The effect of orchard sanitation and predatory ants on the eclosion of the internal feeding pests and Oriental fruit fly, in South Africa

by

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ABSTRACT

There are several pests of phytosanitary concern in the citrus industry in South Africa. Orchard sanitation can play an important role in suppressing the populations of these pests, however there are little data on the efficacy of sanitation techniques. Therefore, the current study investigated the effect of fruit disposal techniques and burying depths on the eclosion of the most important pests of citrus in South Africa, false codling moth *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), Mediterranean fruit fly or Medfly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), Natal fruit fly *Ceratitis rosa* Karsh (Diptera: Tephritidae), and Oriental fruit fly *Bactrocera dorsalis* Hendel (Diptera: Tephritidae). Abscised *C. sinensis* fruits were inoculated with larvae of *T. leucotreta*, and eggs of *C. capitata*, *C. rosa*, and *B. dorsalis,* before being disposed as pulped, or whole, and buried at different depths (0 cm, 5 cm, 25 cm, and 50 cm). Abundance and richness of predatory ants were monitored using pitfall traps to ascertain their effect on the mortality of the immature stages of these pests. *Ceratitis capitata* and *C. rosa* failed to eclose from the inoculated fruits disposed at different depths, however, *T. leucotreta* and *B. dorsalis* adults did eclosed. Significantly fewer *B. dorsalis* eclosed from fruits that were pulped in comparison to eclosion where the fruit were left whole ($F_{(3, 16)} = 11.45$, $P < 0.01$). Furthermore, depth of burial had a significant effect on the number of eclosed adults of *Drosophila* sp ($F_{(3, 112)} = 3.43$, $P < 0.01$). Burying fruits at 50 cm suppressed the eclosion of all the internal feeding pests tested. Twenty-seven thousand seventy-three individual ants (Hymenoptera: Formicidae) were sampled from the same plots as used above, with at least 47% and 53% sampled from plots where pulped and whole *C. sinensis* fruits were disposed of, respectively. The ants were identified to morphospecies which included *Pheidole*1, *Pheidole*2, Formicinae1, Formicinae2, Formicinae3, and Myrmicinae1. The disposal of the inoculated *C. sinensis* fruits either as pulped or whole and burying at different depths significantly suppressed and/or delayed the eclosion of either of the tested internal

feeding pests of citrus. Although, predacious ants were sampled from the same treatment plots they did not affect the survival or eclosion of the tested pests, and this could be attributed to the application of the slow toxic ant bait. Therefore, based on the observed results *B. dorsalis* adults showed the ability to eclose from 50 cm depth where fruit was either disposed as pulped or whole, thus, citrus farmers are advised to use hammer mill that will finely crush sanitised fruit, and/or bury fruit beyond 50 cm depth to prevent the adult od this pest from eclosing.

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DECLARATION

I Mr Tshepang Makitla, confirm that this MSc thesis has only been submitted to Rhodes University. This work was done under the supervision of Prof. M. Hill, Mr L. Mukwevho, Dr's A. Manrakhan and S. Moore. Sources of information and other material have been fully acknowledged.

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DEDICATION

I dedicate this work to my co-supervisor Mr Ludzula Mukwevho, thank you for believing in me even when I doubted myself you have really improved and impacted my life.

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“A GO LEHLOKWA LA GO HLOKA THEKO, LEHLOKWA LE HLOKA BABAPATSI BA LONA”

CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background to the study

South African citrus industry is an export-oriented entity with up to 126-million cartons (15 kg equivalent) of citrus exported in 2019 and approximately R20-billion in revenue was made in this specific financial year (CGA, 2020). Globally, South Africa is the second largest exporting country of fresh citrus after Spain (Edmonds, 2016; CGA, 2020). Among different citrus species, sweet orange, *Citrus sinensis* (L.) Osbeck (Rutaceae) is the most cultivated cultivar in South Africa and elsewhere in the world (Pieterse et al., 2020). Approximately 64% (i.e. 1 018 487 of 1 590 463 tons) of fresh citrus exported from South Africa in 2019 was *C. sinensis* (Edmonds, 2016). However, fresh *C. sinensis* consignments can be denied access to the international markets due to infestation of insect pests and diseases of phytosanitary concern (Manrakhan & Addison, 2007; Manrakhan & Pterandrus, 2016; Moore, 2019; Pieterse et al., 2020). The common pre and post-harvest insect pests of phytosanitary concern for *C. sinensis*, include false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae); Mediterranean fruit fly or Medfly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae); Natal fruit fly, *Ceratitis rosa* Karsh (Diptera: Tephritidae); and Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) (Grout, n.d.; Manrakhan & Addison, 2007; Moore & Kirkman, 2009; Manrakhan & Pterandrus, 2016; Moore, 2019; Pieterse et al., 2020). These internal feeding insect pests cause blemishes and rotting that results from oviposition and internal feeding and require considerable pre-harvest intention to ensure population are kept at low level (Manrakhan & Addison, 2007; Manrakhan & Pterandrus, 2016; Moore, 2019; Pieterse et al., 2020).

Various management measures are implemented to mitigate pest impact in the orchards. Control measures include cultural, chemical, biological, behavioural, and mechanical control which are implemented either solely or in an integrated approach (Manrakhan & Addison, 2007; Dias et al., 2018; Malan et al., 2018; Haran et al., 2019; Hatting et al., 2019). The use of synthetic chemicals presents challenges due to the development of resistance by the insect pests and accumulation of chemical residue beyond the acceptable limits for export markets, therefore, there is pressure to use environmentally safe practices as an alternative to synthetic compounds (Manrakhan & Pterandrus, 2016; Dias et al., 2018; Malan et al., 2018). The current study measured the efficiency of different orchard sanitation practices, including disposal techniques and burying depths on the eclosion of four internal feeding insect pests (i.e., *T. leucotreta*, *C. capitata*, *C. rosa*, and *B. dorsalis*) of *C. sinensis* in South Africa.

1.2. Botany and production of *Citrus sinensis*

Cultivated citrus species belong to a single subgenus *Citrus*, in the family Rutaceae (Velasco & Licciardello, 2014; Coombes et al., 2017; Wang et al., 2017; Gmitter et al., 2020). *Citrus sinensis* is a hybrid that resulted from crossing a *Citrus maxima* Merr, pomelo and *C. reticulata* Blanco, mandarine (Etebu & Nwauzoma, 2014). Other closely related species includes the *C. paradise* Macfad grapefruit, *C. limon* (L.) Osbeck lemons (e.g. which were developed through a cross between bitter orange *C. aurantium* (L.) and a citron *C. medica* (L.), *C. aurantifolia* (Christm) lime, and *C. reticulata* and *C. tangerina* (Tanaka) all known as soft citrus (Coombes et al., 2017; Wang et al., 2017; Gmitter et al., 2020).

Citrus sinensis is a perennial, evergreen tree plant, with oval shaped leaves, and can grow up to 10 m in height, although some cultivars can reach up to 15 m (Etebu & Nwauzoma, 2014). Moreover, under field conditions tree development and reproduction is dependent on the type of rootstock the scion is budded or grafted onto. Compatible *C. sinensis* rootstocks include trifoliate hybrid rootstocks (i.e. Carrizo citrange, Swingle citrumelo, and Troyer citrange), and rough lemon (Lee et al., 2009; Grout, 2015; Noichinda et al., 2019). *Citrus sinensis* trees take two to three years to flower. Flowering occurs in spring provided adequate water and mineral nutrients are available. *Citrus sinensis* trees produces white fragrant hermaphroditic flowers. Potent pollination agents include honeybees and wind to facilitate the process of fertilisation resulting in fruit development (Etebu & Nwauzoma, 2014; Grout, 2015; Stander, 2015).

Citrus sinensis fruits undergoes three growth stages: cell division, cell enlargement and maturation (Verreynne, 2010; Stander, 2015). *Citrus sinensis* fruit varies in shape and size, from spherical to oblong (Etebu & Nwauzoma, 2014). Anatomically, *C. sinensis* fruits are divided into two distinct regions, which include the pericarp or rind, followed by the fruit pulp with the juice segments. The skin consists of the epidermis layer, with epicuticular wax containing oil glands. The rind consists of the outer epicarp or flavedo and the inner mesocarp or albedo. The pulp or flesh of the fruit is divided into 10 to 14 sweet, juicy segments, which may vary in colour depending on the type of *C. sinensis* cultivar, from orange, to red colouration. The sweet juice inside the segments is contained in the endocarps (Etebu & Nwauzoma, 2014).

Although, climatic requirements vary between varieties of *C. sinensis*, the trees performs well in the subtropical and temperate regions (Stander, 2015). The two varieties of *C. sinensis* that are extensively cultivated in South Africa are the Valencia and Navel oranges (CGA, 2020). Valencia cultivars are mainly cultivated in the warmer subtropical region of Limpopo and Mpumalanga provinces, hence, the Navel cultivars are mainly grown in the cooler temperate region of the Eastern Cape and Western Cape provinces (CGA, 2020). However, citrus trees prefer frost free areas where temperature do not fall below -2 °C (Verreynne, 2010; Etebu & Nwauzoma, 2014; Stander, 2015).

1.3. Internal feeding insect pests of citrus

Any organisms that inflict direct damage to the plant parts (i.e. roots, stems, leaves, blossoms or fruits), as a result reducing the production capacity of the citrus plant is considered a pest

(Gendall, 2007) (Table 1.1.). Subsequently, such organisms pose serious economic losses, and if not controlled, can inflict over 90% damage in the production of fresh citrus (Dias et al., 2018; Malan et al., 2018; Grové et al., 2019; Moore, 2019). False codling moth (FCM) (*T. leucotreta*) and the tephritid fruit flies (*C. capitata, C. rosa* and *B. dorsalis*) are amongst serious pests of citrus in South Africa and are widely distributed throughout the citrus production regions (Manrakhan & Addison, 2007; Grout, 2015; Onah et al., 2016; Grové et al., 2019). In South Africa and elsewhere in the world, each of these four internal feeding pests of citrus are polyphagous and have greater than 30 hosts ranging from cultivated to the naturally occurring plants (Manrakhan & Addison, 2007; Opoku-Debrah et al., 2013; Onah et al., 2016).

Table 1.1. Naturalised pests of economic importance which directly affect the quality and quantity of *Citrus sinensis* South Africa.

Order and a scientific name	Origin/native range	Symptoms or damage
Diptera		
***Bactrocera dorsalis* (Hendel)**	Asia	Ovipositional punctures, blemishes and rotting on the fruit
***Ceratitis capitata* (Wiedemann)**	Sub-Sahara Africa	Ovipositional punctures, blemishes and rotting on the fruit
***Ceratitis rosa* (Karsch)**	Southern Africa	Ovipositional punctures, blemishes and rotting on the fruit
Hemiptera		
***Aphis spiraecola* (Patch)**	Asia	Sap-sucking pest which depletes the plant reserves and reduce the fruit load leads to leaf discolouration
***Aphis gossypii* (Glover)**	Unknown	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
***Aonidiella aurantii* (Maskell)**	Southern China	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
***Icerya purchasi* (Maskell)**	Australia	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
***Paracoccus burnerae* (Brain)**	South and Eastern Africa	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration

[1] Unites States of America (USA)

Table 1.1 (Continued). Naturalised pests of economic importance which directly affect the quality and quantity of *Citrus sinensis* fruits in South Africa.

