara & Laing (2019), in an earlier study on 'Pinkerton' avocado in KwaZulu-Natal, observed an infestation level of 4.82 %. 'Carmen®-Hass' is probably able to sustain high populations of *S. aurantii* owing to its indeterminate flush phenology (Illsley-Granich *et al.* 2011). The percent fruit infestation levels appear to be proportional to the number

of *S. aurantii* larvae that emerge from the fruitlets. The higher the fruit infestation, the greater the number of *S. aurantii* individuals that emerge from those fruitlets.

Kirk (1997) highlighted the role weather plays in controlling populations of thrips in field crops. The temperature range during the study was 17.51-21.15°C with a mean of 19.50°C. The effect of temperature on the dynamics of *S. aurantii* populations was not evident. In citrus, *S. aurantii* is known to inflict the greatest damage in summer when temperatures are generally high (Bedford 1943). At 15-20°C, *S. perseae* thrips exhibit their greatest fecundity (39.6 eggs per female) and larval to adult survivorship (41 %) (Hoddle 2002), whilst the optimum temperature for *S. citri* is reported to be 31°C (Munger 1942).

Rainfall may have played a more significant role. The highest rainfall (10.16 mm) recorded in the week ending 17$^{th}$ March 2020 coincided with the lowest fruit infestation index (34.72 %) and the number of larvae (1.15 ± 0.27) that emerged from the fruitlets. A sharp decline in the number of *S. aurantii* individuals caught in the D/E traps was also recorded during this period. Heavy rainfall has been shown to be detrimental to thrips populations (Kirk 1997), with Hall (1930) suggesting that the physical effect of rainfall action can either kill or dislodge *S. aurantii* individuals from the tree. Kirk (1997) further suggested that the mechanical action may also interfere with the movement and feeding of thrips because quiescent pupae may be drowned or trapped by moisture films and soil caking, while some of the washed-off larvae may not be able to return to the leaves and fruits. This may explain the abrupt reduction in fruit infestation levels during this week.

Acheampong *et al.* (2019) noted that *Metarhizium anisopliae* (Metchnikoff) Sorokin and *B. bassiana* were promising agents against soil dwelling stages of *S. aurantii* in citrus, whilst Lee *et al.* (2017) reported *B. bassiana* to be effective on tomato and cucumbers against soil dwelling *F. occidentalis.* In 2019 and 2020, field evaluations of the effectiveness of synthetic and biological control agents on soil dwelling stages of *S. aurantii* revealed that some control was possible as evidenced by slightly reduced populations. However, the overall *S. aurantii* populations were too low to draw any meaningful conclusion. The discrepancy between the low numbers caught in D/E traps is more apparent when compared to the estimated above ground populations. Whilst the fruit infestation derived estimates served only as a guide, the actual numbers of *S. aurantii* thrips in the canopy should likely be far greater, because other already existing individuals and life stages not captured by the fruit infestation index, such as the 1$^{st}$ and 2$^{nd}$ instar larvae on foliage, pupae and adults, will undoubtedly impact the population dynamics.

Dispersal/Emergence traps were not effective in monitoring *S. aurantii* populations in avocadoes during the early fruit development period, because the numbers collected in the traps were not representative of above ground populations. It is possible that *S. aurantii* modifies its behaviour according to the phenology of the host tree on which it develops. This would explain why more *S. aurantii* individuals were caught in D/E traps in citrus (Gilbert & Samways 2018) than in avocadoes. This is the first time D/E traps were trialled for use in monitoring *S. aurantii* populations in avocadoes. Fruit infestation indices and yellow sticky traps (Bara & Laing 2020) give a better indication of the population dynamics and the threat of thrips to the avocado crop. Further studies may be necessary to create a cultivar specific model that calculates the economic threshold levels from the fruit infestation indices.

One possible reason for the discrepancy in population numbers recorded using D/E traps and the fruit infestation index could be the site of pupation. Previous studies have varied in their estimates of where *Scirtothrips* spp. pupate (Gilbert & Samways 2018), and it is possible that the primary sites of pupation may be a function of the host species, host phenology and available pupation niches. Grout *et al.* (1986) estimated that only 33 % of *S. citri* adults emerged from the orchard floor, while Schweizer & Morse (1996) put the figure at 49–90 %. Bedford (1943) suggested that *S. aurantii* rarely pupated in the tree canopy, noting that mature larvae dropped from the tree into the leaf-litter, a view held by many scholars. However, under 'ideal' laboratory conditions, only 18.40 % pupated on the floor of the incubation units, while 81.60 % pupated away from the floor (67.20 % on the roof and 14.40 % in crevices/shaded areas on the fruit), demonstrating that *S. aurantii* actively seeks out dark, sheltered areas to pupate. It is possible that avocado trees provide favourable pupation niches in the canopy, due to their thicker foliage, larger leaves and rough tree bark, which encourage pupation in the canopy. The laboratory experiment strongly indicated that the prepupal and pupal stages largely take place in the tree canopy. The low percentage of *S. aurantii* pupating on the ground means that soil application of non-systemic insecticides would result in poor control, a view also shared by Grout *et al.* (1986) and Tanigoshi *et al.* (1982) on the control of *S. citri* in citrus.

