Biology and ecology of *Ceratitis rosa* and *Ceratitis quilicii* (Diptera: Tephritidae) in citrus

J Daneel

orcid.org 0000-0001-9854-7896

Dissertation accepted in fulfilment of the requirements for the degree *Master of Science in Environmental Sciences* at the North-West University

Supervisor:	Prof J van den Berg
Co-supervisor:	Dr A Manrakhan

Graduation May 2020

25006754

DECLARATION

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

Date: 25 November 2019 Sign: 

ACKNOWLEDGEMENTS

There are several people without whom this project would not have been possible. Therefore, I would like to give special thanks to:

- My supervisors, Prof Johnnie van den Berg and Dr Aruna Manrakhan, for their guidance and dedication to this project.
- Prof Suria Ellis for her patience and assistance with statistical analyses.
- Dr Massimiliano Virgilio for the genetic determination of the female flies.
- Bianca Greyvenstein for assistance with, and creation, of the maps.
- Citrus Research International for technical staff who assisted with field- and lab work, with particular thanks to Catherine Savage who helped to proofread this manuscript.
- Citrus Research International for funding and allowing the project to be conducted.
- All the growers who allowed us to work in their orchards and who supplied the project with fruit.
- My family and friends for their support and encouragement.

ABSTRACT

Ceratitis rosa Karsch s. l. (Diptera: Tephritidae), an indigenous pest of commercial fruit including citrus in South Africa, belongs to a complex of cryptic species (*Ceratitis* FAR). *Ceratitis rosa* s.l. was recently split into *C. rosa* and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio. The recent description of a new species in the FAR complex impacts the pre- and postharvest management of this species complex in South Africa. In light of the species split and the lack of specific information on each of these species, this study was conducted to determine: (1) the relevant abundance of these species in citrus in the northern parts of South Africa, (2) how effective attractant-based traps in citrus orchards are to these species, and (3) the rate of larval development in fruit of different citrus types. Traps baited with three types of attractants (EGO Pherolure, Capilure (male lures), and three-component Biolure (food-based attractant)) were set out in orchards on nine farms, for a period of one year, in the northern parts of South Africa. Males of the two species were distinguished morphologically, whereas the females were identified using microsatellite markers. Larval development of *C. rosa* and *C. quilicii* were compared in *Citrus limon*, *C. paradisi*, *C. reticulata* and *C. sinensis*. Eggs were artificially inoculated into fruit of each citrus type and larval development assessed daily over 15 days, by dissecting sub-samples of the infested fruit. *Ceratitis quilicii* were more abundant than *C. rosa* through almost all of the study areas and *C. quilicii* appeared to tolerate a wider temperature regime than *C. rosa*. *Ceratitis rosa* was negatively affected by low temperatures. EGO Pherolure and Biolure were effective in trapping both fly species. The ratio between the two fruit fly species was similar, when comparing the male and female catches in *C. quilicii* dominated areas. The development of these two fruit fly species did not differ in each of the citrus types. There were however differences in larval and pupal survival rates between the species depending on citrus type. For both species, larval development was optimal in *C. sinensis* and poor in *C. reticulata*. *Ceratitis rosa* had higher larval survival rates than *C. quilicii* in *C. limon* and *C. sinensis*. Survival was also highest in *C. sinensis* and it is the most suitable host to conduct further cold sterilization trials with. The different instars of both species can be exposed on similar days when exposing the fruit to a cold treatment to determine which life stage is the most cold tolerant. Findings in this study contribute to improved management of *C. rosa* and *C. quilicii* which are of quarantine importance in Africa by determining: the northern distribution in South Africa, the effectiveness of commercially available traps, and the most susceptible citrus cultivar to use for future cold sterilization work.

Key words: *Ceratitis quilicii*, *Ceratitis rosa*, attractants, relative abundance, larval development.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1.1. *Ceratitis rosa* s.l. a pest of quarantine importance

Ceratitis rosa s.l. Karsch (Natal fruit fly) belongs to the subtribe Ceratitidina (tribe Dacini), an Afrotropical group of fruit flies with populations also occurring in Mauritius and Reunion (De Meyer 2001). *Ceratitis rosa* s.l. attacks a wide variety of indigenous and commercial fruit (White & Elson-Harris 1992; De Meyer 2001) and is considered a pest of quarantine (phytosanitary) importance (Badii *et al*. 2015). In South Africa, *C. rosa* s.l. is distributed in the northern and eastern parts and along most of the coastal areas (including the south western areas) of the country, while it is largely absent in the drier inland areas and arid regions (De Villiers *et al*. 2013). *Ceratitis rosa* s.l. prefers higher elevations and a wetter climate (Normand *et al*. 2000). In commercial citrus orchards in South Africa, *C. rosa* s.l. is managed by means of aerial and ground insecticidal bait applications, bait stations, male annihilation techniques (MAT), and orchard sanitation (Manrakhan 2019). For some export markets, additional phytosanitary risk mitigation assurances must be provided with mandatory postharvest cold treatment of the fruit being required by official bilateral trade protocols (Grout *et al*. 2011).

1.2. A taxonomic review of *C. rosa* s.l.

The taxonomy of *C. rosa* s.l was recently reviewed and new insights established. *Ceratitis rosa* s.l. is one of the species in the *Ceratitis* FAR-complex (acronym for *fasciventris*, *anonae* and *rosa*) which consists of three species: *Ceratitis fasciventris* (Bezzi)*, C. anonae* Graham and *C. rosa* s.l. (Hendrichs *et al.* 2015). Through the use of microsatellites, it was established that the FAR-complex consisted of five genotypic clusters, with *C. anonae* having a single cluster, *C. fasciventris* consisting of two clusters *(*F1 and F2) with allo- and parapatric distributions, and *C. rosa* with two clusters (R1 and R2), with an allo- and sympatric distribution (Virgilio *et al*. 2013). The sympatric clusters of *C. rosa* were reported to both occur in South Africa (Virgilio *et al.* 2013). Laboratory studies showed differences in thermal biology of the two *C. rosa* types (Tanga *et al*. 2015). Trapping studies in Tanzania also confirmed different climatic requirements of the two *C. rosa* types with R1 being more abundant in hot areas and R2 being more abundant in cooler areas (Mwatawala *et al.* 2015). The R1 and R2 clusters were further differentiated morphologically using feathering on the midtibia of the males (De Meyer *et al.* 2015). After it was suggested that the *C. rosa* R2 should be considered a new species (De Meyer *et al*. 2015), De Meyer *et al*. (2016) subsequently described morphotype R2 as *Ceratitis* (*Pterandrus*) *quilicii* De Meyer, Mwatawala & Virgilio and R1 was referred to as the true type of *C. rosa*.

1.3. Practical management implications

There is currently a gap of knowledge regarding whether *C. rosa* or C. *quilicii* might be problematic to the citrus industry in South Africa, if not both. It is also not known if there is a differential response of the two species to currently available fruit fly attractants. Furthermore, since these species are of quarantine importance, the efficacy of currently used postharvest cold treatment schedules for each species will have to be determined. In this study, aspects of the biology and ecology of *C. rosa* and *C. quilicii* will be quantified in order to provide a rational basis for pest management and the biological information required for further efficacy evaluation of postharvest cold treatments.

1.4. Problem statement

Ceratitis rosa s.l. is of economic importance to the South African fruit industry. Recent studies have demonstrated that there are two morphotypes and genotypes of *C. rosa* (Virgilio *et al.* 2013). These two types have been split into two species (De Meyer *et al*. 2016). *Ceratitis rosa* and *C. quilicii* occur sympatrically in South Africa (Virgilio *et al.* 2013). The implications of the species split are that previously collected data on pre- and postharvest treatments for *C. rosa* s.l. might now be questionable for use on either species. The research described below addresses the biology and ecology of *C. rosa* and *C. quilicii* and identifies similarities and differences between the two species. The focus of the study will be on citrus and aspects of their biology and ecology that have a direct implication for practical pest management and phytosanitary risk mitigation of relevance to international trade of citrus fruit.