Order and a scientific name	**Origin/native range**	**Symptoms or damage**
Hemiptera:		
***Planococcus citri* (Risso)**	Asia	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
***Pseudococcus longispinus* (Targioni-Tozzetti)**	North America (Hawaii)	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
Hemiptera: *Toxoptera aurantii* (Fonscolombe)	East Asia	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
Hemiptera: *Toxoptera citricidus* (Kirkaldy)	[1]USA Florida	Sap-sucking pest which depletes the plant reserves, reduce the fruit load, and leads to leaf discolouration
Lepidoptera: *Helicoverpa armigera* (Hubner)	Europe	Damaged fruitlets and the inflorescence
Lepidoptera: *Thaumatotibia leucotreta* (Meyr)	Sub-Sahara Africa	Damaged fruit due to larval boring, blemishes, rotting and excreted fras
***Scirtothrips aurantii* (Faure)**	Southern Africa	Lesion or marks on the fruit, reducing its marketability although the fruit is edible
Trombidiformes: *Aceria sheldoni* (Ewing)	North America	Deformed leaves, flowers, and fruit

[1] Unites States of America (USA)

Due to the climatic adaptability and distribution of host plants, the spread of the internal feeding insect pests has emulated the distribution of hosts and has spread throughout Africa, Asia, Europe, North and South America, and Oceania (Virgilio et al., 2014; Grové et al., 2019; Pieterse et al., 2020). Out of the four internal feeding insect pests of citrus, *C. capitata* is the most adaptive and widely spread fruit fly species, occurring in six continents, except Antarctica (Manrakhan & Addison, 2007; Opoku-Debrah et al., 2013; Onah et al., 2016). *Bactrocera dorsalis* is considered the second most distributed fruit fly, moreover, it currently occurs in Africa and Asia (Meyer et al., 2016). Furthermore, *T. leucotreta* and *C. rosa* occur mainly in Africa, particularly southern and eastern parts of the continent (Ekesi et al., 2016; Malan et al., 2018; Mutamiswa et al., 2021).

Amongst commonly cultivated varieties of *C. sinensis*, Navel oranges are highly susceptible to *T. leucotreta*, compared to Valencia (Love et al., 2014; Hattingh et al., 2020; Moore, 2021). Of the Navel cultivars, Palmer is the most susceptible, whereas, Cambria, Glen Ora and Fischer Navel are less susceptible to *T. leucotreta* (Love et al., 2014). Thus, the impact and population densities of *T. leucotreta* is higher in the Eastern and Western Cape provinces of South Africa, where Navel cultivars are extensively cultivated (Moore et al., 2016; Grové et al., 2019; Moore, 2019). The fruit fly pests prefer mature ripped *C. sinensis* fruit in which they may oviposit their eggs. Hence, fruit fly pests are distributed around the country where *C. sinensis* is cultivated (Manrakhan & Addison, 2007; Manrakhan & Pterandrus, 2016). Although, Valencia cultivars are highly prone to *T. leucotreta* and fruit flies infestations, since they mature late in the season, serving as overwintering propagules (Manrakhan & Addison, 2007; Moore & Kirkman, 2009; Manrakhan & Pterandrus, 2016).

1.4. Taxonomy, biology, and impact of the internal feeding pests of citrus

1.4.1. False codling moth (FCM) (*Thaumatotibia leucotreta*)

False codling moth (FCM) *Thaumatotibia leucotreta* Komai (Lepidoptera: Tortricidae) is a sexually dimorphic insect pest, adult males have a wingspan of 15-16 mm, while female wingspan is 19-20 mm in length (Love et al., 2014). Similarly, both sexes forewing has a mixture of grey, brown, black, and orange-brown markings, while there is a visible triangular marking on the outer part of the wing against the hind margin, and a crescent shaped marking above it. However, male *T. leucotreta* is distinguishable from the female, males have a specialized hindwing, which is slightly reduced, and has a circular pocket of fine hair-like scales overlaid with brown weakly shining whitish scales in the anal angle. Furthermore, males have a heavily tufted hind tibia (Love et al., 2014; Malan et al., 2018).

Thaumatotibia leucotreta is a serious polyphagous pest of citrus in southern Africa (Moore, 2019). Damage to fruit ranges from 42% to 90% in South Africa. Specific fruit losses caused by *T. leucotreta* on *C. sinensis* varieties in the country ranges from 1.6% in Navels and 0.3% in Valencia oranges, respectively (Begemann & Schoeman, 1999; Love et al., 2014; Adom et al., 2021).

Adult *T. leucotreta* are nocturnal, and normally mate during the night (Zagatti & Castel, 1987). A fertilised adult female will lay 100 to 400 flattened, oval shaped eggs, measuring 0.9 mm in diameter, on a surface of the preferred host fruit such as citrus. Newly hatched larvae are yellowish white with dark spots. Larvae undergoes five larval instar stages feeding inside the host fruit before they may pupate. The wandering larvae (length: 15 mm, bright red or pink head prothoracic plate and with yellow brown pinacula) drops to the ground to form a tough silken cocoon using soil and debris for pupate. Eclosion from the cocoon occurs after a 12 to 14 days depending on the environmental conditions. The life cycle takes approximately 30 days in the summer months, while in the winter months it may take 60 days. Typical of insect species *T. leucotreta* is ectothermic, the insect depend on the surrounding environmental temperatures for body function and development (Daiber, 1980).

1.4.2. Mediterranean fruit fly (*Ceratitis capitata*)

The Mediterranean fruit fly or medfly [*Ceratitis capitata* Wiedemann (Diptera: Tephritidae)] is among a group of nine pestilent fruit fly species that belong to the genus *Ceratitis* (Manrakhan & Pterandrus, 2016; Meyer et al., 2016). Adult *C. capitata* is 3 mm to 5 mm in body length. Their thorax is creamy white to yellow and has a distinctive pattern of black blotches. The abdomen is tinted brown with fine black bristle on the dorsal surface and two light bands on the basal half. Both male and female wings have a band across the centre with dark streaks and spots in the wing cell. Male *C. capitata* have their own distinguishable features that separates them from the female, these include a lower orbital setae, and a diamond shaped black apex (Meyer et al., 2016).

Ceratitis capitata is the most widely spread fruit fly pest worldwide (Malacrida et al., 2007). Due to its ability to tolerate different temperature ranges *C. capitata* can survive in both natural and cultivated habitats, with over 300 host plants (Ekesi et al., 2016; Meyer et al., 2016). It is a pest of high economic importance affecting production, control costs and market access especially in fresh citrus produce. While trading of fresh produce such as citrus has enabled the pest to establish in other parts of the world. If not controlled the pest may inflict 100% damage in fruit crops including citrus (Ekesi et al., 2016; Meyer et al., 2016).

Typical of a fruit fly species adult female *C. capitata* has an ovipositor which it uses to mate and oviposit eggs (Ekesi et al., 2016; Meyer et al., 2016). The fertilised adult female will oviposit 1 mm long eggs underneath the skin of the preferred host fruit, as a result creating blemishes which may render the fruit unmarketable. *Ceratitis capitata* eggs are curved, shiny white in colour and have a smooth feature. The eggs may take two to four days or 16 to 18 days in cool weather before they may hatch. Hatched larvae are creamy white with a cylindrical shape, a narrow anterior end, and a flattened caudal tail. Larvae undergoes three instar stages feeding inside the fruit for 11 days before pupation. The third final instar larvae measuring 7 mm or 9 mm in body length drops to the ground to pupate. Pupation may last for six to 11 days or longer in cool conditions, and after which the adult eclose. An adult *C. capitata* has a life-span of over two months, and females require a constant 25°C for five days to develop an ovary (Ekesi et al., 2016; Meyer et al., 2016).

1.4.3. Natal fruit fly (*Ceratitis rosa*)

Natal fruit fly *Ceratitis rosa* Karsh (Diptera: Tephritidae) belongs to the subgenus *Pterandrus*. Adult *C. rosa* is 4.25-5.30 mm in body length, while their wingspan is 4.50-5.75 mm in length. Typical of *Ceratitis* species, adult *C. rosa* has banded wings and a swollen scutellum which is yellow and black in colour. Furthermore, adult *C. rosa* has the distinctive grey flecks in the basal wing cell which separates *Ceratitis* species from most tephritid genera (Ekesi et al., 2016; Meyer et al., 2016). However, recent research in the taxonomy of the species has found that *C. rosa* can be separated into two species, and the second species is *C. quilicii* (Manrakhan & Addison, 2007; Ekesi et al., 2016; Meyer et al., 2016).

Ceratitis rosa is a polyphagous fruit fly species native to southern and eastern Africa. This pest is tolerant to arid environmental conditions than *C. capitata*, as a result the pest has the potential to establish in other parts of Africa and the world, hence, it is quarantine listed. In South Africa *C. rosa* is a serious pest of citrus and deciduous fruits (Tanga et al., 2015; Ekesi et al., 2016; Meyer et al., 2016).

Adult female *C. rosa* may begin to oviposit their eggs seven days post pupation. The eggs may be laid at intervals of 10 to 20 at a time underneath the fruit surface. *Ceratitis rosa* eggs may take four days, or longer in cold weather before they may hatch. Newly hatched larvae are creamy white like those of *C. capitata*. Larval development undergoes three instar stages feeding inside the host fruit. The last instar larvae drops to the ground to pupate underground, and pupation may last for 12 days depending on the surrounding weather conditions (Tanga et al., 2015; Ekesi et al., 2016; Meyer et al., 2016).

1.4.4. Oriental fruit fly (*Bactrocera dorsalis*)

Oriental fruit fly *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) was initially treated as a single species until Ekesi et al. (2016), and Meyer et al. (2016) described other species within the same *Bactrocera* genus, which include *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae), *Bactrocera papayae* Drew & Hancock (Diptera: Tephritidae), and *Bactrocera philippinesis* Drew & Hancock (Diptera: Tephritidae).

Adult *B. dorsalis* has a clear wing membrane, except at the narrow costal band, a black scutum with a present lateral vittae and an absent medial vitta. The scutellum is yellow except at the basal band which is usually very narrow, and the abdomen has medial dark stripe on the third and firth segments, respectively. However, *B. dorsalis* has distinctive features which separates it from other *Bactrocera* species. These include a yellow postpronotal lobes, parallel lateral vittae, and a femor that is not extensively marked. Other distinguishable features include a short to long aculeus, a tomentum with no gap, and a narrow costal band (Ekesi et al., 2016; Meyer et al., 2016).

Bactrocera dorsalis is endemic to the Asian continent and is the second most widely spread fruit fly pest after *C. capitata.* If left uncontrolled *B. dorsalis* can cause a 100% damage rate on their host plants. Furthermore, due to its polyphagous characteristics this pest reduces production, increase control costs, and affect market access. In South Africa this pest occurs in the north-eastern areas of the Limpopo and Mpumalanga provinces. Moreover, in recent research findings revelled that this pest may outcompete *C. capitata* on deciduous fruits in the Western Cape Province of South Africa (Ekesi et al., 2016; Meyer et al., 2016; Pieterse et al., 2020; Manrakhan, 2020).