## 7.5 Conclusion

The study confirmed a high incidence of *S. aurantii* populations in the early fruiting phase of 'Carmen®-Hass' and the effect of rainfall in modifying the thrips populations. However, the observed incidence of *S. aurantii* did not correlate with the low catches in dispersal/emergence traps and consequently, soil drenches of biologicals and synthetic insecticides did not provide good control of thrips. Under laboratory conditions, 81.60 % of mature larvae pupated away

from the floor of the incubation units, a result that suggests that most of the *S. aurantii* larvae pupate in the avocado tree canopy, making the efficacy of non-systemic soil insecticides unlikely to be satisfactory.

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# Chapter 8: Thesis overview

## 8.1 Introduction

The South African avocado industry is a multibillion-rand (ZAR), export-orientated industry employing tens of thousands of people. Several biotic and abiotic factors work to limit the production and export of quality avocado fruit. Understanding the role played by biotic and abiotic factors (particularly *Scirtothrips aurantii* Faure and *Bracharoa mixta* (Snellen)) in constraining production were the broad objectives of this study. The information generated in this study will contribute to the knowledge and management of *S. aurantii* Faure in avocado orchards. The main findings of the study, implications and gaps for future research are summarised in this chapter.

## 8.2 Determination of the natural host status of avocado fruit to pestiferous thrips (Thysanoptera: Thripidae) in KwaZulu-Natal, South Africa

Unsightly, 'alligator skin' scars were observed on avocado fruit and thrips were suspected. A study was conducted to investigate the thrips spectrum in avocado flowers and the role thrips play in scarring avocado fruit. *Frankliniella occidentalis* (Pergande), *Scirtothrips aurantii* Faure, *Thrips gowdeyi* (Bagnall), *Thrips pusillus* Bagnall, *Thrips tenellus* Trybom, *Haplothrips gowdeyi* (Franklin), *Haplothrips bedfordi* Jacot-Guillarmod and *Megalurothrips sjostedti* (Trybom) were consistently collected from flowering panicles from May to September 2018. The minute size of thrips (<1 mm) warranted an investigation on materials that could be used to contain the thrips for laboratory and field experiments. Insect screen (149 μm pore size), nylon netting (250 μm pore size), chiffon (210 μm pore size), voile (250 μm pore size), organza (500 μm pore size), tea filter paper (74 μm pore size) and coffee filter paper (53 μm pore size) were evaluated as thrips exclusion screens. Only coffee filter paper and tea filter paper successfully retained these thrips species and can therefore be used as thrips exclusion screens requiring confinement of thrips from avocado. Fruit sampling of naturally (field) infested avocado fruitlets revealed a 4.86 % infestation by *S. aurantii*. This is the first report to confirm the host status of avocado to *S. aurantii*. The confirmation of *S. aurantii*, an established and well-studied pest of citrus in South African avocado fruit, forms the basis for tailor made IPM programs in avocadoes and allows for parallels and comparisons to be drawn on its biology, ecology and management from the citrus industry.

### 8.3 Attractiveness of different coloured sticky traps to the South African citrus thrips (*Scirtothrips aurantii* Faure) in avocado, KwaZulu-Natal, South Africa

Monitoring an insect pest's presence and abundance is the first step in any integrated pest management program and coloured sticky traps provide a rapid and cost effective monitoring tool for various insect pests (Bashir *et al.* 2014). Yellow sticky traps were found to be effective in monitoring *S. aurantii* in avocado orchards. Attractiveness followed the order yellow > blue, white, clear > green, red, black, purple and orange. The incidence of this species was not restricted to the border or the interior regions but was randomly distributed in avocado orchards. This means when monitoring for the presence of *S. aurantii*, yellow sticky traps can be set-up randomly in an avocado orchard. *Scirtothrips aurantii* numbers were high during the spring flush and in particular, during the economically critical early fruit development period. The numbers gradually declined after the spring flush only to pick up in summer. Therefore, the spring flush and early fruiting period present a small window of opportunity when control measures can be implemented (if necessary) to prevent scarring damage on the young developing fruit.