1.5. General objective

This study aims to quantify similarities and differences in the biology and ecology of *C. rosa* and *C. quilicii* in citrus.

1.6. Specific objectives

The specific objectives of this study are:

- to determine relative abundance of *C. rosa* and *C. quilicii* in citrus orchards using attractant-based traps and through fruit collection;
- to compare the response of *C. rosa* and *C. quilicii* to two male attractants and one food-based attractant;
- to compare the developmental rates of all life stages of *C. rosa* and *C. quilicii* in citrus fruit under constant temperature rearing conditions.

1.7. References

BADII, K.B., BILLAH, M.K., AFREH-NUAMAH, K., OBENG-OFORI, D. & NYARKO, G. 2015. Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae). *African Journal of Agricultural Research* **10**: 1488-1498.

DE MEYER, M. 2001. On the identity of the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **71**: 55-62.

DE MEYER, M., DELATTE, H., EKESI, S., JORDAENS, K., KALINOVÁ, B., MANRAKHAN, A., MWATAWALA, M., STECK, G., VAN CANN, J., VANÍČKOVÁ, L., BŘÍZOVÁ, R. & VIRGILIO, M. 2015. An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 405-427.

DE MEYER, M., MWATAWALA, M., COPELAND, R.S. & VIRGILIO M. 2016. Description of new *Ceratitis* species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy* **233**: 1-23.

DE VILLIERS, M., MANRAKHAN, A., ADDISON, P. & HATTINGH, V. 2013. The distribution, relative abundance, and seasonal Phenology of *Ceratitis capitata, Ceratitis rosa,* and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa*. Environmental Entomology* **42**: 831-840.

GROUT, T.G., STEPHEN, P.R., DANEEL, J.H. & HATTINGH, V. 2011. Cold treatment of *Ceratitis capitata* (Diptera: Tephritidae) in Oranges using a larval endpoint. *Journal of Economic Entomology* **104**: 1174-1179.

HENDRICHS, J., VERA, M.T., DE MEYER, M. & CLARKE, A.R. 2015. Resolving cryptic species complexes of major tephritid pests. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 5-39.

MANRAKHAN, A. 2019. Fruit fly. In T.G. Grout (Ed.) *Integrated Production Guidelines for export citrus. Integrated pest and disease management.* 1-10. Nelspruit, South Africa: Citrus Research International.

MWATAWALA, M.W., VIRGILIO, M., JOSEPH, J. & DE MEYER, M. 2015. Niche partitioning among two *Ceratitis rosa* morphotypes and other *Ceratitis* pest species (Diptera, Tephritidae) along an altitudinal transect in Central Tanzania. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 429-442.

NORMAND, F., QUILICI, S. & SIMIAND, C. 2000. Seasonal occurrence of fruit flies in strawberry guava (*Psidium cattleianum* Sabine) in Reunion Island: host phenology and fruit infestation. *Fruits* **55**: 271-281.

TANGA, C.M., MANRAKHAN, A., DANEEL, J.-H., MOHAMED, S.A., FATHIYA, K. & EKESI, S. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis rosa* Karsch (Diptera, Tephritidae) populations from Kenya and South Africa. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 467-487.

VIRGILIO, M., DELATTE, H., QUILICI, S., BACKELJAU, T. & DE MEYER, M. 2013. Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa, Ceratitis fasciventris* and *Ceratitis anonae* (Diptera: Tephritidae). *Molecular Ecology* **22**: 2526-2539.

WHITE, I.M. & ELSON-HARRIS, M.M. 1992. *Fruit fly of economic significance: their identification and bionomics.* C.A.B. International, London, UK and Australian Centre for International Agricultural Research, Canberra, Australia. xii + 601 pp.

CHAPTER 2: LITERATURE REVIEW

2.1. Background

The aim of this thesis is to elucidate the biology and ecology of two closely related species, *Ceratitis rosa* Karsch and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio, previously considered as one species, *C. rosa* (De Meyer 2001b) within the same cryptic species complex, i.e. the *Ceratitis* FAR complex (Virgilio *et al*. 2013).

2.1.1. Species delimitation and cryptic species complexes

Species are a fundamental unit in biology, however, "species delimitation has been confused by the concept of species itself" (de Queiroz 2007; Mallet 2007). Hausdorf (2011) discusses seven different species concepts, namely: lineage-based species concept, biological species concept, phylogenetic species concept, the genotypic cluster definition, cohesion species concept and the differential fitness species concept. A common element is present in all the species concepts, the primary property, however, studies tend to focus on different aspects, the secondary properties, of each concept (intrinsic reproductive isolation etc.) (de Queiroz 2007). A common problem amongst all the concepts is that they focus on a different temporal aspect during the speciation process and that is why they differ from each other (de Queiroz 2007). In other words, some concepts concentrate more on the start of the speciation process while others' research focuses more on the end of the speciation process (de Queiroz 2007). With genetic tools and data becoming more readily available, several approaches have been used to study diversity at the species level and investigate species boundaries (Carstens *et al.* 2013). An integration of genetic and non-genetic data such as life history, geographical distribution, morphology and behaviour is recommended before boundaries between species are concluded (Carstens *et al*. 2013).

Cryptic species are those that are morphologically indistinguishable and that are erroneously classified as one species (Bickford *et al*. 2006). Modern DNA sequencing has aided the discovery of cryptic species over a whole range of taxa (Bickford *et al*. 2006). For cryptic species that occur in sympatry, differences in their ecological niches can occur. For instance, Scriven *et al*. (2016) studied a complex of cryptic bumblebee species, *Bombus* (*Bombus*) *cryptarum* (Fabricius), *B.* (*B.*) *lucorum* (Linnaeus) and *B.* (*B.*) *magnus* (Vogt) throughout their flight season in Scotland and compared the variation of the bumblebees along different niche dimensions in an attempt to establish how they partitioned their niches in order to avoid exclusion. Divergent thermal and host preferences were found between these species which allowed them to co-exist (Scriven *et al*. 2016). For some cryptic species, subtle changes in

morphological characters are required to enable co-existence. Darwell & Cook (2017) determined the geographic distribution of cryptic species in a fig wasp community, consisting of four genera, *Sycoscapter, Philotrypesis*, *Watshamiella* (all Sycoryctinae), *Eukobelea* (Sycophaginae), and studied their ovipositor length as a key morphological character. The authors found that congeneric species which co-existed sympatrically differed in their ovipositor lengths while those with similar ovipositor lengths lived parapatrically with little overlap (Darwell & Cook 2017).

2.2. Tephritidae and species complexes

2.2.1. Tephritidae

True fruit flies belong to the family Tephritidae, which contains about 4000 species, arranged in 500 genera, of which 35 % attack soft fruit (White & Elson-Harris 1992). Tephritids are further divided into the subfamilies: Dacinae (larvae that develop in fruit), Trypetinae (larvae that develop in fruit, leaves or stems), and Tephritinae (larvae that develop in flowers). The subfamilies containing economically important fruit fly species are Dacinae and Trypetinae (White & Elson-Harris 1992). The important fruit fly species of the Dacinae belong to the tribes Ceratitini and Dacini. The Ceratitini contains members such as the genera *Ceratitis* MacLeay and *Trirhithrum* Bezzi, whereas *Bactrocera* Macquart and *Dacus* Fabricius belong to the tribe Dacini (White & Elson-Harris 1992). Genera of economic importance in the subfamily Trypetinae belongs to the tribe Toxotrypanini which includes *Anastrepha* Schiner, and the tribe Trypetini which includes *Rhagoletis* Loew (White & Elson-Harris 1992; White 2006).