Bactrocera dorsalis adult females will oviposit 0.8 mm long and 0.2 mm wide eggs underneath the host fruit skin. The eggs have a slightly protruding micropyle at the anterior end, a reticulated chorion, and are white to yellow-white (Ekesi et al., 2016). With respect to temperature *B. dorsalis* eggs may take a day or 20 days in cool conditions to hatch. Hatched larvae is white to yellowish in colour with a maggot appearance and the third instar larvae measures 7.5-10 mm long and 1.5-2.0 mm wide (Ekesi et al., 2016; Meyer et al., 2016). Larvae will feed inside the fruit for another six to 35 days, after which the third final instar larvae will drop to the ground to pupate. The pupa is barrel shaped and white to yellow brown in colour. Pupation in *B. dorsalis* may also take place inside the host fruit, whereby the process requires a constant 25°C temperature and 80% relative humidity for a period of 10 to 25 days, however, under cool conditions pupation may last for 90 days. Adult *B. dorsalis* has a lifespan of one to three month in warmer conditions, while during cooler conditions the adult may live up to 12 months. Mature adult will begin to mate eight to 12 days after eclosing from pupae (Ekesi et al., 2016; Meyer et al., 2016).

1.5. Management of the internal feeding pests of citrus

Pest management involves measures used to regulate pest populations to a level below thresholds (Manrakhan & Addison, 2007; Dias et al., 2018; Malan et al., 2018). Pest management involves an array of different management measures including chemical, biological, behavioural, and cultural control (Dias et al., 2018; Malan et al., 2018; Moore, 2019). Citrus farmers in South Africa implement these management strategies either solely or in integration (Moore, 2019). Trapping and monitoring practices are among the baseline practices used to predict the abundance and impact of pests in the orchards (Manrakhan & Addison, 2007; Dias et al., 2018; Malan et al., 2018; Moore, 2019). Specialist traps are baited with either sex pheromones, protein lures or chemical insecticides to capture different groups of herbivorous insects in citrus orchards (i.e. *T. leucotreta*; *C. capitata*; *C. rosa*, and *B. dorsalis*) (Manrakhan & Addison, 2007; Moore, 2019). Pest monitoring also enables a farmer to determine the time for applying the management technique, and the choice of a technique (Moore, 2019).

1.5.1. Physical control

Physical control is a cultural control practice that involves the manipulation of the agroecosystem to minimize the establishment of pests in the farming systems such as orchards (Moore & Kirkman, 2009). Orchard sanitation involves the picking and removal of all fallen fruits or fruits left on the trees post-harvest from the orchard to minimize food resources and overwintering propagules of the internal feeding pests of citrus (Moore & Kirkman, 2009). In field experiments weekly orchard sanitation in South Africa has proven that it can remove 75% of *T. leucotreta* larvae from the orchards. Hence, in the warmer areas of the Limpopo and Mpumalanga provinces sanitation is recommended to take place twice a week since larvae grow faster due to favourable weather conditions. Fruit disposal include pulping with a hammer mill and burying infested fruits 40 cm underground and/or collecting fruits in black refuse plastic bags and exposing them to the sun for a week (Moore & Kirkman, 2009; Moore, 2019).

1.5.2. Chemical control

Chemical control involves the use of synthetic chemicals and bio-chemicals to eradicate and/or suppress the population of pests in the agroecosystems (Malan et al., 2018). Among the pests of citrus whose population is significantly reduced by chemicals include *T. leucotreta*; *C. capitata*; *C. rosa* and *B. dorsalis* (Manrakhan & Addison, 2007; Manrakhan & Pterandrus, 2016; Moore, 2019). Synthetic chemicals are non-selective, killing non-target organisms such as pests' natural enemies and beneficial insects (Malan et al., 2018; Mansour et al., 2018; Haran et al., 2019). Other undesirable effects of synthetic insecticides include the inflated economics of control, ecotoxicology, and development of resistance by the insects towards the compounds (Dias et al., 2018; Malan et al., 2018). Four chemical groups used in citrus orchards including organophosphates; carbamates; pyrethroids; and oxadiazine indoxacarb these compounds were reported to be lethal towards natural enemies of citrus pests, namely *Aphytis melinus* DeBach, (Hymenoptera: Aphelinidae); and *Orius insidiosus* Say, (Hemiptera: Anthocoridae), the natural enemy of citrus mealybugs *Planococcus citri* Risso (Hemiptera: Pseudococcidae) and *T. leucotreta*, respectively (Mansour et al., 2018).

Registered insecticides recommended to control *T. leucotreta* in citrus orchards include two chitin inhibitors belonging to the benzolyl urea group, Alsystin (triflumuron) (Bayer, Germany) and Nomolt (teflubenzuron) (BASF, Germany). These products work by disrupting embryonic development of *T. leucotreta* larvae in the eggs. However, application of these products has been restricted pending development of resistance by the pest. Another insect growth regulator Runner (methoxyfenozide) ecdysone agonist used to control *T. leucotreta* in the orchards, whereby affected larvae will undergo a lethal premature moult (Moore, 2019).

There are two larvicides synthetic pyrethroids used to control *T. leucotreta*, which include cypermethrin and meothrin (fenpropathrin) (Philagro, South Africa). Other registered insecticides include Coragen (chlorantraniliprole) a novel anthranlic diamide, is a ryanodine receptor activator, which prevents muscle contraction, killing the target pest by paralysis. Delegate (Spinetoram) part of the spinosyns group, is a nicotinic acetylcholine receptor. Warlock (emamectin benzoate) an emulsified concentrate (EC) emamectin benzoate kills the target pest by disrupting the nerve signal (Table: 1.2) (Moore, 2019).

As for fruit fly management citrus farmers implement bait sprays and stations (Manrakhan & Pterandrus, 2016; Dias et al., 2018; Haran et al., 2019). In bait sprays farmers calibrate a mixture of protein lures with a registered insecticides and apply the mixture throughout the orchard canopy, fruit fly adults feeding on the residue of the mixture are killed by the insecticide (Manrakhan & Pterandrus, 2016). Available bait spray mixtures include Hym-lure (protein hydrolysate) (Biotrap Australia Pty Ltd), Lok-lure and Buminal lures mixed with either Trichlorfon or Dipterex (dimethyl 2, 2, 2-trichloro-1-hydroxythylphosphonate) or Malathion EC and/or Malathion WP. In terms of bait stations farmers distribute specialist traps baited with either protein or pheromone lures and an insecticide, this is a form of mass trapping, attract and kill method (A&K), such as Male Annihilation Technique (MAT) implemented against *B. dorsalis*, whereby pest males are mass trapped using Methyl Eugenol (ME) inserted alongside an organophosphate dichlorvos 2, 2-dichlorobinyl dimethyl phosphate (DDVP) (Manrakhan & Pterandrus, 2016).

1.5.3. Biological control

Biological control involves the use of natural organisms such as insects, mites and pathogens (e.g. bacteria, fungi, nematodes, viruses, and protozoans) to reduce populations of citrus pests in the orchards (Sharma et al., 2013). Amongst the natural enemies are the host specific and generalist agents of either native or exotic origin that preys on the target pest (Sharma et al., 2013; Haran et al., 2019). These organisms are mass reared and introduced in the citrus orchards to suppress the population of a specific insect pest (Sharma et al., 2013; Moore, 2019). The success of biological control agents is however dependent on the regional climate, and the synergy and/or compatibility with other implemented management control measures in the orchard (Sharma et al., 2013). Thus, for a biocontrol agent to establish in the introduced environment, climatic conditions such as temperature need to be favourable (Sharma et al., 2013). Furthermore, majority of biocontrol agents are sensitive to synthetic chemicals used in citrus orchards, hence they need to be incorporated with environmentally friendly management measure of integrated pest management (IPM) (Moore & Kirkman, 2009; Sharma et al., 2013).

Among the naturalized biological agents of *T. leucotreta* found in citrus orchards is the egg parasitoid *Trichogrammatoidae cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae); the larval parasitoids *Agathis bishopi* Nixton (Hymenoptera: Braconidae) and *Apophua leucotreta* Wilkinson (Hymenoptera: Ichneumonidae); and the larval predators from the *Orius* species (Hemiptera: Anthocoridae) (Malan et al., 2018; Moore, 2019). Their efficacy as biocontrol agents of *T. leucotreta* in the field ranges from 40% to 80%, respectively (Malan et al., 2018; Moore, 2019). The three native parasitoids of fruit flies are; *Fopius ceratitivoras* Wharton, (Hymenoptera: Braconidae); *Psyttalia humilis* Silvestri, (Hymenoptera: Braconidae); and *Tetrastichus giffardii* Silvestri, (Hymenoptera: Tetrastichinae) (Dias et al., 2018; Haran et al., 2019). Their efficacy as fruit flies parasitoids in the field ranges from 0 to 54% mortality rate (Haran et al., 2019). *Fopius ceratitivoras* is common in the Nelspruit region of Mpumalanga Province, while *P. humilis* occurs in both Limpopo and Mpumalanga provinces of South Africa, and *T. giffardii* occur in the Western Cape Province (Dias et al., 2018; Haran et al., 2019). Emulating the distribution of natural plant hosts of tephritids fruit flies such as *Syzygium cordatum* (Hochst and Krauss) and *S. jambos* (L.) (Alston), which occur in the Limpopo and Mpumalanga areas (Grové et al., 2019; Haran et al., 2019) (Table 1.2).

Entomopathogenic nematodes that parasitize both *T. leucotreta* and tephritids fruit fly's immature stages (e.g., larvae and pupae) include *Heterorhaditis* spp. (Rhabditida: Heterorhabditidae) and *Steinernema* spp. (Rhabditida: Steinernematidae) are common, and under laboratory assays their impact ranges between 14% and 96%, respectively (Dias et al., 2018). Potent entomopathogenic fungi include two fungal isolates, namely *Beauveria bassiana* Balsamo, (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* Sorokin, (Hypocreales: Clavicipitaceae) which under laboratory assays their impact on the population of *T. leucotreta* ranges from 84.6% and 94.2%, respectively (Mkiga et al., 2019; Acheampong et al., 2020). Although, *B. bassiana* and *M. anisopliae* may be used as fruit fly pathogens in citrus orchards reducing the eclosion of adult fruit flies by 90% to 100% in laboratory assays (Toledo et al., 2006), while the efficacy of *B. bassiana* in the field is about 65% (Dias et al., 2018).

The entomopathogenic virus, *Cryptophlebia leucotreta* granulovirus (CrleGV) virulent had been identified and commercially produce to control *T. leucotreta* in citrus orchards. These include, Cryptogran, Cryptex and Gratham which cause significant mortality rate of up 70% against *T. leucotreta* (Mkiga et al., 2019; Moore, 2019). Although, the two entomopathogenic fungi and the virus isolates and/or viral base are sensitive to Ultra-Violate (UV) radiation respectively, thus reducing the effectiveness in the field (Moore, 2019; Mkiga et al., 2019).