### 8.4 An investigation into the role played by endophytic *Cladosporium* spp., wind abrasion and thrips (Thysanoptera: Thripidae) in scarring avocado fruit

Several diseases are known to inflict serious economic damage to avocado fruit. Among them is avocado scab (*Sphaceloma perseae* Jenkins) which shows as corky, raised, oval or irregular shaped brown to purplish-brown spots similar to those caused by thrips damage. Fruits were subjected to abrasion and fungal inoculation treatments to determine fruit response and the results compared to typical field damaged fruit signs. Judging by the fruit scars, *Sphaceloma perseae* was suspected as it has been recorded as being present in South Africa (Jenkins 1934) and the documented signs were consistent with scarring damage observed on the fruits. However, morphological and DNA fingerprinting using universal primers did not confirm presence of the fungus. Several endophytic *Cladosporium* spp. were isolated from scarred avocado fruit and pathogenicity tested. Pathogenicity by endophytic *Cladosporium* spp. was not demonstrated under field conditions when inoculated directly on bruised and intact fruit. Wind induced abrasion and insects, particularly thrips (Thysanoptera: Thripidae) were confirmed to be the main agents responsible for scarring avocado fruit in South Africa. Growers are advised to use wind breaks and minimise thrips damage.

### 8.5 Susceptibility of avocado fruit to *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and wind scarring damage in Limpopo and KwaZulu-Natal Provinces of South Africa

Thrips and wind abrasion were identified as major quality constraints, accounting for levels of 30 % scarring damage, a loss factor of 13.72 % and a combined revenue loss of 5.57 %. Revenue losses in the order of 1.49 % are made annually to *S. aurantii* downgrading (3.86 % loss factor). Avocado growers are therefore advised to take steps to minimise wind damage by siting avocado orchards away from prevailing and dominant winds, as well as putting in place suitable windbreaks. Cultivar differences were also observed, with the cultivar 'Pinkerton' showing the greatest susceptibility to scarring damage by both wind and *S. aurantii*, whilst the cultivar 'Carmen®-Hass' showed a natural predisposition to higher levels of thrips damage. The presence of macadamia trees near avocado trees exposes avocado fruit to higher levels of *S. aurantii* attack, and therefore damage. Growers are advised to take cognisance of the cultivar susceptibility in selecting cultivars, and to avoid growing avocado trees in close proximity to macadamia trees.

### 8.6 First report of tussock moths (*Bracharoa mixta* (Snellen) (Lepidoptera: Erebidae)) scarring avocado fruit in KwaZulu-Natal, South Africa

This is the first report of a tussock moth scarring avocado leaves and fruit in South Africa and adds to the current knowledge base of the avocado industry. *Bracharoa mixta* (Lepidoptera: Erebidae) was identified morphologically. A DNA barcode was deposited with GenBank (GenBank MN527963), and voucher specimens (voucher I.D AcP 9636) were deposited with the PPRI, ARC for future referencing. Potential revenue losses of up to ZAR1,352.90/t (2.26 % revenue loss) through downgrading are possible because this insect is capable of causing economic loss and sporadic, isolated outbreaks. The implications of these findings are that the insect has been identified, characterized and its biology documented. This will aid in rapid detection and implementation of IPM should there be an outbreak in the future.

### 8.7 Biological and chemical control of soil-dwelling stages of the South African citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae), in avocado, *Persea americana* Mill. (Lauraceae)

*Scirtothrips aurantii* individuals were consistently collected from naturally infested fruitlets and dispersal/emergence traps in two avocado seasons, 2019 and 2020, confirming the host status of avocado to this thrips species. A high prevalence of *S. aurantii* populations in the early fruiting phase of 'Carmen®-Hass', and the effect of rainfall in modifying the thrips populations,

were documented. This study confirmed that the early fruiting period is a critical period for controlling the insect. Dispersal/emergence traps were not effective in monitoring *S. aurantii* populations during the early fruit development period because the numbers collected in the traps were not representative of above ground populations. Fruit infestation indices and yellow sticky traps give a better indication of the population dynamics, and growers and extension officers are encouraged to use these for monitoring. Attempts to control the population of thrips in avocado orchards using soil drenches of biologicals and synthetic insecticides was not successful, probably because only a small percentage of pupae dropped to the soil and pupated in the ground (18.40 % under laboratory conditions). The majority of the thrips completed their life cycle in the tree canopy. The low percentage of *S. aurantii* pupating on the ground suggests that soil applications of non-systemic insecticides will result in poor control, a view also shared by Grout *et al.* (1986) and Tanigoshi *et al.* (1982) on *S. citri* in citrus.