The genera of economically important fruit flies have naturally restricted distributions (White & Elson-Harris 1992), although with some species such as *Ceratitis capitata* (Wiedemann), the origin of a species and the areas to which it expanded its range, is debatable (De Meyer *et al.* 2002). Some species in some of these genera have, however, invaded new areas mainly due to anthropogenic activities (White & Elson-Harris 1992; Karsten *et al.* 2016). The *Anastrepha* genus is present in South and Central America and the West Indies. *Rhagoletis* species are distributed in South and Central America, but with wider distribution into the temperate areas of North America and Europe (White & Elson-Harris 1992). *Dacus* spp. are mainly restricted to Africa (White & Elson-Harris 1992). *Dacus ciliatus* Loew has spread to the Indian Ocean Islands and the Indian subcontinent (White & Elson-Harris 1992). *Bactrocera* species have native ranges in Asia, Australia and the South Pacific. A few species have spread into new areas such as Hawaii, French Guiana, Suriname and Brazil (White & Elson-Harris 1992: Van Sauers-Muller 2005; Marchioro 2016). However, some species, like *Bactrocera zonata* (Saunders) and *B. latifrons* (Hendel), are now also found in Africa (OEPP/EPPO 2005;

Mwatawala *et al.* 2007). *Bactrocera dorsalis* Hendel invaded new areas in Australia, Central America, Oceania, continental United States (Clarke *et al.* 2005) and Africa (Lux *et al*. 2003). *Ceratitis* spp. are native to the African continent but *C. capitata* has successfully invaded many other areas in the world with the exception of Asia and certain areas in North America (Carey 1991; White & Elson-Harris 1992; Malacrida *et al*. 2007). *Ceratitis rosa* s.l. has spread to the Indian Ocean Islands of Reunion and Mauritius (White *et al.* 2000).

Fruit flies are pests of agricultural importance causing direct crop losses with quarantine implications (White & Elson-Harris 1992). Globally the total damage caused by fruit flies is estimated to amounts over US$ 2 billion per annum (Shelly *et al.* 2014). Potential fruit fly invasions are therefore of great concern for regulatory authorities, as well as domestic growers. Evidence of potential infestation of fruit shipments requires expert, rapid, and accurate identification (Barr *et al*. 2006), with the correct mitigating measures to avoid trade bans. For instance, *B. dorsalis* invaded the African continent during 2003 (Lux *et al*. 2003) and quickly displaced the indigenous *C. cosyra* (Ekesi & Billah 2006), becoming the most important fruit fly pest in parts of Africa (Ekesi *et al*. 2009). This resulted in the banning of import of several fruit and vegetable species from African countries to the USA, Europe and even to South Africa, Seychelles and Mauritius (Badii *et al*. 2015).

2.2.2. Life cycle and life history strategies of frugivorous fruit flies

Females lay eggs just under the skin of the fruit (Christenson & Foote 1960). The hatching larvae feed on the fruit pulp (Grout & Moore 2015). Most Tephritidae have three larval instars (White & Clement 1987). Third instar larvae leave the host and crawls and jump until a suitable place is found, sometimes digging into the ground, to form a puparium in the soil (Christenson & Foote 1960). Developmental times of immatures vary depending on temperature (Grout & Stoltz 2007; Grout & Moore 2015). Males and females reach sexual maturity a few days after adult emergence (Grout & Moore 2015). The egg to egg period of some *Ceratitis* species range from 20.2 days for *C. capitata*, 22.7 days for *C. cosyra* to 24.4 days for *C. rosa* s.l. at 26 °C (Grout & Stoltz 2007).

Comparing three *Ceratitis* species in South Africa, Grout *et al.* (2011b) found that *C. capitata* had the widest distribution. *Ceratitis. rosa* s.l. were more common in the eastern and northern parts of the country, while *C. cosyra* was more common in the northern parts and absent from the cooler southern parts. According to Grout & Stoltz (2007) *C. capitata* had an advantage over the other two species by having the shortest development period between adult eclosion and oviposition allowing them a better ability to withstand low relative humidity, wind, predators or toxic applications. According to De Villiers *et al.* (2013) *C. rosa* s.l. is better adapted to areas

with higher rainfall, but *C. cosyra* were more closely associated with the distribution of its host plants such as *Sclerocarya birrea* (A. Rich.) Hochst. (Marula tree). In South Africa fruit flies overwinter in commercial crops or home gardens and can move between crops as host fruit becomes available (De Villiers *et al.* (2013).

Fruit flies have different host utilisation strategies. Some fruit flies are oligophagous, meaning all of its hosts are usually from one family, or stenophagous, only attacking a small range of plants, largely from the same genus (White & Elson-Harris 1992; De Meyer 2001a). However, many species of Tephritidae are generalists, with larvae utilizing hosts across two or more plant families (White & Elson-Harris 1992; De Meyer *et al*. 2002; Barr *et al*. 2006; Copeland *et al*. 2006; Badii *et al*. 2015). Polyphagy was discussed as being an evolutionary outcome in the *Bactrocera* genus based on the (1) absence of negative effects of larval feeding on plant fitness, (2) common odour stimuli found in plants, (3) low *Bactrocera* diversity in native rainforest ecosystems and (4) advantage of new host, providing escape from parasitoids and competitors (Clarke 2017).

Fruit flies from the tropics and subtropics, which are multivoltine (multiple life cycles in a year), do not undergo diapause (Christenson & Foote 1960). Diapause is common with fruit flies (e.g. *Rhagoletis* spp. from North America) that have a univoltine life stage (one life cycle per year) because they are exposed to more extreme climatic variations between seasons such as in the temperate areas (Christenson & Foote 1960). These diapause periods, occurring at the pupal stage, usually last for only one year, but it has been recorded that this can last up to four years (Boyce 1931). The pupae usually overwinter in the soil underneath the trees (Feder & Bush 1989). In a study conducted by Feder *et al.* (2010) the authors hypothesised that the diapause response of *Rhagoletis pomonella* (Walsh) was influenced by pupal energy reserves that in turn influences the duration of the diapause. In *R. cerasi* Linnaeus two dormancies were recognised: 1) prolonged dormancies due to insufficient chilling, and 2) facultative dormancies due to extended exposure to chilling (Moraiti *et al.* 2010). These two dormancies were caused by local climatic conditions (adaptive response) and interannual climatic variability (plastic responses) (Moraiti *et al.* 2010).

2.2.3. Economically important species complexes within Tephritidae

2.2.3.1. The *Anastrepha fraterculus* Complex

This complex of 11 species, occurs from Mexico to northern Argentina and is a severe pest in some areas (Hernández-Ortiz *et al.* 2015; Schutze *et al*. 2017) and causes quarantine restrictions for export fruit (Steck 1999). Originally, all 11 species of *Anastrepha*, eight from Brazil, two from Peru, as well as *Anastrepha fraterculus* (Wiedemann) itself, were considered

as synonyms of *Anastrepha fraterculus.* A study by Schutze *et al.* (2017) which used morphometrics and molecular techniques to distinguish between these species identified seven possible species, but was largely inconclusive. Reproductive isolation was reported between certain strains at the pre- and post-zygotic level, such as between the species from Peru and Argentina. Cáceres *et al*. (2009) attributed this pre-zygotic isolation to differences in the sex pheromone of the males, with females prefering the pheromone of their own males. Even hybrid females prefered their own hybrid males, indicating a rapid step in incipient speciation (Cáceres *et al*. 2009; Segura *et al*. 2011). Assortative mating might explain the specific preference of the females to only mate with males that release the exact pheromone blend that they prefer (Segura *et al*. 2011).