Table 1.2. Economically important internal feeding pests of citrus in South Africa, including their native naturalised biological control agents.

Internal feeding insect of Citrus sinensis	Pesticides	Established biological control agents in South Africa	Introduction mechanism
Diptera			
***Ceratitis capitata* (Wiedemann)** ***Ceratitis rosa* (Karsch)** ***Bactrocera dorsalis* (Hendel)**	Trichlorfon or Dipterex (dimethyl 2, 2, 2-trichloro-1-hydroxythylphosphonate) Malathion EC and/or Malathion WP	*Fopius ceratitivoras* Wharton, (Hymenoptera: Braconidae) *Psyttalia humilis* Silvestri, (Hymenoptera: Braconidae) *Tetrastichus giffardii* Silvestri, (Hymenoptera: Tetrastichinae)	Native to South Africa
Lepidoptera: ***Thaumatotibia leucotreta* (Meyr)**	Benzolyl urea group, Alsystin (triflumuron) (Bayer, Germany) and Nomolt (teflubenzuron) (BASF, Germany). pyrethroids cypermethrin and meothrin (fenpropathrin) (Philagro, South Africa). Coragen (chlorantraniliprole). Delegate (Spinetoram); Warlock (emamectin benzoate); (EC) emamectin benzoate	*Trichogrammatoidae cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) *Agathis bishopi* Nixton (Hymenoptera: Braconidae) *Apophua leucotreta* Wilkinson (Hymenoptera: Ichneumonidae)	Native to South Africa

Another form of biological control includes sterile insect technique (SIT), whereby sterilized male insect pests of citrus importance are released to compete for mates with the fertile wild males of the same species (Ekesi et al., 2016). Successful copulation between the sterile males and the wild females will result in underdeveloped eggs which will not mature rather deteriorate, as a result, the pest population is reduced. The sterile males of *T. leucotreta* and/or fruit flies (i.e., *C. capitate*, *C. rosa* and *B. dorsalis*) are released at a ratio of 10:1, and 80:1, respectively, in relation to the wild males (Dias et al., 2018; Malan et al., 2018)

1.6. Problem statement

Citrus orchard sanitation has been qualified as a phytosanitary measure implemented to remove pest propagules and reducing pest populations during and between the citrus seasons (Moore & Kirkman, 2009; Moore et al., 2016). In addition orchard sanitation is one of the prerequisites in the systems approach developed to control populations of *T. leucotreta* at the orchard (Moore et al., 2016; Hattingh et al., 2020). However, the complimentary disposal techniques, in particular pulping and burying of fruit propagules had not been quantified in terms of their efficacy in suppressing pest eclosion of *T. leucotreta* and tephritid fruit flies (Moore & Kirkman, 2009). Therefore, this study seeks to quantify the efficacy of citrus orchard sanitation disposal techniques to suppress the eclosion of *T. leucotreta* and three tephritid fruit fly species (e.g., *C. capitata, C. rosa* and *B. dorsalis*) infesting *C. sinensis* varieties in South Africa.

1.7. Aims of the study

The main objective of this study was to quantify the impact of two fruit disposal methods (i.e., pulping or whole) and burial depths on the eclosion of the internal feeding pests of citrus in the Lowveld region of Nelspruit Mpumalanga, South Africa. As well as monitoring the activity of predatory ants foraging within the same treatment plot where citrus fruit had been disposed of.

Specific objectives

To determine the effect of the disposal techniques and burying depth on the eclosion of the internal feeding pests of citrus

To monitor the role of predatory ants in plots where *C. sinensis* fruits were disposed as pulped or whole and buried at different depths to ascertain their effects on pest's immatures, and teneral adults

CHAPTER TWO: THE EFFECT OF FRUIT DISPOSAL METHODS AND BURIAL DEPTH ON THE ECLOSION OF INTERNAL-FEEDING PESTS OF CITRUS IN SOUTH AFRICA

2.1. Introduction

Edaphic factors such as soil texture, soil depth, temperature and soil moisture influence the pupation period and eclosion of soil pupating arthropods (Alyokhin et al., 2001; Dimou et al., 2003; Bohua et al., 2006; Love, 2015; Love et al., 2019). The pupation behaviour of wandering late instars and eclosion rate of *T. leucotreta* and tephritids fruit flies is dependent on the soil type, and moisture regimes (Alyokhin et al., 2001; Bohua et al., 2006; Dimou et al., 2003; Love, 2015; Love et al., 2019). Although the wandering larvae of *B. dorsalis* and *C. capitata* is capable of successfully pupating and eclosing from 15 cm to 30 cm in sandy soil, however, in most times Tephritidae species have been found pupating in shallow depths between 0 and 5 cm (Alyokhin et al., 2001; Klungness et al., 2005; Bohua et al., 2006; Hulthen & Clarke, 2006; Yee, 2013; El-Gendy & AbdAllah, 2019). As result eclosed teneral adults can easily ascend through the soil to the ground surface in porous soils dominated by sandy particles (Dimou et al., 2003; Hulthen & Clarke, 2006; Bohua et al., 2006; Quesada-Moraga et al., 2012; Love, 2015; Love et al., 2019).

Citrus orchard sanitation is an on-farm management practice implemented to subdue the population of the internal feeding pests of *C. sinensis*, including *T. leucotreta*, *C. capitata*, *C. rosa* and *B. dorsalis* in South Africa (Moore & Kirkman, 2009). Orchard sanitation is incorporated in the integrated pest management (IPM) programme combating pests of economic importance (Moore & Kirkman, 2009; Moore, 2019). Orchard sanitation involves regular picking of fallen fruits from the floor of the orchard and the removal of fruits left on trees after harvesting, and such fruits are disposed of either by pulping with a hammer mill or by burying them 40 cm underground in compact soil (Moore & Kirkman, 2009). Other sanitation practices employed to dispose fruit include covering of infested fruits with augmentorium nets and plastic bags to capture eclosed adults (Moore & Kirkman, 2009; Jang et al., 2010). Field sanitation has been shown to be effective and has contributed to a significant reduction of tephritid pest populations in the state of Hawaii in the United States of America, and La Reunion Island (Deguine et al., 2005; Klungness et al., 2005; ; Jang et al., 2010). Orchard sanitation practices such as fruit pulping and burying disrupts the development of the internal feeding pest of citrus within the host fruit and as a result pupation may not occur (Klungness et al., 2005; Moore & Kirkman, 2009; Moore, 2019). Although, the impact of orchard sanitation on the efficacy of internal feeding pests of *C. sinensis* from orchards has been studied in isolation (Moore & Kirkman, 2009), the combined impacts of disposal techniques (whole fruit of pulped) and burying depths on populations of internal feeding pests of citrus has not been assessed. The current study assessed the impact of fruit burying depths on the pupation and eclosion of internal feeding pests of *C. sinensis* fruits at Mpumalanga Province of South Africa.

2.2. Materials and methods

2.2.1. Source of Citrus fruits, larvae, and eggs of internal feeding pests of citrus

Abscised sweet orange fruits (i.e., Turkey Valencia, Valencia, and Palmer Navel) were sourced from the Crocodile Valley Estate (Komati) farm (25°28’04.93” S; 31°02’40.60” E) and the Crocodile Valley Farms (The Valley Farm) (25°27’50.91” S; 31°04’54.59” E). Collected fruits were stored at the University of Mpumalanga storage room at 10 °C for two days before use. Approximately 10 000 eggs of *T. leucotreta* were sourced from the Citrus Research International (CRI) - Port Elizabeth campus, whilst 102 900 (±5 ml), 41 601 (±3 ml) and 21 000 (±1 ml) eggs of *C. capitata*, *B. dorsalis* and *C. rosa* respectively were sourced from CRI-

Nelspruit campus. *Thaumatotibia leucotreta* eggs were incubated at 27°C for 24 hours to allow hatching. Larvae of *T. leucotreta* were inoculated onto the fruits, while eggs of *C. capitata, B. dorsalis* and *C. rosa* were used during inoculation.

2.2.2. Inoculation and incubation of *C. sinensis* fruits

The collected citrus fruits were treated with a mixture of IMAZICURE fungicide: water mixture (500 ml/*l*: 40 *l* of water) to disinfect the fruits from common fungal spores. The mixture was done four times as result of soil accumulation. Flower stalks (pedicel) of *C. sinensis* fruits were punctured onto the central core and white fibres were removed for all inoculated fruits. Larvae of *T. leucotreta* were observed from the hatching paper using a hand lens, picked, and put on the fruits using a small wet paint brush, thus the larvae can drill into the fruit pulp same way as they do in the orchard. The volume of undiluted eggs used for *C. capitata*, *B. dorsalis* and *C. rosa* were 5 ml, 3 ml, and 1 ml, respectively. Egg-water concentrations were calculated using the formula: $C_1V_1 = C_2V_2$, where C_1 is the number of eggs in a fixed volume of undiluted eggs, V_1 the volume of undiluted eggs available, C_2 number of eggs required per volume of egg water mixture, and V_2 the volume of water to be added to the mixture. To achieve a concentration of approximately 50, 30 and 20 eggs in a 25µl aliquot the volume of water added into the volume of undiluted eggs was 51.45 ml, 34.67 ml, and 27.41 ml for *C. capitata*, *B. dorsalis* and *C. rosa*, respectively. Prior to inoculation, wounds were treated with nature's choice brewer's yeast (50g per 100ml of water) as a source of protein for larvae. A 25µl of egg-water mixture of *C. capitata*, *B. dorsalis* and *C. rosa* was inoculated on each of the 600 (±135kg) abscised *C. sinensis* fruits, whilst the fruits used as controls were neither inoculated with larvae nor eggs of internal feeding pests of citrus. The punctured wounds were closed with the cotton to avoid egg spillage. Inoculated and control fruits were incubated for nine days at

27 °C prior to fruit disposal (i.e., pulped, or whole) and burying at different depths. Fruit pulping was done using a hammer mill and the pulp was collected using refuse plastic bags.

2.2.3. Experimental layout and fruit disposal

The study was conducted at the University of Mpumalanga experimental farm (25°42’50.88’’ S; 30°96’98.99’’ E), Mbombela campus. Treatment plots were demarcated at the University of Mpumalanga experimental farm (measuring 50 m x 20 m) in randomised complete block design (RCBD). Each block had 40 treatment plots [five treatments (four pests + control); two disposal techniques (Pulping and whole); and four burying depths (0, 5, 25 and 50 cm)]. Each treatment was replicated three times. The blocks were 10 meters apart from each. Pulped or whole fruits were disposed and buried at soil depths of 5 cm, 25 cm, and 50 cm, whereas fruits disposed on the ground surface was labelled as 0 cm. Approximately 5.5kg (±25) of either pulped or whole citrus fruits were disposed at an area of 0.25 m^2 demarcated at the centre of each of the treatment plots (1 m^2) covered with augmentorium nets (Figure 2.1) (Deguine et al., 2005; Klungness et al., 2005; ; Jang et al., 2010). Impenetrable mesh materials were used to make the augmentorium nets to prevent the eclosed adults from escaping the nets (Jang et al., 2010). White sticky traps were used to monitor the eclosion of the internal feeding pests of citrus and these boards were mounted at the top corner of each of the augmentorium net.