### 8.8 Further research

1) Prior to this study, *H. haemorrhoidalis* and *S. rubrocinctus w*ere described as the main thrips species in avocado fruit in South Africa (Steyn *et al.* 1993). However, they were not collected throughout the study. The fate of these species in avocado orchards is unknown and needs further investigation. It is possible that *S. aurantii* has displaced these two thrips species and has established itself as the dominant thrips species of avocado in South Africa.
2) Studies on the determination of economic threshold levels using fruit infestation indices and yellow sticky traps will be beneficial. This will enable growers to rapidly determine *S. aurantii* populations and action appropriate management measures when economic threshold levels are breached. Fruit infestation indices have the additional advantage of giving levels of infestation based on the number of larvae emerging from the fruits - the primary focus of the monitoring protocol.
3) The fruit scars observed on naturally scarred fruit are visually similar to those described for the disease avocado scab caused by *Sphaceloma perseae. S. perseae* was reported as being present in South Africa in the 1930's (Jenkins 1934) but has not been isolated since. This is a phytosanitary issue hampering export of South African avocado fruit to some markets. It would be worthwhile to investigate the presence or absence of the pathogen in South Africa using advanced molecular techniques, and culture-free techniques such as real-time PCR.

4) The practical application of biologicals in the tree canopy is a research area that could provide an IPM solution for the management of thrips in avocado orchards. This is paramount as avocado fruit are subject to strict pesticide MRL's and conventional chemical control has undesirable consequence for the environment, especially honeybees, which are crucial to the pollination of avocado crops. Studies on how to operationalize field application of environmentally friendly alternatives in the canopy is thus worthwhile.

**References**

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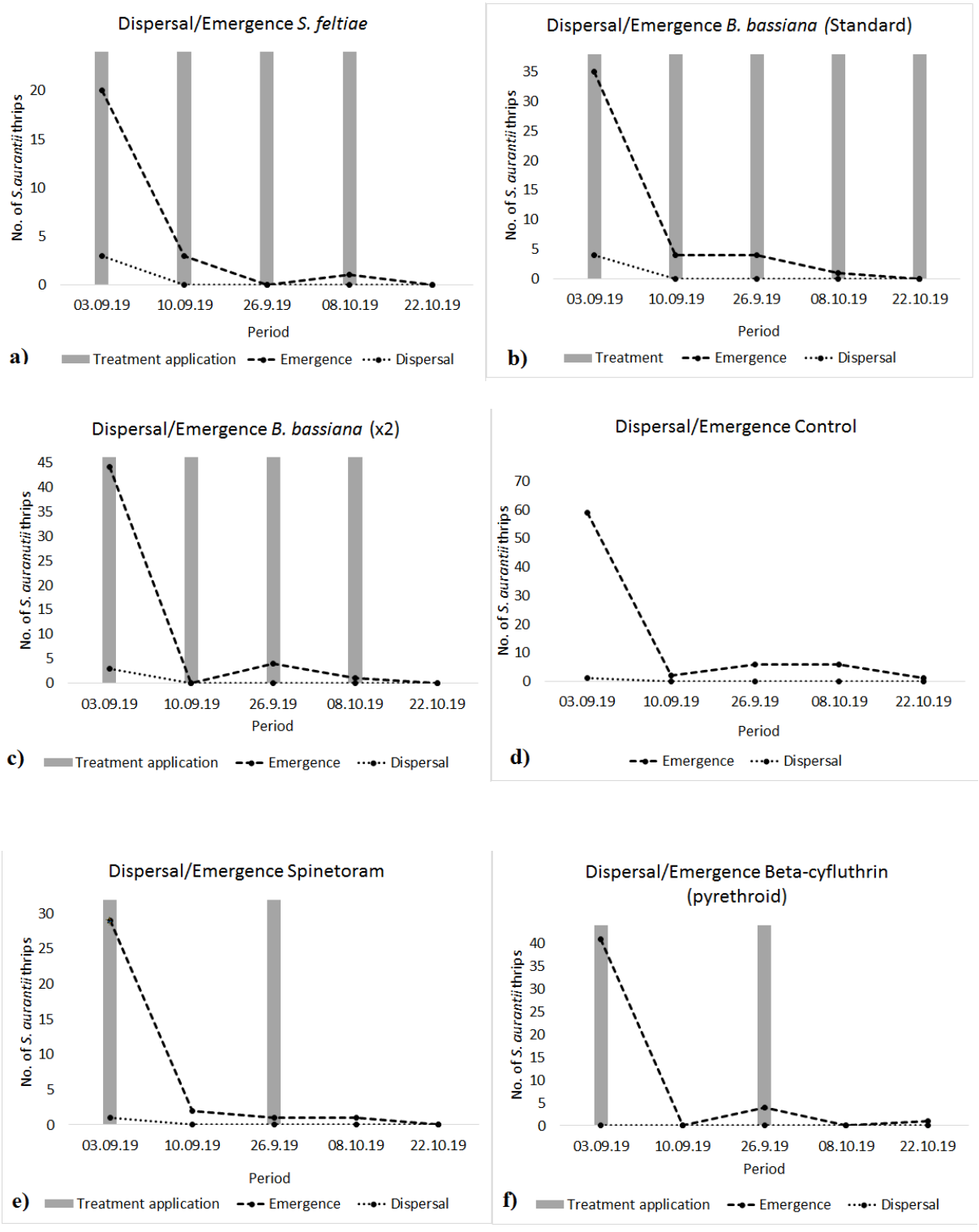
TANIGOSHI, L.K., MORENO, D.S., NISHIO-WONG, J.Y. & FARGERLUND, J. 1982. Soil applications of insecticides for control of *Scirtothrips citri* (Moulton)(Thysanoptera, Thripidae) on citrus. *Zeitschrift für Angewandte Entomologie* **93**: 17-24.