Evidence of post-zygotic isolation become evident after inter-specific or inter-subspecific crosses indicated a sex ratio distortion of the F_2 and the following generations (Cáceres *et al*. 2009). According to Haldane's rule, sterility is found in the heterogamete offspring, usually males, after reciprocal crosses, indicating that sterility in hybrids are always preceded by sterility in males (Coyne & Orr 1989). Haldane's rule was validated by Selivon *et al*. (1999, 2005) for *A. fraterculus*. In the *A. fraterculus* complex, Segura *et al*. (2011) found that males from Argentina and Peru had different lekking positions (a lek being a gathering of males during courtship) in a tree and that the maternal lineage determined this location. Different temperatures, and even different light conditions in the tree, affected the lekking positions in the tree, thus resulting in pre-zygotic isolation between the two strains (Segura *et al*. 2011).

2.2.3.2. The *Bactrocera dorsalis* Complex

The *Bactrocera dorsalis* complex is an important group of agricultural and phytosanitary pests and is a example of many attempts of delineation of a species (Hendrichs *et al*. 2015). In order to apply field treatments such as Sterile Insect Technique (SIT), and to overcome phytosanitory barriers to export trade, it is important to identify species correctly (Hendrichs *et al*. 2015). Consensus on the species-limits of five species within the *B. dorsalis* complex could previously not be reached. These species were *B. dorsalis* s.s., *B. papayae* Drew & Hancock, *B. philippinensis* Drew & Hancock*, B. carambolae* Drew & Hancock and *B. invadens* Drew, Tsuruta & White (Hendrichs *et al*. 2015). Allozymes, chemical ecology, DNA barcoding, morphology, morphometrics and phylogenetic tools have been used in an attempt to gain insight into the *B. dorsalis* Complex (Schutze *et al.* 2017).

Pre- and post-zygotic isolation was not evident between the above mentioned species with the exception of *B. carambolae* (Chinvinijkul *et al*. 2015). Similarly, four species had identical sex pheromone profiles after feeding on methyl eugenol (ME), but not *B. carambolae* (Tan *et*

al. 2011, 2013). The use of cytogenetic techiques also did not detect any differences in the mitotic karyotypes of these five members, however, *B. dorsalis* s.s. x *B. carambolae* crosses indicated small differences, but not enough to distinguish different species (Augustinos *et al*. 2014). Microsatellite DNA markers showed different genetic clusters between *B. dorsalis* s.s. and *B. carambolae* (Hendrichs *et al*. 2015) and that common haplotypes existed between four species, but not *B. carambolae* (Schutze *et al*. 2012, 2015b). *Bactrocera carambolae* was also indicated to be a monophyletic clade which differed from the other four species (Boykin *et al*. 2014; Schutze *et al*. 2015b). A decision was therefore made to synomize *B. papayae*, *B. philippinensis* and *B. invadens* with *B. dorsalis* (Schutze *et al.* 2015a; Schutze *et al*. 2017). *Bactrocera carambolae* continues to exist as a separate species (Hendrichs *et al*. 2015). This recommendation was endorsed by national and international governments and non-governmental organisations (Schutze *et al*. 2017).

2.2.3.3. The *Rhagoletis pomonella* Complex

This sibling (cryptomorphic) species complex comprises of four members: *Rhagoletis pomonella* s.s., *R. mendax* Curran, *R. zephyria* Snow and *R. cornivora* Bush, with species infesting different sizes of artificial fruit (Berlocher *et al*. 1993). *Rhagoletis* species are univoltine and undergo diapause, usually during the winter months (Feder & Bush 1989). Adults of *Rhagoletis* species generally mate on their respective host plants (Prokopy *et al*. 1971). Field-hybridization tests conducted by Feder & Bush (1989) confirmed that *R. mendax* and *R. pomonella* can mate and that the hybrid larvae are genetically identifiable. The lack of such hybrid larvae from nature is indicative of how seldom adults meet on their respective hosts with *R. mendax* preferring blueberries and *R. pomonella* preferring apples (Feder & Bush 1989). In the absence of a geographical barrier, rapid ecological speciation of these sympatric species appeared to happen as the species shifted to newly discovered hosts (Bush 1966, 1994; Feder & Bush 1989; Feder *et al.* 2010).

2.2.3.4. The *Ceratitis* FAR Complex

Ceratitis fasciventris (Bezzi), *C. anonae* Graham, and *C. rosa* were grouped into a cryptic species complex referred to as the *Ceratitis* FAR complex (De Meyer *et al*. 2015b). All three members of the FAR complex are highly polyphagous and potentially invasive (Barr *et al*. 2006). In addition to this, these three species have sexual dimorphism and are morphologically similar. The only identifiable characteristic that could be used to distinguish between species is the different leg feathering combinations (colour and placement) on the midtibia of males. Females, however, are morphologically completely indistinguishable (Virgilio *et al*. 2013; De Meyer *et al*. 2015b; De Meyer *et al*. 2016). Although these species have largely different distribution regions, there are areas where they co-exist sympatrically (Hendrichs *et al*. 2015).

Due to the morphological similarities of the three species, molecular methods are employed to distinguish between the species (Virgilio *et al*. 2008).

2.2.4. Tephritidae of economic importance on commercial fruit in South Africa

2.2.4.1. *Bactrocera* (*Bactrocera*) *dorsalis*

Bactrocera dorsalis became established in South Africa in 2013 and is now present in the north and north eastern areas of South Africa (Manrakhan *et al*. 2015). In surveys carried out in northern areas of South Africa in 2012 and 2013, the host range of the new pest was found to be largely limited (Manrakhan *et al.* 2015; Grove *et al*. 2017; Theron *et al*. 2017). The pest is still absent from the Free State, Eastern Cape, Northern Cape and Western Cape Provinces (Manrakhan 2019). In a study conducted by Theron *et al.* (2017), *B. dorsalis* was reared from seven plant species which included two commercial crops: *Mangifera indica* (L.) (mango) and *Citrus sinensis* (L.) Osbeck (Valencia) but concluded that the fly had not yet reached its full host range potential.

2.2.4.2. *Bactrocera* (*Daculus*) oleae (Rossi)

Bactrocera oleae is a sporadic pest (Costa 1998) associated with the distribution of cultivated olive trees in the Western Cape Province and Northern Cape Province, but also with naturally occurring wild olive trees in South Africa, Lesotho and Namibia (White 2006). Sporadic outbreaks of this pest are ascribed to be due to climatic changes rather than to biotic factors (Caleca *et al*. 2015). This species differs from other native African *Bactrocera* spp. in that it does not have any prescutellar acrostichal setae and it can be confused with certain *Dacus* spp. that also lack these setae (White 2006). *Bactrocera oleae* infests up to one third of the cultivated olive crop, however this is not a problem at harvest since most of the fruit are produced for oil production (White & Elson-Harris 1992; DAFF 2010b).

2.2.4.3. *Ceratitis* (*Ceratitis*) *capitata*

Ceratitis capitata belongs to the subfamily Dacinae under the tribe Ceratitini and it is highly polyphagous. It attacks crops such as apple, apricot, coffee, guava, figs, granadilla, litchi, mango, oranges, pear, plums, and grapes (White & Elson-Harris 1992). Liquido *et al*. (2015) reported *C. capitata* on 304 plant species from 57 families. Compared to other fruit fly species it has the widest distribution in South Africa (De Villiers *et al*. 2013).

2.2.4.4. *Ceratitis* (*Ceratalaspis*) *cosyra*

Ceratitis cosyra, previously known as *Pardalaspis cosyra* (Walker) or *Trypeta cosyra* Walker, is of phytosanitary concern since it attacks cultivated crops such as mango, guava, and avocado (White & Elson-Harris 1992). It is closely associated with the distribution of the Marula

tree in South Africa (Hancock 1987; De Villiers *et al.* 2013) which is more prominent in the northern and eastern parts of South Africa in the Limpopo, Mpumalanga, KwaZulu-Natal and Eastern Cape Provinces (DAFF 2010a).