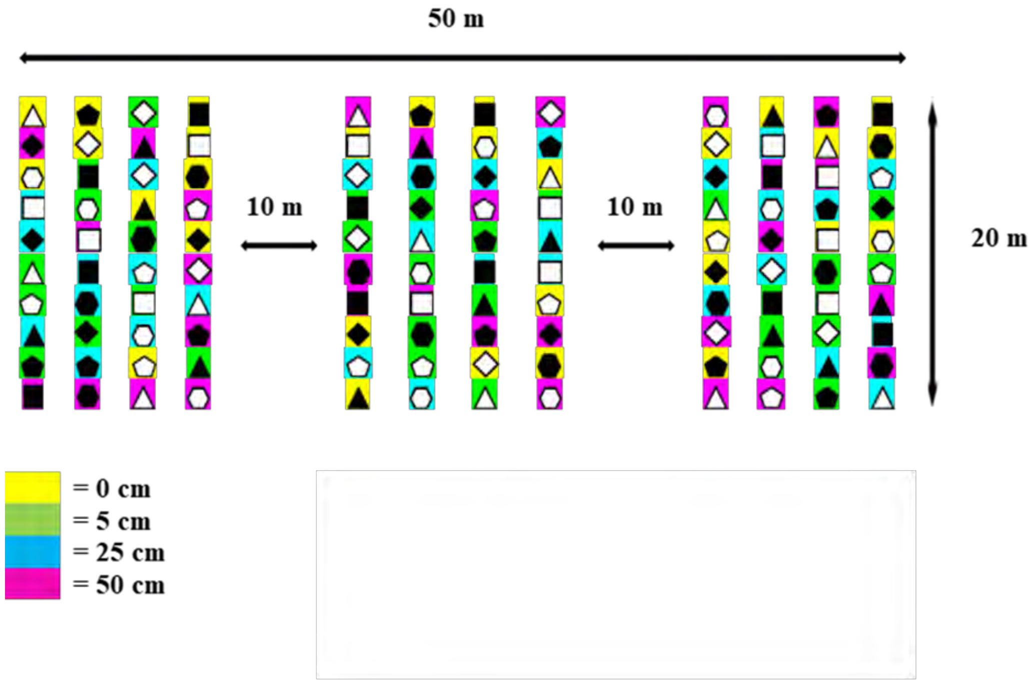

Figure 2.2. Layout of treatment plots in three blocks demarcated at the University of Mpumalanga experimental Farm where C. sinensis fruits inoculated with larvae of *T. leucotreta* △, eggs of *C. capitata* □, *C. rosa* ⬡, *B. dorsalis* ⬠ and the non-inoculated control fruits ◇ were disposed of. Open shapes show the plots where pulped fruits were disposed, hence, the shaded ones show plots where whole fruit were disposed.

2.2.4. Eclosion of internal feeding pests of *C. sinensis*

The eclosion period of the known internal feeding pests (i.e., inoculated and uninoculated) of *C. sinensis* were recorded at each treatment plot where the disposed fruits were buried at different depths (i.e., 0, 5, 25 and 50 cm). Emulating Bohua et al., (2006), the eclosion percentage of internal feeding pests of *C. sinensis* was determined by dividing the number of plots where adult pests eclosed with the total number of treatment plots, and multiply by 100.

2.2.5. Statistical analysis

The number of eclosed adults of the internal feeding pests of *C. sinensis* from each treatment plot was converted into percentages. Factorial analysis of variance (ANOVA) was used to determine the average number of eclosed adult pests at each treatment plot where fruits were disposed as pulped or whole and buried at different depths. The analysis was followed by Tukey's honesty significant difference (HSD) to measure statistical differences in eclosion of pests on pulped and whole *C. sinensis* buried at different depths. In addition, the same analysis was used to determine the average number of weeks taken for the internal feeding pests of *C. sinensis* to eclose from the fruits disposed and buried at different depths. Spearman's Rank order correlations was used to determine if whether the eclosed non-inoculated pests affected the eclosion of the inoculated internal feeding pests of *C. sinensis*. All statistics tests were done using Statistica, version 13.3.0.

2.3. Results

2.3.1. Eclosion of the internal feeding pest of *C. sinensis* inoculated

Of the four inoculated internal feeding pests of citrus, only *T. leucotreta* and *B. dorsalis* eclosed from both pulped and whole *C. sinensis* fruits disposed at the different depths tested, whilst *C. capitata* and *C. rosa* both failed to eclose. The percentage eclosion of *T. leucotreta* was 33.3% at 0, 5 and 50 cm from pulped fruits and at 5 and 25 cm from whole fruits, respectively. Furthermore, the percentage eclosion of *B. dorsalis* was 33.3% at 0 and 5 cm from pulped fruits, while 100%, 66.7%, 100%, and 33.3% percentage eclosion was recorded at depths of 0, 5, 25, and 50 cm from whole fruit, respectively (Figure 2.2). At least one adult insect of *T. leucotreta* eclosed from each of the treatment plots where *C. sinensis* fruits were disposed as pulped and buried at the depth of 0 cm, 5 cm and 50 cm, hence, eclosion was recorded at plots where whole *C. sinensis* fruits were buried at a depth of 5 cm and 25 cm. Furthermore, at least one *B. dorsalis* eclosed from pulped *C. sinensis* fruits buried at 0 cm and 50 cm. Although there were no significant differences on the number of adult *T. leucotreta* ($F_{(3, 16)} = 0.73$, $P = 0.54$) eclosed, significantly more *B. dorsalis* ($F_{(3, 16)} = 11.45$, $P < 0.01$) eclosed from whole than pulped *C. sinensis* fruits. The numbers of adult *B. dorsalis* eclosed from whole *C. sinensis* fruits gradually declined (i.e., from 14 to three) with the burying depth, thus, more adults were recorded from plots where fruits were at the shallower depths (e.g., 0 cm) compared to the plots where the fruits were disposed at a depth of 50cm (Table 2.1).

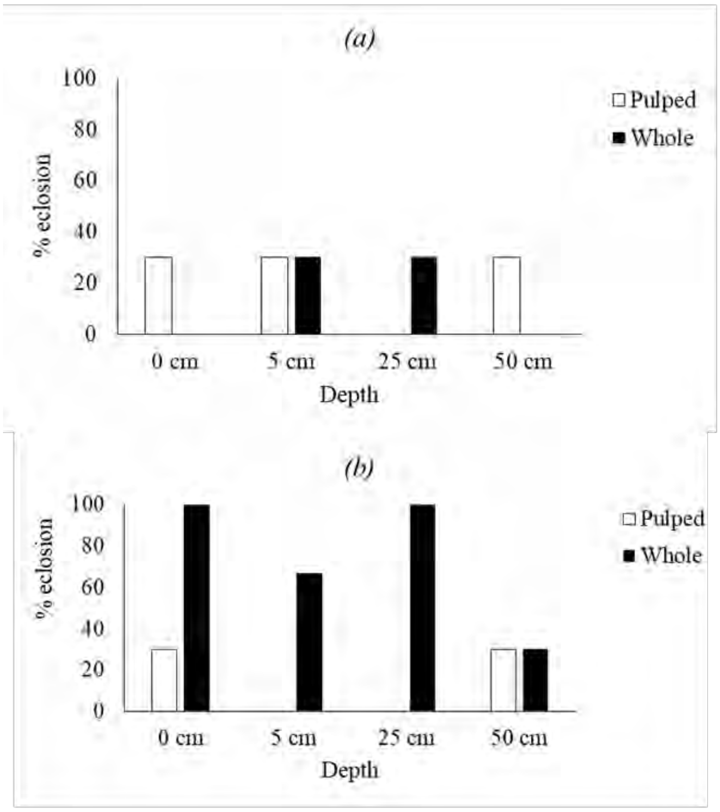

Figure 2.2. Percentage eclosion of *T. leucotreta (a)* and *B. dorsalis (b)* from inoculated *C. sinensis* fruits disposed as either pulped or whole, and buried at the depths of 0 cm, 5 cm, 25 cm, and 50 cm.

Table 2.1. Number (mean ± SE) of adult *T. leucotreta*, *C. capitata*, *C. rosa*, and *B. dorsalis* eclosed from pulped and whole *C. sinensis* fruits buried at 0, 5, 25, and 50 cm depths at the University of Mpumalanga experimental Farm.

Disposal techniques	Depths	Number (mean ±SE) of adults eclosed[1]			
		T. leucotreta	***C. capitata***	***C. rosa***	***B. dorsalis***
Pulping	0 cm	1 ± 0.33a	-	-	1 ± 0.33a
	5 cm	1 ± 0.33a	-	-	-
	25 cm	-	-	-	-
	50 cm	1 ± 0.33a	-	-	2 ± 0.67a
Whole	0 cm	-	-	-	14 ± 1.20b
	5 cm	1 ± 0.33a	-	-	10 ± 2.85b
	25 cm	1 ± 0.08a	-	-	10 ± 0.33b
	50 cm	-	-	-	3 ± 1.00ab

[1] Columns with the same letter shows that there were no significant differences.

2.3.2. Disposal technique and depth on the eclosion period

Thaumatobia leucotreta took an average of two weeks to eclose from pulped fruits buried at 0 and 5 cm, and three weeks at 50 cm. In addition, the pests took two weeks to eclose from whole fruits buried at 5 cm and three weeks at 25 cm. Adults of *B. dorsalis* took an average of two weeks to eclose from pulped fruits on the surface of the soil (i.e., 0 cm), and three to four weeks to eclose at 50 cm. Furthermore, adults of *B. dorsalis* took two weeks to eclose from whole fruits buried at 0, 5 and 25 cm, while it took three to four weeks to eclose at 50 cm. There were significant differences ($F_{(3,16)} = 1.00$, $P < 0.01$) in the time taken for the first eclosion and total eclosion period for both *T. leucotreta* or *B. dorsalis* where *C. sinensis* fruits were disposed as either pulped or whole and buried at different depths. Notably, pest eclosion took longer at plots where *C. sinensis* fruits were buried at 50 cm delaying the rate of eclosion (Table 2.2).

Table 2.2. Number (mean ± SE) of weeks taken for first and overall eclosion by *T. leucotreta* and *B. dorsalis* from pulped or whole *C. sinensis* fruits buried at 0, 5, 25, and 50 cm depths.