# Appendices

## Appendix 1

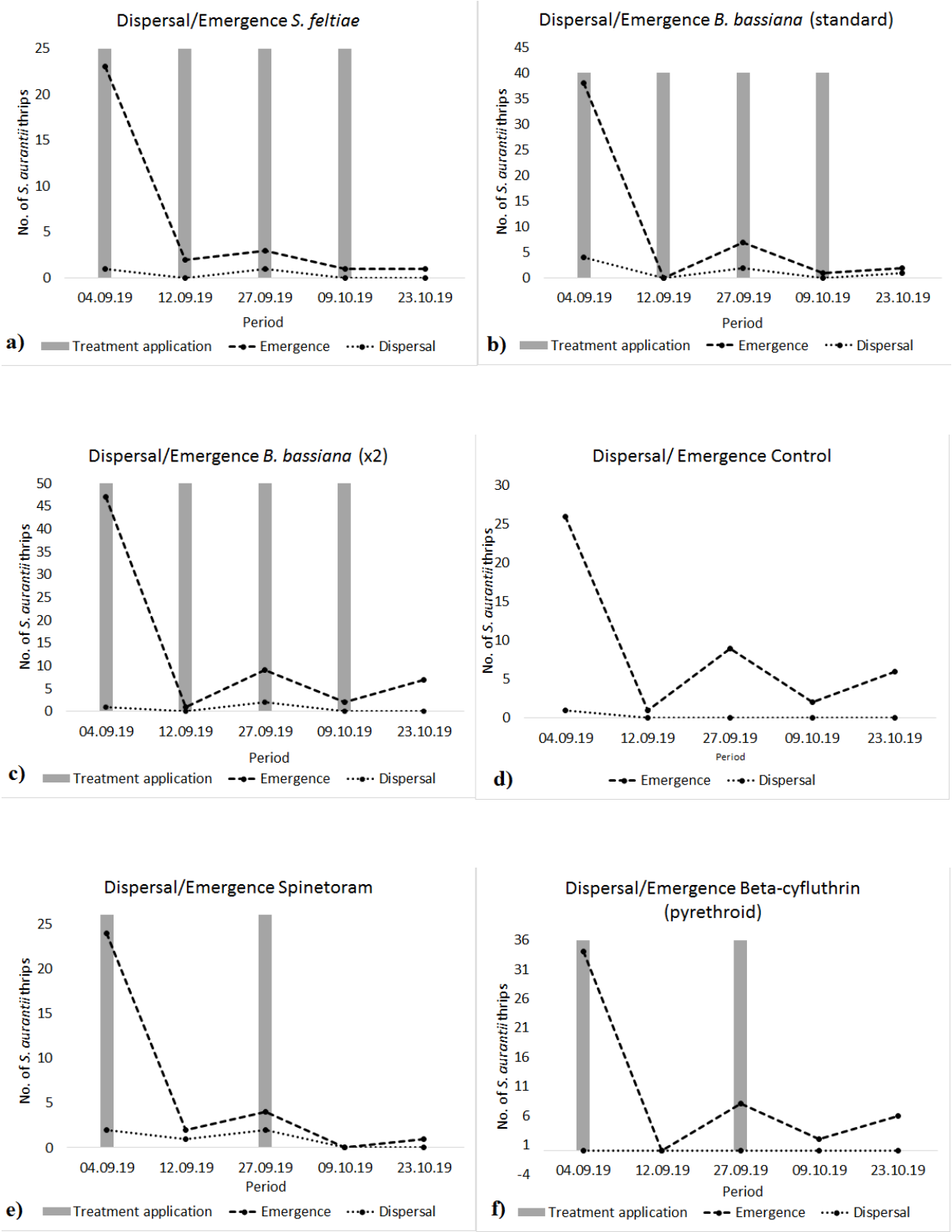
**Baynesfield Dispersal/Emergence Summary (No. of *S. aurantii* per 4 traps per week).**