2.2.4.5. *Ceratitis* (*Pterandrus*) *rosa* s.l.

Ceratitis rosa s.l. is highly polyphagous attacking a wide range of indigenous and commercial fruit including oranges (White & Elson-Harris 1992). In South Africa *C. rosa* s.l. is distributed from the Western Cape, along the coastal regions to the wetter northern parts of the country. It is not common in the drier inland areas (De Villiers *et al*. 2013).

2.2.5. Changes in taxonomy of *C. rosa* s.l.

2.2.5.1. Split of *C. rosa* s.l. and *C. fasciventris*

The holotype of *C. rosa* s.l. is a single male collected from Delagoabai (an area close to modern day Maputo, Mozambique) and described by Karsch (1887) in De Meyer (2001b) who emphasized the importance of only two characters, the brownish bands over the abdomen, and the feathering of the midtibia which have a silvery shine. Karsch failed to mention the black coloration associated with the midtibia (De Meyer 2001b; De Meyer *et al*. 2015b). Unfortunately, from early on, there existed ambiguity between *C. rosa* s.l. and *C. fasciventris* since Bezzi (1920) described *C. fasciventris* only as a variant, and not as a separate species at the time, and similarly to Karsch, also failed to mention the difference in colouration of the midtibia (De Meyer 2001b). De Meyer (2001b) reported on a study conducted by G. Franz (Seibersdorf) that *C. rosa* s.l. and *C. fasciventris* could interbreed under laboratory conditions and that the offspring have a different feathering variation on the midtibia than the parents. However, there are no records of hybridization between wild populations (De Meyer 2001b).

De Meyer (2001b) mentioned the misidentification between *C. rosa* s.l. and *C. rosa* var. *fasciventris* and that Hering (1935) described *C. flavotibialis* as a separate species. After studying the type-material it was confirmed that *C. rosa* var. *fasciventris* is a species on its own and that *C. flavotibialis* and *C. fasciventris* were in fact the same species (Cogan & Munro 1980: De Meyer 2001b).

2.2.5.2. Split of *C. rosa* s.l. into two species

Virgilio *et al*. (2008) used mitochondrial and nuclear markers to resolve species differences in the complex but these methods failed to provide a clear answer. Delatte *et al*. (2013) collected material from eleven localities in African countries: *C. fasciventris* from Benin, Kenya, Rwanda and Zambia; *C. anonae* from Democratic Republic of Congo, Kenya and Uganda; and *C. rosa* s.l. from Kenya, Mozambique and South Africa to recover a set of polymorphic nuclear neutral

markers. The latter study tested those markers on six field populations, namely, *C. fasciventris* (Benin and Mali), *C. anonae* (Benin and Cameroon), and *C. rosa* s.l. (Kenya and Tanzania). Delatte *et al*. (2013) showed that most of the primers amplified all three species and were highly polymorphic for all three species, with a mean of five alleles for *C. fasciventris,* and seven alleles each for *C. anonae* and *C. rosa* s.l.

Virgilio *et al*. (2013) expanded on this study by surveying allelic variation at 16 polymorphic microsatellite loci in 27 African populations of the three morphospecies of the *Ceratitis* FAR complex. Five genotypic clusters were identified using inter-population distances and individual Bayesian assignments (Virgilio *et al*. 2013). Two clusters involved *C. fasciventris* (F1 and F2; with parapatric distributions), one *C. anonae* cluster, and the remaining two clusters belonging to *C. rosa* (R1 and R2, occurring in sympatry) (Virgilio *et al.* 2013). The authors found that the genetic distances between the conspecific clusters, F1-F2 and R1-R2, were comparable with differentiation between heterospecific clusters such as F1-A or R2-A. The gene flow observed among morphospecies or heterospecific genotypic clusters were different from zero, indicating an absence of reproductive isolation. Secondary morphological characteristics (*a posteriori*) of male flies (the extent of the feathering on the midtibia) partly supported the genetic differentiation between genotypic clusters. A reinterpretation of the FAR complex thus required an integrated approach using both morphological and molecular evidence. Virgilio *et al*. (2013) concluded that major revision was essential to further inform current models of ecological niche requirements and the invasion risk of *C. rosa* s.l. This provided a basis for taxonomical re-interpretation of the FAR complex.

2.2.5.3. Differences between the two genotypic clusters of *C. rosa*

2.2.5.3.1. Cuticular hydrocarbons

In a study on the cuticular hydrocarbons of two *C. rosa* populations in Kenya (highland (R2) versus lowland (R1)), Vaníčková *et al*. (2015) found significant differences in the quantities/ratios of a number of hydrocarbons between the populations, although the types of hydrocarbons were similar in both populations. The authors concluded that their study supported other studies' findings that the two *C. rosa* populations are two different biological species in Kenya, but recommended that this study must be extended to include other geographical areas and host ranges of the two species.

2.2.5.3.2. Temperature related differences

A laboratory study by Tanga *et al*. (2015) showed that the two genotypic clusters of *C. rosa* responded differently to different temperature regimes. *Ceratitis rosa* R2 was better adapted to low temperatures, based on its lower developmental thresholds.

2.2.5.3.3. Split of *C. rosa* s.l. and description of *C. quilicii*

In view of the differences found between the two genotypic clusters of *C. rosa*: R1 and R2, *C. rosa* was split in two species. *Ceratitis rosa* R2 or the 'cold type' was described as *Ceratitis* (*Pterandrus*) *quilicii* De Meyer, Mwatawala & Virgilio (De Meyer *et al*. 2016). *Ceratitis rosa* R1 was considered as *C. rosa* s.s. (De Meyer *et al*. 2016).

De Meyer *et al*. (2016) gave a thorough description of *C. quilicii* which included its distribution in Africa. A DNA barcode was provided for this species but the authors warned that DNA barcoding, on its own, is not a completely reliable method of identifying the different members of the FAR complex, since *C. quilicii* clusters with specimens of *C. rosa* s.s. that were collected in Kenya and Mozambique. The identification of *C. rosa* s.s. in the latter case was confirmed morphologically. The authors distinguish *C. quilicii* morphologically from the other FAR members by using the male midtibia where the black colour normally does not reach the dorsal and ventral margins, especially the basal part. The black feathering is similar to *C. rosa* s.s. (De Meyer *et al*. 2015b) stretching dorsally along distal 0.75 to ventrally along distal 0.66, sometimes to distal 0.75 (De Meyer *et al*. 2016).

Correct identification of Tephritidae flies are vital for further research and management of these pests, since the presence of certain species in a fruit exporting country has quarantine implications as well as economic and political implications such as instituting of trade barriers (De Meyer *et al*. 2015a; Hendrichs *et al*. 2015).

2.3. Citrus production in South Africa

Citrus prefers subtropical or tropical climates where frost does not occur and temperatures do not go below -2 °C, and preferably never rising above 39 °C (Bijzet 2006). Specific periods of cold and warm weather, and similarly dry and rainy spells, are required in the phenology of a citrus tree for it to survive and bear fruit (Bijzet 2006). In South Africa, the citrus production areas lie between the latitudes 17 °E and 34 ° S (Bijzet 2006; Davis 1928). Barry (1996) divided the commercial citrus production areas of southern Africa into six climatic zones: Hot-Dry (less than 600 km from the sea and below 600 m a.s.l.), Hot-Humid (up to 200 km from the sea and below 300 m a.s.l.), Intermediate (between 600 - 900 m a.s.l.), Cool-Inland (above 900 m a.s.l.), Cold/Semi-Coastal (semi-coastal, between 32° 30‘ E and 34° 30‘ S) and Semi-Desert (hot summers, cold winters). These zones are not only based on altitude or climatic factors such as temperature, but also on the performance of various cultivars in the specific production areas (Barry 1996).