Name of the internal feeding pest of *C. sinensis*	**Technique**	**Depth**	**First eclosion in weeks**	**Eclosion period in weeks**
T. leucotreta	**Pulped**	**0 cm**	2 ± 0.67a	2 ± 0.67a
		5 cm	2 ± 0.67a	2 ± 0.67a
		25 cm	-	-
		50 cm	**3 ± 1.00b**	**3 ± 1.00b**
	Whole	**0 cm**	-	-
		5 cm	2 ± 0.67a	2 ± 0.67a
		25 cm	3 ± 1.00b	3 ± 1.00b
		50 cm	-	-
B. dorsalis	**Pulped**	**0 cm**	2 ± 0.67a	2 ± 0.67a
		5 cm	-	-
		25 cm	-	-
		50 cm	**3 ± 1.00b**	**4 ± 1.33b**
	Whole	**0 cm**	2 ± 0.00a	2 ± 0.00a
		5 cm	2 ± 0.67a	2 ± 0.67a
		25 cm	2 ± 0.00a	2 ± 0.00a
		50 cm	**3 ± 1.00b**	**4 ± 1.33b**

[1] Columns with the same letter shows that there were no significant differences.

2.3.3. Eclosion of non-inoculated pests of *C. sinensis*

Adults of *T. leucotreta*, *B. dorsalis*, *C. rosa*, *Drosophila* sp. and *Silba* sp. eclosed from treatment plots where neither the pest larvae nor eggs were inoculated (i.e. background population level in the controls). At least one adult *C. rosa* and *B. dorsalis* eclosed from whole *C. sinensis* fruits buried at 5 cm. In addition, at least 14 adults of *T. leucotreta* eclosed between the depths of 0, 25 and 50 cm from pulped fruits and one adult at 25 cm from whole fruits. The average number of *T. leucotreta* ($F_{(3,\ 112)} = 1.04$, $P = 0.44$), *B. dorsalis* ($F_{(3,\ 112)} = 1.00$, $P = 0.40$) and *C. rosa* ($F_{(3,\ 112)} = 1.00$, $P = 0.40$) eclosed, between the disposal techniques and depths of burying were not significantly different. The average number of eclosed adult *Drosophila* sp. between the depth levels was significantly different ($F_{(3,\ 112)} = 3.43$, $P = 0.01$), thus, eclosion declined with burying depths. There were no significant differences in the number of eclosed *Silba* sp. ($\mathrm{F}_{(3,\ 112)} = 0.61$, $P = 0.61$) between the disposal techniques and depth of burying (Table 2.3).

Table 2.3. Number (mean ± SE) of adult internal feeding pests of *C. sinensis* eclosed from treatment plots where neither the pest's larvae nor eggs were not inoculated into the disposed fruits.

Disposal techniques	**Depths**	**Number (Mean ±SE) of adults eclosed[1]**					
		Thaumatotibia leucotreta	***Ceratitis capitata***	***Ceratitis rosa***	***Bactrocera dorsalis***	***Drosophila* sp**	***Silba sp***
Pulped	0 cm	0.07 ± 0.07a	-	-	-	52.40 ± 14.09a	5.33 ± 4.67a
	5 cm	-	-	-	-	12.40 ± 4.49b	9.20 ± 6.00a
	25 cm	0.13 ± 0.09a	-	-	-	8.33 ± 2.41b	3.40 ± 1.02a
	50 cm	0.27 ± 0.15a	-	-	-	19.87 ± 8.20b	3.33 ± 1.25a
Whole	0 cm	0.07 ± 0.07a	-	-	-	49.53 ± 20.95a	17.00 ± 10.79a
	5 cm	0.07 ± 0.07a	-	-	-	15.27 ± 8.87b	12.13 ± 7.64a
	25 cm	0.27 ± 0.21a	-	-	-	12.47 ± 5.98b	4.40 ± 1.71a
	50 cm	0.07 ± 0.07a	-	-	-	8.00 ± 3.15b	0.87 ± 0.62a

[1] Columns with the same letter shows that there were no significant differences.

2.3.4. Relationship between internal feeding pests of *C. sinensis* sharing a host

The presence of *B. dorsalis* (r = 0.29, *P* = 0.48), *Drosophila* sp. (r = 0.39, *P* = 0.33) and *Silba* sp. (r = 0.17, *P* = 0.69) in the fruit did not significantly affect the eclosion of adults of *T. leucotreta*. Furthermore, presence of *T. leucotreta* (r = -0.30, *P* = 0.46), *C. rosa* (r = 0.33, *P* = 0.42), *Drosophila* (r = 0.31, *P* = 0.44), and *Silba* sp. (r = 0.36, *P* = 0.38) also did not significantly affect the eclosion of *B. dorsalis*.

2.4. Discussion

The main aim of this chapter was to ascertain the effect of orchard sanitation disposal techniques and burying depth on the survival of the internal feeding pests of *C. sinensis*, including *B. dorsalis*. As observed from the chapter's results the employed disposal techniques and burying depths suppressed the eclosion of the tested pests in particular *T. leucotreta*, *C. capitata*, and *C. rosa*. Although, *B. dorsalis* had the highest eclosion rate of all tested pests, however, the number of the eclosed adults were still very low in comparison to the number of eggs inoculated per fruit. Furthermore, in a study conducted in Hawaii *B. dorsalis* larvae have been reported to successfully pupate and eclose at depths 20 cm to 25 cm in sandy soil with intermediate soil moisture (Alyokhin et al., 2001)

As mentioned earlier in the introduction exposure to unfavourable environmental conductions during pupation negatively affects the rate of pupation and eclosion eventually affecting the population increase of either *T. leucotreta* or tephritids fruit flies (Alyokhin et al., 2001). Thus, the low rate of eclosion by adults of *B. dorsalis*, and *T. leucotreta*, or the non eclosion of *C. capitata* and *C. rosa* from the inoculated fruits may be attributed the combined effect of the employed disposal techniques (i.e., pulping or whole), depths of fruit burial and edaphic factors such as soil texture, hence, the rate of eclosion decreased with the increase in the depth of burial. Similarly, Bohua et al. (2006) reported that the rate of eclosion of *C. capitata* decreases with the increase in depth level and Love et al. (2019) also reported similar results in *T. leucotreta*.

In contrast to our results, Klungness et al. (2005) conducted a similar study in United States of America in the state of Hawaii where it was found that smashing (i.e., pulping) and burying of infested fruit propagules between the depths of 15 and 30 cm partially prevents pest eclosion and does not sufficiently reduce the rate of eclosion of melon fruit fly *B. cucurbitae* (Diptera: Tephritidae) while burying at 46 cm depth prevented adult eclosion completely. While in our study treatment plots where pulped fruits were disposed recorded the lowest rate of eclosion in all depth levels (Deguine et al., 2005; Klungness et al., 2005; Jang et al., 2010).

Thaumatotibia leucotreta and tephritid pests prefer to pupate between the depths of 0 to 5 cm in sandy soil with a moisture of 10 to 30%. Sandy soil is more porous allowing eclosed teneral adult pests to ascend to the ground surface (Alyokhin et al., 2001; Bohua et al., 2006; Love et al., 2019). Similarly, other Tephritidae species from the genera *Anastrepha*, *Ceratitis* and *Rhagoles*, including *Bactrocera* species such as peach fruit fly *B. zonata* (Diptera: Tephritidae), Queensland fruit fly *B. tryoni* (Diptera: Tephritidae) and Olive fruit fly, *B. oleae* (Diptera: Tephritidae) also prefer to pupate between the depth of 0 to 5 cm in soil with low bulk density (Hulthen & Clarke, 2006; Yee, 2013; El-Gendy & AbdAllah, 2019). In a study by Love et al. (2019) it was discovered that *T. leucotreta* larvae can optimally pupate and eclose successfully at the depth of 4 mm (i.e., 0.4 cm), and pupation success decreases with increasing soil depth beyond that.

Thaumatotibia leucotreta and tephritids larvae and pupae are susceptible to desiccation and the highest larval and pupal mortality is attributed to lack of, or too much moisture (Alyokhin et al., 2001; Bohua et al., 2006; Love et al., 2019). Soil moisture of between 80 to 100% also inhibits pupation and may induce surface pupation by *T. leucotreta* larvae (Love et al., 2019), while 0% soil moisture causes 85% pupal mortality rate on *B. tryoni*, and high moisture regimes reduce pupal eclosion affecting negatively on the emergence of *B. zonata* (Hulthen & Clarke, 2006; El-Gendy & AbdAllah, 2019).

With the South Africa citrus industry being export oriented (CGA, 2020), compulsory phytosanitary measures such as orchard sanitation implemented to reduce the impact of the internal feeding pests on citrus fruits must be efficient to ensure that fruits are pest free. The economic significance of these results is that the disposal techniques proved to be effective in disrupting the development of larvae and pupae of *T. leucotreta* and tephritids fruit flies resulting in a low adult eclosion. Thus, their practice in citrus orchards will help improve fruit production.

CHAPTER THREE: THE EFFECT OF ANT PREDATION ON ECLOSION OF INTERNAL FEEDING PHYTOSANITARY PESTS OF *CITRUS SINENSIS* IN SOUTH AFRICA

3.1. Introduction

Integrated Pest Management (IPM) involves the use of multiple methods to suppress the population of economic and phytosanitary pests in agriculture (Manrakhan & Pterandrus, 2016; Moore, 2019). The incorporated control measures needs to be compatible and may include biological, chemical, cultural or behavioural measures (Manrakhan & Pterandrus, 2016; Moore, 2019). Although the techniques are applied to suppress the population and development of a target pest, some non-target pests and beneficial organisms inhabiting the agroecosystems may also be affected (Coombes et al., 2017).

Although IPM measures are effective (Manrakhan & Pterandrus, 2016; Moore, 2019), naturally occurring predators and parasitoids compliment these control measures by also attacking the vulnerable immature stages of insects (i.e., eggs, larvae, pupae and teneral adults) (Urbaneja et al., 2006; Bownes et al., 2014). In citrus orchards in South Africa and Spain predatory arthropods have been found complementing the primary cultural and behavioural practices used were from the families such as Formicidae, Staphylinidae, Aranea, Carabidae, Cicindelidae, and Dermaptera prey on the immatures (i.e., larvae and pupae) of soil pupating pest at the floor of the orchards (Urbaneja et al., 2006; Bownes et al., 2014). Other natural enemies include the eggs parasitoid *Trichogrammatoidae cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) which attacks eggs of *T. leucotreta*, also attacking *T. leucotreta* larvae include the larval parasitoids *Agathis bishopi* Nixton (Hymenoptera: Braconidae) and *Apophua leucotreta* Wilkinson (Hymenoptera: Ichneumonidae), and the larval predators from the genera *Orius* (Hemiptera: Anthocoridae) (Malan et al., 2018; Moore, 2019).

Among different predatory arthropods ants are the most diverse species and are widely distributed group (Niu et al., 2014). The abundance and predation intensities of ants directly influences the diversity of both pests and beneficial arthropods, thus, disrupting their contribution towards the ecological functions (Nyamukondiwa & Addison, 2014; Calabuig et al., 2015). Predatory activities by ants, especially on pests of agricultural or phytosanitary importance aids in the reduction of the undesired species at different agroecosystems (Wang et al., 2017). In South Africa, the native pugnacious ant *Anoplolepis custodiens* F. Smith (Formicidae: Formicinae) and exotic brown house ant *Pheidole megacephala* Fabricius (Formicidae: Myrmicinae) are amongst the common and widely distributed predators that predate on the immatures (i.e., larvae and pupae) of soil pupating pests such as bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) and Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) at the floor of citrus orchards (Bownes et al., 2014). Similarly, populations of African weaver ant *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) augments in the management of the wandering larvae stage of *Bactrocera dorsalis* (Diptera: Tephritidae) on the floor of Mango orchards in western African (Ekesi et al., 2016).