Summary of Dispersal/Emergence data at Baynesfield 'Pinkerton' in 2019: a) *S. feltiae;* b) *B. bassiana* (Standard); c) *B. bassiana* (x2 conc.); d) Control; e) Spinetoram; f) Beta-cyfluthrin.

## Appendix 2

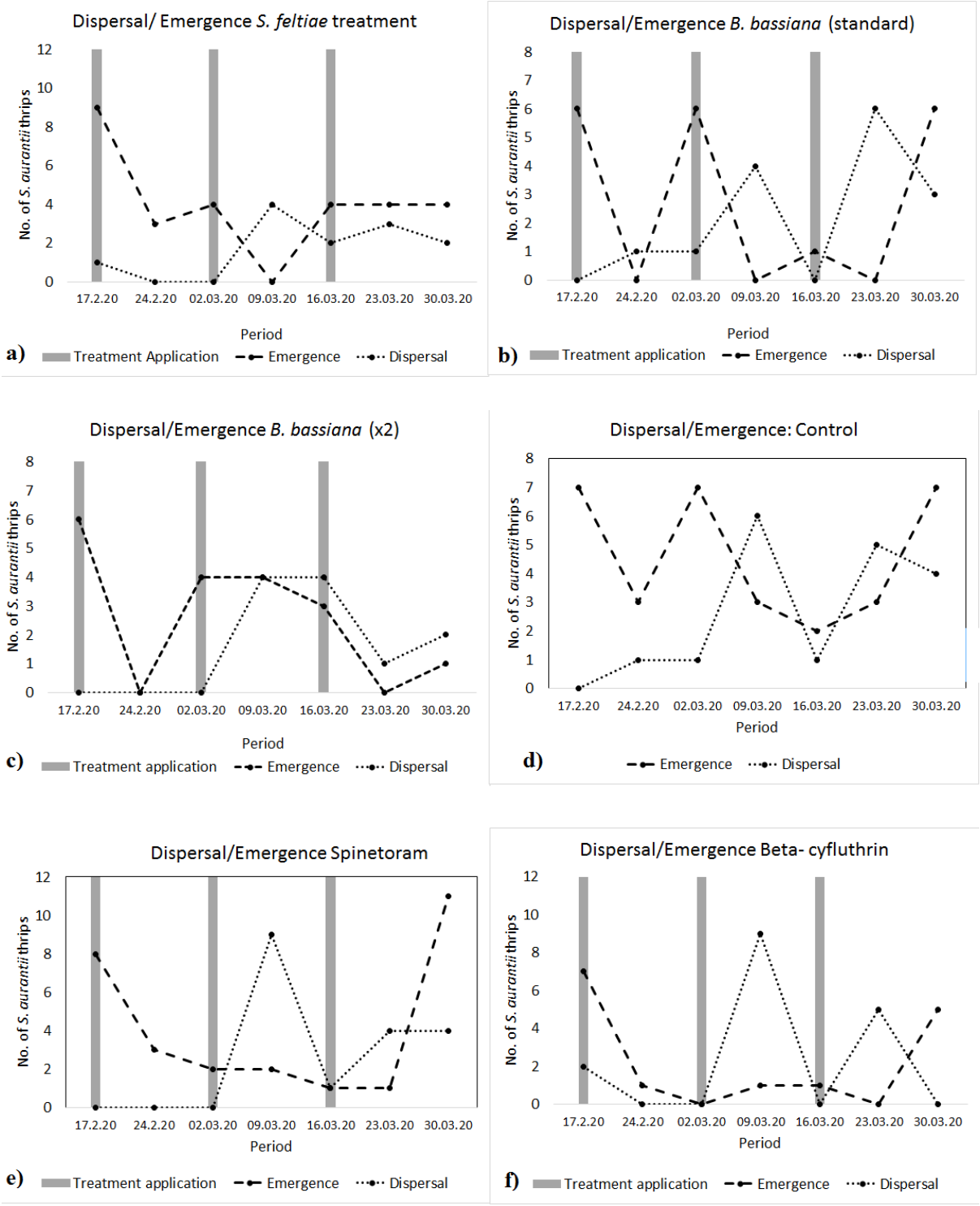
**Conlink Dispersal/ Emergence Summary (No. of *S. aurantii* per 4 traps per week).**




Summary of Dispersal/Emergence data at Conlink 'Pinkerton' 2019: a) *S. feltiae;* b) *B. bassiana* (Standard); c) *B. bassiana* (X2 conc.); d) Control; e) Spinetoram; f) Beta-cyfluthrin.