More than half (68%) of all citrus production in South Africa is in the Limpopo (34763 ha) and Eastern Cape provinces (21157 ha). The other provinces, in order of the respective size to

their production areas are Western Cape (14883 ha), Mpumalanga (6363 ha), KwaZulu-Natal (1853 ha), Northern Cape (1773 ha), and North West (13 ha). Citrus is also produced in two neighbouring countries, Zimbabwe (2131 ha) and Swaziland (554 ha) (CGA 2018).

The five most commonly produced citrus species and cultivars in South Africa are: *Citrus sinensis* (L.) (Osbeck) cv. Valencia/Midseason (28455 ha), *C. sinensis* cv. Navel (16285 ha), *C. reticulata* Blanco, soft citrus (16234 ha), *C. limon* (L.) Osbeck, lemon and *Citrus aurantiifolia* (Christm.) Swingle, lime (14740 ha), and *C. paradisi* Macfad, grapefruit (7743 ha) (CGA 2019). South Africa is currently the 14th largest producer of citrus worldwide and the 2nd largest exporter, only surpassed by Spain (CGA 2019). The bulk of South African fruit are exported (76%), the rest are processed (18%) or sold on the local market (6%). According to the Southern Africa Customs Union (SACU), citrus fruit is the single biggest horticultural crop to be exported, valued at R14.818 billion in 2015/ 2016 (DAFF 2017). In total, the volume of fruit (export, local market and processed) produced in southern Africa was 1.845 million tons, valued at +/- R17.7 billion, for the 2017 citrus season (CGA 2018).

Citrus is attacked by various pests belonging to different orders and families such as the Diptera (Tephritidae), Hemiptera (Cicadellidae, Triozidae, Aphididae, Diaspididae and Pseudococcidae), Thysanoptera (Thripidae), Coleoptera (Curculionidae), Lepidoptera (Tortricidae, Papilionidae and Pyralidae) and Hymenoptera (Formicidae) (Grout & Moore 2015). Some species are a threat to citrus production, such as *Aonidiella aurantii* (Maskell) (Family: Diaspididae) and *Trioza erytreae* (Del Guercio) (Family: Triozidae) (Grout & Moore 2015). Certain of these pests are of quarantine importance such as *Thaumatotibia leucotreta* (Meyrick) (Family: Tortricidae) and *B. dorsalis*, *C. capitata* and *C. rosa* s.l. (Family: Tephritidae) (Grout & Moore 2015).

2.4. Fruit fly pests of citrus

The fruit flies: *B. dorsalis*, *C. capitata*, *C. rosa* s.s. and *C. quilicii* are pests of citrus in South Africa (Georgala 1964; Manrakhan 2019). There is a zero tolerance of their presence in export fruit from southern Africa, with certain export markets requiring quarantine protocols such as cold disinfestation treatments to ensure fruit fly free fruit (Georgala 1964; White & Elson-Harris 1992; Barnes 2000; Grout & Stoltz 2007; Grout *et al*. 2011a,c; Grout & Moore 2015; Manrakhan *et al*. 2018).

Bactrocera dorsalis has been recorded from the eastern part of its native range (China, Japan and Taiwan) on orange, and in the Hawaiian Islands on orange, tangerine and Valencia orange (White & Elson-Harris 1992). In South Africa, *B. dorsalis* was successfully reared from Valencia oranges (Theron *et al*. 2017) and *C. capitata* from navel and Valencia oranges (White

& Elson-Harris 1992; De Meyer *et al.* 2002). Similarly to *C. capitata*, *C*. *rosa* s.l. was also reared from navel and Valencia oranges (White & Elson-Harris 1992; De Meyer *et al.* 2002). The species split between *C. rosa* s.s. and *C. quilicii* raised uncertainty on the previously collected host data, and therefore also which species infests citrus and which are the preferred cultivars.

2.5. Fruit fly ecology

2.5.1. Attractants

Fruit fly populations are generally studied using traps and attractants targeting the adult stages. The trap design and the different tephritid target species determine which attractants are deployed inside orchards (IAEA 2003; Manrakhan *et al.* 2017). Traditionally fruit fly attractants are divided into three categories: pheromones, male lures (parapheromones), and food-based attractants (Cunningham 1989; Epsky *et al.* 2014; Tan *et al.* 2014; Manrakhan *et al.* 2017).

Sex pheromones are typically very specific, not as effective as male lures and its long-range capabilities have weak empirical support (Tan *et al*. 2014). Sex pheromones are used commercially for very few economically important tephritids and none is in use for any *Ceratitis* species (Tan *et al.* 2014).

Male lures are categorized as either anthropogenic or plant borne. Examples of anthropogenic lures are Cuelure [CL], trimedlure [TML], fluorinated methyl eugenol [ME] analogues and raspberry ketone-formate [RKF]. Plant borne lures are for example, α-copaene, ME, raspberry ketone [RK], and Zingerone (Tan *et al*. 2014). Male lures are more effective than sex pheromones and are commonly used in baited traps for early detection, surveys, delimitation, and eradication by means of the male annihilation technique (Tan *et al*. 2014). *Bactrocera* spp. respond to CL/RK, ME and Zingerone attractants, *Ceratitis* spp. to α-copaene and natural oils, TML and Ceralure, and *Dacus* spp. to CL, ME and Vert-lure (methyl paraben) (Tan *et al*. 2014). In a study conducted by Mwatawala *et al*. (2013, 2015) and Manrakhan *et al*. (2017) enriched ginger root oil (EGO) was found to be a good attractant to *Ceratitis* spp. Manrakhan *et al*. (2017) also found that Zingerone was an effective attractant for *Dacus frontalis* Becker and attracted other *Dacus* spp. as well as *B. dorsalis*, but not for *Ceratitis* spp. No male lures currently exist for *Anastrepha* spp. and *Rhagoletis* spp. (Light & Jang 1996, White & Elson-Harris 1992; Tan *et al*. 2014).

Food-based attractants are divided into two groups: 1) liquid protein hydrolysates, and 2) synthetic analogue lures for protein hydrolysates (IAEA 2003; Epsky *et al*. 2014). Typical

examples of food-based attractants that mimic adult food sources other than host fruit include sugar from sugarcane (Molasses), active yeast from Brewer's yeast, autolyzed protein from Brewer's yeast or yeast extract, enzomatic hydrolysis from yeast, acid hydrolysis from corn (NuLure [Miller Chem & Fert Corp, Hanover, PA, USA]) (Epsky *et al*. 2014). Although not as strong as male lures, food-based attractants catch the first flies in the season, which is important for orchard management and for detecting potential invaders, and the trap catches can be males or females of a species, although there is a bias towards females (Epsky *et al*. 2014). In a study conducted by Papadopoulos *et al.* (2001) in Greece, it was found that the food-based attractants caught *C. capitata* females first in the season due to ripening fruit in the orchard.

Other volatiles such as fruit volatiles and bacterial odours have been investigated and used as fruit fly attractants. Gravid females of fruit flies such as *R. pomonella* and *R. cerasi* use both olfactory and visual cues to locate suitable hosts (Prokopy 1968a; Boller *et al*. 1970). Even bacterial odours can attract fruit flies (Drew 1989), and lures which are releasing very similar odours, are being manufactured and used in pest management (Sivinski & Calkins 1986; Robacker 2007).