Although different management procedures (e.g., orchard sanitation, disposal techniques and chemicals) are employed to control orchard pests such as *T. leucotreta* and tephritids fruit flies, they remained amongst the most destructive pests of *C. sinensis* in South Africa (Moore & Kirkman, 2009; Manrakhan & Pterandrus, 2016; Moore, 2019). Naturalized ants and other predatory arthropods on the floor of the orchard may be complementing the commonly applied sanitation techniques to minimize the population of citrus pests. In this study, we hypothesise that the predatory ants contribute to the suppression of the soil pupating pests of citrus, especially the wandering larval and immobile pupal stages of *T. leucotreta* and tephritids fruit flies. Thus, the current study aimed at determining the contribution of predatory ants on the suppression of *T. leucotreta*, *C. capitata*, *C. rosa* and *B. dorsalis* on disposed *C. sinensis* fruits.

3.2. Materials and methods

3.2.1. Site details

The study was conducted at the University of Mpumalanga experimental farm (25°42’50.88’’ S; 30°96’98.99’’ E), Mbombela campus. The selected experimental plot (Area: Length 50 m × Breath 20 m) has sandy loamy soil and is frequently used for the cultivation of cash crops such as maize and groundnuts. The inter and intra row spacing between the augmentorium nets was one meter ($1m^2$). The experimental site is within the Lowveld region of Mpumalanga Province which experiences subtropical climates with warm (min 20 ºC and max 30 ºC temperatures) and rainy (average annual rainfall: 620 mm) summer and dry cooler winters (min 13 ºC and max 26 ºC) (Stander, 2015).

3.2.2. Fruit disposal

Citrus sinensis fruits inoculated with the larvae of *T. leucotreta*, eggs of *C. capitata*, *C. rosa* and *B. dorsalis* were disposed at different depths as either pulped or whole (See more details in Chapter 2). At each disposal plot, ants were excluded to minimize the predatory activities by these common predatory ants which are widely distributed across citrus producing regions in South Africa (Samways et al., 1982).

3.2.3. Ant exclusion and sampling

A recommended dosage of $6g/m^2$ of the ant bait Maxforce (IUPAC name: hydramethylnon) was applied weekly for a period of five months to exclude common ants and minimize their predatory impacts on the wandering larvae and pupae of the soil pupating pests of *C. sinensis*. Ants were monitored using pitfall traps as jars (i.e., 8 cm height × 7 cm diameter). Jars were buried with their rims flushing with the ground surface and were half-filled with propylene glycol (IUPAC name: propane-1,2-diol). Jars were buried at each of the four corners of the augmentorium net (0.25 m²) and were left open for five consecutive days during ant collection (Bownes et al., 2014; Mauda et al., 2018). Data from the four traps at each treatment plot was pooled for analysis. Sampling was done at 21day interval (i.e., between April, and August 2021). Sampled ants were preserved in a 70% ethyl-alcohol to maintain their morphological features before identification (Bownes et al., 2014). Sampled ants were identified to morphospecies using Ant of Southern Africa- Ant Catalogue: Southern Africa (Slingsby, 2017).

3.3.3. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the ant abundance and species richness from each treatment plot where *C. sinensis* fruits were disposed as pulped or whole and buried at different depths. While a Mann Whitney U test was used to compared significant difference between where pulped or whole fruits were disposed and buried at different depths. Furthermore, Spearman's Rank order correlations was used to determine if whether there was any interaction between the ants and the eclosed adults of the internal feeding pest of *C. sinensis*, by correlating the average number of the eclosed adults of the internal feeding pests of *C. sinensis* with the sampled ants from each treatment plot to ascertain the ant's role on the predation of the pest immatures or teneral adults. While principal components analysis was used to determine the distribution of sampled ants. All statistics tests were done using Statistica, version 13.3.0.

3.4. Results

A total of 27 073 individual ants (Hymenoptera: Formicidae) were collected in the study, with at least 47% and 53% sampled from plots where pulped and whole fruits of *C. sinensis* were disposed, respectively. Among the sampled morphospecies of ants were *Pheidole*1 (72% of ants collected), *Pheidole*2 (18%), Formicinae1 (4%), Formicinae2 (2%), Formicinae3 (2%), and Myrmicinae1 (2%). There were no significant differences on the number of ants sampled from treatment plots where *C. sinensis* fruits were disposed as either pulped or whole (Table 3.1).

Table 3.1. Abundance and richness (mean ± SE) of ants sampled by pitfall trapping from treatment plots where *C. sinensis* fruits inoculated with larvae of *T. leucotreta*, eggs of *C. capitata*, *C. rosa*, and *B. dorsalis* were disposed of as pulped or whole fruits and buried at different depths, at the University of Mpumalanga experimental Farm.

Technique	**Depth**	**Ant abundance (mean ± SE)**[1]		**Statistics**		
		Pulping	Whole	DF	Mann Whitney U	P- value
Abundance	0 cm	72.04 ± 8.54a	89.76 ± 9.79a	3	862.00	0.22
	5 cm	75.33 ± 8.83a	69.02 ± 7.96a	3	964.00	0.70
	25 cm	69.38 ± 8.27a	79.07 ± 10.02a	3	980.00	0.79
	50 cm	67.84 ± 7.79a	79.07 ± 10.45a	3	971.50	0.74
Richness	0 cm	4.00 ± 0.26a	4.33 ± 0.22a	3	913.50	0.41
	5 cm	4.11 ± 0.25a	3.60 ± 0.23a	3	825.50	0.12
	25 cm	4.00 ± 0.25a	3.71 ± 0.24a	3	923.50	0.46
	50 cm	3.69 ± 0.25a	3.89 ± 0.26a	3	952.00	0.62

[1] Columns with the same letters shows that the was no significant difference

Although the abundance of sampled ants was not statistically significant, further analysis showed that the number of sampled Formicinae1 at 5 cm ($U = 162.5$, $P < 0.03$), Formicinae2 at 0 cm ($U = 248.5$, $P < 0.001$) and 5 cm ($U = 187.0$, $P < 0.01$) and Myrmicinae1 at 0 cm ($U = 201.5$, $P < 0.02$) were significantly different between plots where *C. sinensis* fruits were disposed as either pulped or whole (Table 3.2).

Table 3.2. Ants (mean ± SE) sampled by pitfall trapping from treatment plots where *C. sinensis* fruits inoculated with larvae of *T. leucotreta*, eggs of *C. capitata*, *C. rosa*, and *B. dorsalis* were disposed of as either pulped or whole and buried at different depths at the University of Mpumalanga experimental Farm.

Ant name	**Depth**	**Ant abundance (mean ± SE)^1**		**Statistics***		
		Pulped	Whole	N	U- value	P
Pheidole1	**0 cm**	89.9 ± 11.0	106.9 ± 13.2	40	597.5	0.20
	5 cm	83.4 ± 10.2	82.2 ± 10.9	39	634.5	0.47
	25 cm	82.1 ± 9.6	118.1 ± 12.4	39	687.5	0.59
	50 cm	81.3 ± 15.0	126.6 ± 9.5	37	620	0.49
Pheidole2	**0 cm**	28.1 ± 4.3	23.7 ± 3.1	41	756.5	0.82
	5 cm	28.7 ± 5.0	22.8 ± 3.0	40	639.5	0.23
	25 cm	28.1 ± 5.3	27.0 ± 2.2	39	707.0	0.73
	50 cm	22.9 ± 2.6	28.3 ± 2.2	37	655.5	0.91
Formicinae1	**0 cm**	4.1 ± 0.5	3.4 ± 0.5	31	359.5	0.25
	5 cm	6.3 ± 2.6	4.8 ± 1.0	28	291.0	0.40
	25 cm	8.8 ± 2.6	5.3 ± 0.6	27	162.5	**0.03**
	50 cm	18.3 ± 4.9	4.9 ± 0.5	30	326.0	0.55
Formicinae2	**0 cm**	3.8 ± 0.6	1.9 ± 0.4	34	248.5	**0.001**
	5 cm	2.3 ± 0.4	2.8 ± 0.6	34	187.0	**0.01**
	25 cm	3.5 ± 0.7	3.4 ± 0.5	26	250.5	0.22
	50 cm	3.3 ± 0.9	3.2 ± 0.6	26	281.5	0.92
Formicinae3	**0 cm**	3.0 ± 0.6	1.6 ± 0.4	28	213.5	0.14
	5 cm	3.4 ± 0.8	3.5 ± 0.8	27	174.0	0.10
	25 cm	4.5 ± 0.8	4.0 ± 0.5	25	281.0	0.70
	50 cm	4.5 ± 0.9	3.7 ± 0.4	24	251.5	0.99
Myrmicinae1	**0 cm**	4.0 ± 0.6	2.2 ± 0.5	27	201.5	**0.02**
	5 cm	3.9 ± 0.7	2.7 ± 0.8	23	245.5	0.86
	25 cm	4.1 ± 0.7	4.6 ± 0.8	26	300.0	0.81
	50 cm	2.5 ± 0.7	5.0 ± 0.8	26	219.0	0.11

*Superscript: Statistically significant values are highlighted in bold (*Mann-Whitney U* test at $p < 0.05$)

3.4.1. Relationship between sampled ants and the eclosed internal feeding pests of *C. sinensis*

The ants did not significantly affect the survival and eclosion of the inoculated immatures or eclosed teneral adults of either *T. leucotreta* (r = -0.45, P > 0.26) or *B. dorsalis* (r = 0.43, P > 0.29). Furthermore, the sampled ants also did not affect the survival and eclosion of non-inoculated eclosed adults of the internal feeding pests of *C. sinensis* such as *T. leucotreta* (r = -0.28, P > 0.50), *C. rosa* (r = -0.41, P > 0.31) *B. dorsalis* (r = -0.41, P > 0.31), *Drosophila* (r = -0.11, P > 0.80) and *Silba* sp (r = 0.23, P > 0.59).

3.5. Discussion

In the current study, more ants were sampled from plots where whole *C. sinensis* fruits were disposed compared to plots where fruits were pulped, but the ants did not affect the survival and/or the eclosion of the internal feeding pests of *C. sinensis*. Slow toxic ant baits such as Maxforce enable ants to collect and transport the bait to the colony where trophallaxis occurs between colony members (Greenberg et al., 2013; Buczkowski et al., 2014; Nyamukondiwa & Addison, 2014). This is supported by Bownes et al., (2014) who reported that pupae of *H. armigera*, *T. leucotreta* and *C. capitata* survived significantly better in plots where the area prevalent with *A. custodiens* and *P. megacephala* predators were poisoned compared to the untreated control. Although, *Pheidole* species mainly prey on smaller items such as nematodes, fruit maggots, root maggots and rootworm eggs (Bownes, 2003). Nyamukondiwa & Addison (2014) reported that for effective management of ants, knowledge about their foraging activity is very important hence such information may help in terms of bait distribution and bait size and formulations (i.e., liquid or granules) which influence bait collection. For example, *L. humile* and *C. peringueyi* ant species are attracted to a 25% liquid concentration of either sugar or honey mixed with water than tuna bait. Although, *A. custodiens* species are mainly attracted to tuna-based baits the ants are also attracted to liquid concentration of honey mixed with water.