## Appendix 3

**Everdon Dispersal/Emergence Summary (No. of *S. aurantii* per 4 traps per week).**




Summary of Dispersal/Emergence data at Everdon 'Carmen®-Hass' in 2020: a) *S. feltiae;* b) *B. bassiana* (Standard); c) *B. bassiana* (x2 conc.); d) Control; e) Spinetoram; f) Beta-cyfluthrin.

## Appendix 4

**Summary of larval emergence pairwise coefficient comparisons for the period.**

| Period | 02.03.20 | 09.03.20 | 17.03.20 | 23.03.20 | 30.03.20 |
|---|---|---|---|---|---|
| 02.03.20 | | $\chi^2 = 1.3$, df = 1, $P(>\chi^2) = 0.26$ | $\chi^2 = 9.0$, df = 1, $P(>\chi^2) = 0.0027$ | $\chi^2 = 6.3$, df = 1, $P(>\chi^2) = 0.012$ | $\chi^2 = 6.7$, df = 1, $P(>\chi^2) = 0.0095$ |
| 09.03.20 | $\chi^2 = 1.3$, df = 1, $P(>\chi^2) = 0.26$ | | $\chi^2 = 9.0$, df = 1, $P(>\chi^2) = 0.0028$ | $\chi^2 = 5.2$, df = 1, $P(>\chi^2) = 0.023$ | $\chi^2 = 5.8$, df = 1, $P(>\chi^2) = 0.016$ |
| 17.03.20 | $\chi^2 = 9.0$, df = 1, $P(>\chi^2) = 0.0027$ | $\chi^2 = 9.0$, df = 1, $P(>\chi^2) = 0.0028$ | | $\chi^2 = 0.63$, df = 1, $P(>\chi^2) = 0.43$ | $\chi^2 = 0.42$, df = 1, $P(>\chi^2) = 0.52$ |
| 23.03.20 | $\chi^2 = 6.3$, df = 1, $P(>\chi^2) = 0.012$ | $\chi^2 = 5.2$, df = 1, $P(>\chi^2) = 0.023$ | $\chi^2 = 0.63$, df = 1, $P(>\chi^2) = 0.43$ | | $\chi^2 = 0.021$, df = 1, $P(>\chi^2) = 0.88$ |
| 30.03.20 | $\chi^2 = 6.7$, df = 1, $P(>\chi^2) = 0.0095$ | $\chi^2 = 5.8$, df = 1, $P(>\chi^2) = 0.016$ | $\chi^2 = 0.42$, df = 1, $P(>\chi^2) = 0.52$ | $\chi^2 = 0.021$, df = 1, $P(>\chi^2) = 0.88$ | |

**Note**. Levels of significance determined using *Wald* $\chi^2$ *test* statistics of the predictors, $P < 0.05$.

## Appendix 5

**Summary of pairwise coefficient comparisons of fruit infestation indices.**

| Period | 02.03.20 | 09.03.20 | 17.03.20 | 23.03.20 | 30.03.20 |
|---|---|---|---|---|---|
| 02.03.20 | | $\chi^2 = 0.28$, df = 1, $P(>\chi^2) = 0.6$ | $\chi^2 = 1.4$, df = 1, $P(>\chi^2) = 0.24$ | $\chi^2 = 0.28$, df = 1, $P(>\chi^2) = 0.6$ | $\chi^2 = 0.55$, df = 1, $P(>\chi^2) = 0.46$ |
| 09.03.20 | $\chi^2 = 0.28$, df = 1, $P(>\chi^2) = 0.6$ | | $\chi^2 = 7.0$, df = 1, $P(>\chi^2) = 0.008$ | $\chi^2 = 2.7e\text{-}32$, df = 1, $P(>\chi^2) = 1.0$ | $\chi^2 = 0.11$, df = 1, $P(>\chi^2) = 0.74$ |
| 17.03.20 | $\chi^2 = 1.4$, df = 1, $P(>\chi^2) = 0.24$ | $\chi^2 = 7.0$, df = 1, $P(>\chi^2) = 0.008$ | | $\chi^2 = 7.0$, df = 1, $P(>\chi^2) = 0.008$ | $\chi^2 = 8.8$, df = 1, $P(>\chi^2) = 0.003$ |
| 23.03.20 | $\chi^2 = 0.28$, df = 1, $P(>\chi^2) = 0.6$ | $\chi^2 = 2.7e\text{-}32$, df = 1, $P(>\chi^2) = 1.0$ | $\chi^2 = 7.0$, df = 1, $P(>\chi^2) = 0.008$ | | $\chi^2 = 0.11$, df = 1, $P(>\chi^2) = 0.74$ |
| 30.03.20 | $\chi^2 = 0.55$, df = 1, $P(>\chi^2) = 0.46$ | $\chi^2 = 0.11$, df = 1, $P(>\chi^2) = 0.74$ | $\chi^2 = 8.8$, df = 1, $P(>\chi^2) = 0.003$ | $\chi^2 = 0.11$, df = 1, $P(>\chi^2) = 0.74$ | |