Visual stimuli are incorporated in traps and stations used for monitoring and control of fruit flies. Prokopy (1968a, b) found that when objects are large, yellow appears to be the better colour, but with smaller objects, red or any darker colour are a good stimulus. The hypothesis is that yellow simulates the colour of the leaves in the tree canopy and the red or darker colour, fruit which serves as oviposition substrate (Prokopy 1968a).

2.5.2. Abiotic factors

2.5.2.1. Diurnal periodicity

Diurnal periodicity in adult emergence has been found by McPhail & Bliss (1933) in their studies on *Anastrepha ludens* (Loew) which emerged in the mornings between 06:00 and 10:00. According to Christenson & Foote (1960), this activity appears to be correlated to sunlight exposure and increasing temperatures. Laboratory studies conducted on *B. dorsalis*, found the highest rate of adult emergence occurred between 08:00 and 10:00 (Christenson & Foote 1960). *Rhagoletis pomonella* had the highest adult emergence between 07:00 and 10:00 (Lathrop & Nickels 1932). These diurnal patterns might be influenced by overcast or rainy days (Christenson & Foote 1960). A study conducted by Nishida & Bess (1957) reported that oviposition decreased during the day for *Zeugodacus cucurbitae* (Coquillett) due to

increased temperatures and sunlight intensity but that it continued during the day if overcast conditions prevailed.

2.5.2.2. Temperature influence

Temperature is the most important factor determining developmental rate in poikilothermic animals (Bateman 1972). Fruit flies are affected by seasonal climate with high abundance during warmer months and low abundance during colder months (Bateman 1972), if no other influences such as a winter crop (citrus) are not considered. Univoltine species may only lay eggs for a short period during the summer months whereas multivoltine species may oviposit over an extensive period, starting in early spring and ending in late autumn with overlapping generations. Bateman (1972) mentioned that most species develop at temperatures ranging between 10 °C and 30 °C but that for survival, a much wider temperature range can be tolerated for short periods of time. Some species may tolerate temperatures as high as 45 °C and as low as -12 °C (Leski 1963). Temperatures between 25 °C and 30 °C are optimal for fecundity, and for oviposition the optimum range are between 9 °C and 16 °C for most species, with egg-laying decreasing during mid-day when temperatures are high (Nishida & Bess 1957; Bateman 1972).

In a study conducted by Nyamukondiwa and Terblanche (2009) on the adult thermotolerence of *C. capitata* and *C. rosa* s.l., it was found that the critical thermal maximum increased with age but only up to 14 days, where after it lowered again. Flies of this age were also the most low temperature tolerant. Twenty-eight day old flies had the poorest thermotolerances. The authors found that feeding prior to the trial increased the thermotolerances of the adult flies. There were no differences between the genders of the two fly species. Both fly species had a similar critical thermal minimum (5.4 -6.6 °C) but *C. capitata* had a higher critical thermal maximum (42.4 – 43.0 °C) than *C. rosa* s.l. (41.8 – 42.4 °C), indicating that *C. capitata* might be more comfortable in the warmer drier areas of South Africa than *C. rosa* s.l. (Nyamukondiwa & Terblanche 2009).

2.5.2.3. Humidity

Humidity strongly influences the survival rate of tephritids especially with species such as *C. rosa* s.l. and *C. catoirii* Guérin-Méneville (Duyck *et al*. 2006b). Environmental moisture has an important effect on the distribution, abundance and survival of fruit flies and for this reason fruit flies are seldom found in dry areas (Bateman 1972; De Villiers *et al*. 2013). However, according to Bateman (1972), this may also be indirectly due to their host's distribution, limited by moisture, rather than the flies' physiologically adaptation alone. Flies such as *Rhagoletis cerasi* (L.) are not affected by low humidity during oviposition but very high humidity drastically

decreases its oviposition rate (Bateman 1972). *Rhagoletis lycopersella* Smyth is reported to survive the hottest months by means of pupal estivation (summer dormancy), delaying eclosion to avoid desiccation for as long as eight months (Smyth 1960). The length of the pupal stage is not affected by moisture but it does affect survival (Christenson & Foote 1960).

Zeugodacus cucurbitae responds to changes in climate by expanding its range during wetter rainy periods and contracting when drier periods sets in (Nishida 1963). In *B. tryoni* (Froggatt), a significant positive correlation was established between peak numbers and the rainy seasons (Bateman 1968). Bateman (1968) also reported lower immigration of *B. tryoni* into areas when it was dry with high adult mortality observed during their emergence from hard dried puparium cases. Mortality was further increased by the inability of flies to crawl through the dry hard soil to a low humidity atmosphere (Bateman 1968). Moisture also affects the depth that the larvae burrow into the soil (Christenson & Foote 1960).

Neilson (1964) reported that *R. pomonella* was abundant during cool wet summers in Canada. A low relative humidity (<60 %) was not conducive for pupal survival. The most vulnerable life stages of fruit flies are the 3rd instar, during which jumping larvae emerge from the fruit and dig into the soil to pupate and the teneral adults (Bateman 1972). Rain has been shown to influence both these life stages and can cause a flush of adult emergence (Smyth 1960). Rain stimulates the emergence of larvae of *A. ludens* (Baker *et al*. 1944) and *B. tryoni* (Bateman 1972) from fruit and also the emergence of *R. pomonella* adults from pupae (Lathrop & Dirks 1945). However, the survival of jumping larvae and pupal stages in the ground that are immersed in water, especially for extended periods, is greatly reduced, indicating that effective irrigation could reduce fruit fly numbers in the orchards (Duyck *et al*. 2006b).

2.5.3. Biotic factors

The niche that a single species occupies is divided into a fundamental niche, which is the niche in the absence of competitors, predators and parasitism, and a realized niche, in which the organism is exposed to restrictive factors such as competition (Begon *et al.* 2006). The distribution of polyphagous fruit flies, is influenced by two types of interspecific competition: 1) exploitation competition, where resources influence the success of the species, and 2) interference competition, where different fruit fly species have a negative physical or chemical influence on each other (Duyck *et al.* 2004). It is important to realize that interspecific competition is only noticeable during the transition period when species are displacing each other, but once the process is completed, interspecific competition is not so noticeable between two co-existing species (Duyck *et al.* 2004).

Living organisms exhibit a range of life history strategies with two extremes known as *r-strategists* and *K*-strategists (Begon *et al.* 2006; Duyck *et al.* 2006b). While *r*-selected species have high fecundity as well as rapid development and growth, which makes them good colonisers of new areas where they move into, *K*-selected species are usually larger and stronger competitors which may displace other weaker species, but they are not as effective as colonisers (Duyck *et al*. 2004). Studies in Réunion followed different fruit fly invasions into the island and it became evident that directional hierarchical competition existed, with one fruit fly species dominating and excluding the other (Duyck *et al*. 2004, 2006a), with the invading species having the competitive superior ability (Juliano 1998; Byers 2000, Vila & Weiner 2004). If no exclusion happened, niche differentiation might have happened, due to a colonisation-competition trade-off, with the weaker competitor being displaced, or having to change host-associations (Duyck *et al*. 2004).

In Hawaii *C. capitata*, the *r*-species, was able to move to higher regions and infest much smaller fruit, after the invasion of *B. dorsalis*, the *K*-strategist (Christenson & Foote 1960; Duyck *et al*. 2004). In an ecosystem where hierarchical competition between fruit flies exists, such as in Réunion, fruit flies follow a specific order of dominance (the *r-K*) gradient, when replacing each other (Duyck *et al*. 2004, 2006a). The authors indicated this order of dominance to be as follows *B. zonata* > *C. rosa* s.l. > *C. catoirii* > *C. capitata*, and that this dominance is not reciprocal. In other words, if *B. zonata* replaced *C. rosa* s.l., the reverse will never happen (Duyck *et al*. 2004, 2006a). In certain areas previously invaded by *Bactrocera species* as far back as the 1920's and 1940's, *Ceratitis* species never managed to invade these areas again (Duyck *et al*. 2004). However, a fly such as *C. rosa* s.l., which is tolerant to high altitudes and cold weather, can exploit a niche (an exploitative competition advantage), where it will not be excluded by other stronger competitive flies (climate-dependent change in competitive hierarchy) (Duyck *et al*. 2004, 2006a). Similar interactions between species were observed with *C. capitata*, which, due to its *r*-selected strategy, colonizes colder temperate areas (Vargas *et al*. 1984, 2000). Climatic conditions may promote co-existing of different species following an invasion (Duyck *et al*. 2006b). Successful invasions by polyphagous species do not require specific hosts and whether an invader successfully excludes the current polyphagous resident fruit fly species in the area, largely depends on climatic conditions and interspecific competition (Duyck *et al*. 2008).