Pheidole sp. are amongst the most efficient predatory ants able to prey and remove approximately 93% of tephritid larvae and pupae from the ground (Fernandes et al., 2012; Bownes et al., 2014). Campolo et al. (2015) also reported that *C. capitata* larvae were severely preyed on by the ant *Tapinoma nigerrimum* (Nylander) on the floor of citrus orchards in the Mediterranean region, although, olfactory cues and larval burying affected prey location and predation by ants. Urbaneja et al., (2006), also reported that the mean survival of *C. capitata* pupae was 35.7 ± 6.2% and 14.3 ± 6.7% where the pest was preyed on by ants and other arthropods from the family Staphylinidae and spider species on the floor of the citrus orchard. Augmentative practices to increase populations of African weaver ants *Oecophylla longinoda* (Latreille) to control the wandering larvae of *B. dorsalis* in Mango orchards in west African countries significantly reduced the pest population (Ekesi et al., 2016).

Burying of inoculated propagules also contributed to the lack of interaction between the ants and the internal feeding pest of *C. sinensis.* Fernandes et al., (2012) showed that larval drilling reduces predation on tephritid fruit fly larvae by predatory ant species. In addition, Aluja et al., (2005) also reported that *Anastrepha* sp. (Diptera: Tephritidae) bury themselves immediately after exiting the fruit in a process that may take 10 minutes, as predatory ant attacks occur mainly within the first five minutes after exiting the fruit. Although, burying of some of *C. sinensis* fruits did not affect the occurrence of the ants, this is because ants are foraging arthropods and some species such as *L. humile*, and *A. custodiens* have a foraging distance of almost hundred meters (Nyamukondiwa & Addison, 2014).

In conclusion, due to the application of the ant bait and burying of fruit propagules the ants did not play a significant role in the mortality of the internal feeding pests of *C. sinensis*. Similar to Bownes et al. (2014), the findings of the current study results showed that poisoning of the area where ants occur negatively affected ant populations and foraging activity resulting in the survival of soil pupating pests such as *T. leucotreta* and tephritids fruit flies. Although ants were deliberately excluded from the current study ant management needs to be less intensive particularly on the floor of the citrus orchards to ensure that adequate ant populations are always present to control ground pupating pests such as *T. leucotreta* and tephritids fruit flies.

CHAPTER FOUR: GENERAL DISCUSSION AND RECOMMENDATIONS

4.1. Introduction

Although IPM suppresses the population of the economic and phytosanitary pests in citrus orchards in South Africa and elsewhere in the world (Moore, 2019; Pieterse et al., 2020). There are still ongoing interception reports of infested fruit consignments by quarantine listed pests (i.e., *T. leucotreta*) from the exporting countries where South Africa trades its citrus fruit, thus threatening the country's citrus industry (Carstens et al., 2021). While phytosanitary measures are meant to detect and discard infested fruit propagules, they are also used as guidelines to grant access to export citrus fruits to the international market. Thus, the industry relies on the phytosanitary measures to detect, control, and ensure that the intended fruit to be exported are pest free particularly of quarantine listed pests.

Although, in South Africa stakeholders such as the Perishable Products Export Control Board (PPECB) and the Department of Agriculture, Land reform and Rural Development (DALRRD) through the Directorate of Food Safety and Quality Assurance participate in the inspection, supervision of the packing line, and certification of citrus fruits to be exported, to mitigate the introduction of unwanted pests to the export countries (Carstens et al., 2021). Field base phytosanitary measures such as orchard sanitation are at the forefront to mitigate pest populations at the orchard, thus, with the constant change of regulations in terms of the exportation of citrus fruit from South Africa these phytosanitary measures need to be regularly assessed to meet the new set of standards required (Moore et al., 2016; Hattingh et al., 2020; Carstens et al., 2021).

Citrus orchard sanitation is a compulsory prerequisite to citrus farmer in South Africa to specifically control the populations of the internal feeding pests of citrus such as *T. leucotreta* and species of tephritids fruit flies naturalised in the country's citrus orchards (i.e., *C. capitata*, *C. rosa* and *B. dorsalis*) (Moore & Kirkman, 2009; Moore, 2019). Although, the impact of orchard sanitation to control the internal feeding pests of citrus from orchards has been studied in isolation (Moore & Kirkman, 2009), the combined impacts of disposal techniques (i.e., disposing fruit either as pulped or whole) and burying depths on populations of these pests has not been assessed. Orchard sanitation practices such as fruit pulping and burying disrupts the development of the internal feeding pest of citrus within the host fruit and as a result pupation may not occur (Klungness et al., 2005; Moore & Kirkman, 2009; Moore, 2019). Although, in other similar studies already conducted such as the work by Klungness et al. (2005) who found that smashing and burying of infested fruit propagules between the depths of 15 and 30 cm partially prevents pest eclosion and does not sufficiently reduce the rate of eclosion of melon fruit fly *B. cucurbitae* (Diptera: Tephritidae) while burying at 46 cm depth prevent adult eclosion. In addition, augmentorium nets covering the disposed of fruits significantly sequester eclosed adult fruit flies and proved to be effective by ensuring that no adult pest escapes (Deguine et al., 2005; Klungness et al., 2005; Jang et al., 2010). Therefore, this study sought to quantify the impact of the disposal methods and burial depth on the eclosion of *T. leucotreta* and three tephritid fruit fly species (e.g. *C. capitata, C. rosa* and *B. dorsalis*) infesting *C. sinensis* varieties in South Africa.

4.2. Disposal methods and burial depths

Chapter two investigated the impact of the disposal techniques and depths of burying on the eclosion of the inoculated internal feeding pests of citrus. The disposal techniques and depth of burying suppressed the eclosion of the internal feeding pests in *C. sinensis* fruit which were disposed as either pulped or whole, and buried at different depths. Although, *B. dorsalis* recorded the highest number of eclosed adults. More adults eclosed from the disposed whole *C. sinensis* fruits buried in shallow depths (i.e., 0, 5 and 25 cm) while few adults eclosed from a depth of 50 cm. More *T. leucotreta* adults eclosed from treatment plots where the disposed fruits were not inoculated with the pest larvae (i.e. the control plots). This suggests a very FCM population pressure in the orchards rom which the fruit were harvested. Adults of *C. capitata* did not eclose from the disposed fruit while one adult of *C. rosa* eclosed from a treatment plot where the disposed fruit were inoculated with eggs of *B. dorsalis*. The suppression on the eclosion of the pests may be attributed to edaphic factors such as soil texture, soil moisture, soil temperature, and depth of burying which are amongst effective abiotic factor causing pupal mortality on either *T. leucotreta* or tephritids fruit flies. Thus, soil such as clay loam, silty loam and silty clay loam do not support eclosion by either *T. leucotreta* or tephritids fruit flies because they have a higher bulk density thus are less porous preventing teneral adults from ascending to the ground surface. The results of this chapter are very important since they showed that the investigated disposal methods and burial depths significantly suppressed the eclosion of the tested pests thus contradicting the finding by Klungness et al. (2005), who found that the disposal methods and shallow burying of fruit propagules did not suppress the eclosion of melon fruit fly *B. cucurbitae* (Diptera: Tephritidae) in the state of Hawaii in the United States of America. Although other variables which may had been investigated in this study include the efficacy of the augmentorium nets to sequester the eclosed adults of the tested pests.

4.3. The role of ants in the control of soil borne stages of citrus pests

With regards to whether the sampled ants affected the survival or eclosion of the tested pests as assessed in Chapter three, due to the application of the ant bait Maxforce ants did not affect the survival or eclosion of either *T. leucotreta* or the three tephritids fruit flies species from this study. Maxforce is a slow toxic ant bait which enables ants to collect and transport the bait to the colony where trophallaxis occurs between colony members as result killing and reducing their populations in the area. Although burying of fruits did not affect the occurrence of the ants, this is because ant species such as those from the genus *Pheidole* have a foraging distance of almost one hundred meters. Foraging activity has to do with the distance the ants travel to locate and collect feed resources (Nyamukondiwa & Addison, 2014). Thus, the results of this study agree with the findings by Bownes et al. (2014) who found that poisoning of ants delimits their foraging activity and reduces their population in the area where they occur. As a result, the ants become less effective as predators. Although known predatory ants such as the *Pheidole* sp group were sampled which have been reported as the most efficient predators able to prey on either *T. leucotreta* and tephritids fruit fly immature stages in citrus orchards. Although, there are other effective predators such as *Tapinoma nigerrimum* (Nylander) in Mediterranean region controlling fruit fly immatures stages in citrus orchards, other ant species such as the African weaver ants *Oecophylla longinoda* (Latreille) are also good predators of other tephritids species such as B. dorsalis (Urbaneja et al., 2006; Fernandes et al., 2012; Bownes et al., 2014; Campolo et al., 2015; Ekesi et al., 2016). However, ants can also be pests if their populations is not controlled. Ants form mutualistic relations with plant sap-sucking honeydew producing homopterans such as mealybugs, whereby they tent and protect the pest against their natural enemies while collecting honeydew from them. Although homopteran species such as red scales do not produce honeydew the presence of ants in the tree canopy prevents the work by their natural enemies to parasitise or prey on the pests. Thus, resulting in the increase the pests populations. Therefore, other variables which could have been investigated in this study include other predatory species which have the potential to prey on either *T. leucotreta* or tephritids fruit flies immature stages. Urbaneja et al. (2006) mentioned that there are other predatory species from the families such as Staphylinidae, Aranea, Carabidae, Cicindelidae, and Dermaptera which prey on the immatures (i.e., larvae and pupae) of soil pupating pests at the floor of the orchards complementing the primary cultural and behavioural practices (Urbaneja et al., 2006; Bownes et al., 2014).

4.4. CONCLUSION AND RECOMMENDATIONS

The results indicate that the disposal methods and deeper burying depths suppressed the eclosion of the tested pests. Although other pests eclosed from treatment plots where neither the pest larvae nor eggs were not inoculated, there were still no significant numbers which may pose an economic threat at the orchards. Ants were effectively controlled by the ant bait and thus there was no relationship between the ants and the eclosed adult pests.

Based on the results of this study citrus farmers are advised to continue to pulp abscised collected citrus fruits from the floor of the orchards with a hammer mill and bury the pulp 50 cm and beyond underground in soil with high bulk density, which is less porous because it prevents eclosed adults of either *T. leucotreta* or tephritids species to ascend to the ground surface. In addition, the area where fruits would be disposed of must also be covered with an augmentorium net to sequester successfully eclosed adult pests from escaping to the orchards. Poisoning of ant around the area where fruit are disposed of must not be practiced. Because predacious ants are effective in preying on the larvae and pupa of either *T. leucotreta* or tephritids fruit fly species.

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