**Note**. Levels of significance determined using *Wald* $\chi^2$ *test* statistics of the predictors, $P < 0.05$.

## Appendix 6

**Summary of pairwise coefficient comparisons of pupation sites under laboratory conditions.**

| Parameter | Floor (without netting) | Floor (with netting) | Fruit (with netting) | Fruit (without netting) | Roof (with netting) | Roof (without netting) |
|---|---|---|---|---|---|---|
| **Floor (without netting)** | | $\chi^2 = 19.0$, df = 1, $P(>\chi^2) = 1.3e\text{-}05$ | $\chi^2 = 15.5$, df = 1, $P(>\chi^2) = 8e\text{-}05$ | $\chi^2 = 3.6$, df = 1, $P(>\chi^2) = 0.059$ | $\chi^2 = 7.9$, df = 1, $P(>\chi^2) = 0.005$ | $\chi^2 = 3.3$, df = 1, $P(>\chi^2) = 0.069$ |
| **Floor (with netting)** | $\chi^2 = 19.0$, df = 1, $P(>\chi^2) = 1.3e\text{-}05$ | | $\chi^2 = 0.024$, df = 1, $P(>\chi^2) = 0.88$ | $\chi^2 = 6.2$, df = 1, $P(>\chi^2) = 0.013$ | $\chi^2 = 7.2$, df = 1, $P(>\chi^2) = 0.0073$ | $\chi^2 = 13.7$, df = 1, $P(>\chi^2) = 0.00021$ |
| **Fruit (with netting)** | $\chi^2 = 15.5$, df = 1, $P(>\chi^2) = 8e\text{-}05$ | $\chi^2 = 0.024$, df = 1, $P(>\chi^2) = 0.88$ | | $\chi^2 = 4.7$, df = 1, $P(>\chi^2) = 0.03$ | $\chi^2 = 5.0$, df = 1, $P(>\chi^2) = 0.025$ | $\chi^2 = 10.0$, df = 1, $P(>\chi^2) = 0.0015$ |
| **Fruit (without netting)** | $\chi^2 = 3.6$, df = 1, $P(>\chi^2) = 0.059$ | $\chi^2 = 6.2$, df = 1, $P(>\chi^2) = 0.013$ | $\chi^2 = 4.7$, df = 1, $P(>\chi^2) = 0.03$ | | $\chi^2 = 0.21$, df = 1, $P(>\chi^2) = 0.65$ | $\chi^2 = 0.3$, df = 1, $P(>\chi^2) = 0.58$ |
| **Roof (with netting)** | $\chi^2 = 7.9$, df = 1, $P(>\chi^2) = 0.005$ | $\chi^2 = 7.2$, df = 1, $P(>\chi^2) = 0.0073$ | $\chi^2 = 5.0$, df = 1, $P(>\chi^2) = 0.025$ | $\chi^2 = 0.21$, df = 1, $P(>\chi^2) = 0.65$ | | $\chi^2 = 2.4$, df = 1, $P(>\chi^2) = 0.12$ |
| **Roof (without netting)** | $\chi^2 = 3.3$, df = 1, $P(>\chi^2) = 0.069$ | $\chi^2 = 13.7$, df = 1, $P(>\chi^2) = 0.00021$ | $\chi^2 = 10.0$, df = 1, $P(>\chi^2) = 0.0015$ | $\chi^2 = 0.3$, df = 1, $P(>\chi^2) = 0.58$ | $\chi^2 = 2.4$, df = 1, $P(>\chi^2) = 0.12$ | |

**Note**. Levels of significance determined using *Wald* $\chi^2$ *test* statistics of the predictors, $P < 0.05$.