The dominance of *B. dorsalis* over *C. capitata* might be the reason why *C. capitata* never managed to successfully invade south eastern Asia (Christenson & Foote 1960). One of the reasons why *B. dorsalis* dominates *C. capitata,* is that *B. dorsalis* utilizes the same puncture holes caused by *C. capitata* females, which has an inhibitory effect on *C. capitata* larvae

(Christenson & Foote 1960). This mechanism has been confirmed by means of laboratory assays (Christenson & Foote 1960). Chemical host marking is documented for some tephritids (Roitberg & Prokopy 1987; White & Elson-Harris 1992). *Ceratitis capitata* and *C. rosa* s.l. leave detectable chemical signals to deter conspecifics from further utilizing the fruit (Duyck *et al*. 2006a). Flies in the genus *Anastrepha* displaced *C. capitata* in Costa Rica, while in Australia, *C. capitata* faced fierce competition from *B. tryoni* (Christenson & Foote 1960). The dominance of *Anastrepha striata* Schiner over many other species in Mexico was confirmed in guavas, which is considered as one of the optimum hosts for fruit flies, indicating that it successfully displaced other potential fruit fly competitors (Christenson & Foote 1960).

This ability to compete and displace certain species from certain hosts or necessitating certain species to move to other less preferred hosts must be viewed with caution when conducting studies on host-arthropod interactions (Christenson & Foote 1960). The absence of a particular species from a known host plant in a specific area might not always be a reliable indicator, if competition is not considered as well, and this is especially applicable in biological races (another description of cryptic complexes). In Hawaii, for example, sampling of known hosts of *C. capitata* indicated the near absence of host utilization by this species, which is inaccurate, since in other parts of the world *C. capitata* would naturally attack these hosts (Christenson & Foote 1960).

Natural enemies play a role in regulating fruit fly populations. The two life stages that are readily attacked by hymenopteran parasitoids, such as the Opiinae (Braconidae), are the larvae and puparia (Christenson & Foote 1960). A koinobiont endoparasitoid egg parasitoid, *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), was evaluated in laboratory trials in Kenya, and observed to prefer *B. dorsalis* eggs for oviposition. The six tephritid species evaluated in the latter study were: *B. dorsalis*, *Ceratitis capitata*, *C. cosyra*, *C. rosa* s.l., *C. fasciventris*, and *C. anonae* (Mohamed *et al*. 2010). Two members of the FAR complex, *C. fasciventris* and *C. rosa* s.l., encapsulated the parasitoid eggs while no parasitoid progeny survived in *C. fasciventris. Ceratitis anonae*, *C. capitata* and *C. cosyra* do however appear to be potential hosts for *F. arisanus* (Mohamed *et al*. 2010).

2.5.4. Distribution and ecology of members of the FAR complex

The three morphometrically similar members of the FAR complex that are considered as major horticultural pests (White & Elson-Harris 1992; De Meyer 2001b) and are of quarantine significance (EPPO/CABI 1997), are highly polyphagous (De Meyer *et al*. 2002). The FAR complex is limited to the Afrotropical biogeographical region including the islands of Réunion

and Mauritius (De Meyer 2000). None of the FAR complex members have invaded further than these islands, although they are potentially invasive (Hendrichs *et al*. 2015).

Virgilio *et al*. (2013) conducted a study across 12 countries collecting adults belonging to the FAR complex: *C. fasciventris, C. anonae*, and *C. rosa* s.l. Both parapatric and sympatric populations of *C. fasciventris and C. anonae* were recorded. This was also the case for *C. fasciventris* and *C. rosa* s.l. They reported that *C. fasciventris* occurred from Mali to Ethiopia, Kenya and Tanzania and its distribution was reported as far south as Zambia. The distribution of *Ceratitis anonae* extended across the middle of the continent but not as far east as Ethiopia and Tanzania or as south as Zambia. *Ceratitis rosa* s.l. is distributed more to the east of the continent: Kenya, Tanzania, Malawi, Mozambique, and South Africa. Flies were also collected from Réunion (Figure 1). Only *C. rosa* s.l. of the FAR complex is currently found in southern Africa (Virgilio *et al*. 2013). *Ceratitis rosa* s.l. and *C. anonae* were not found in the same areas on the African continent. *Ceratitis anonae* occurs over the equatorial belt and *C. rosa* s.l. in the south and eastern parts of the continent (De Meyer *et al*. 2015b). Of the three FAR species, *C. fasciventris* has the widest distribution and occurs throughout western Africa and in isolated regions in Central Africa (De Meyer *et al*. 2015b).

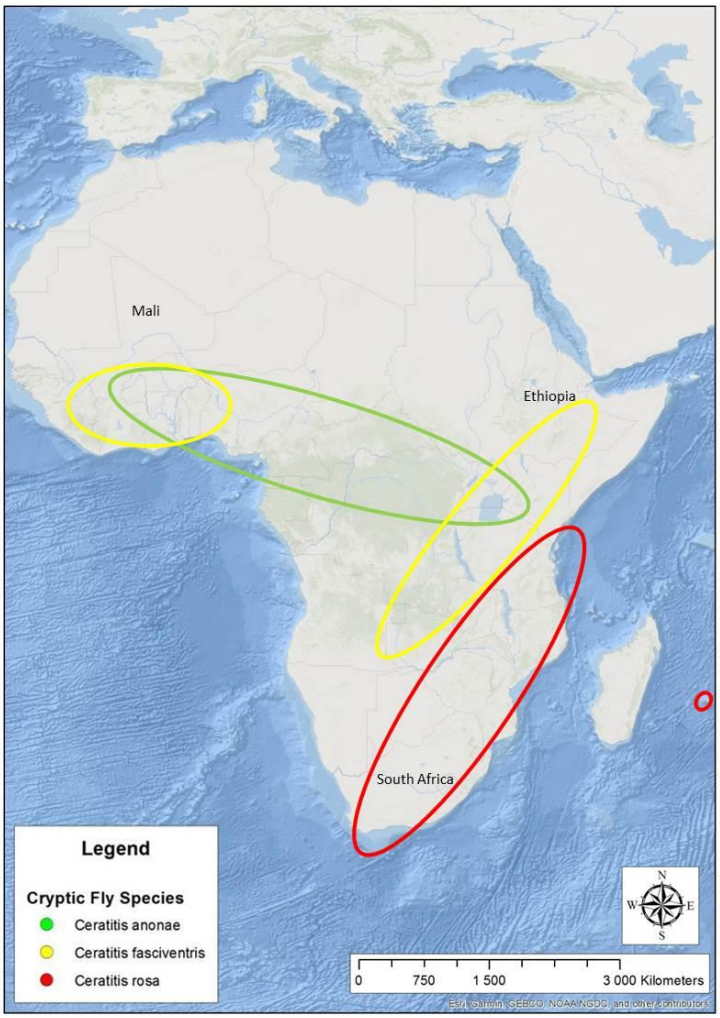

Figure 1. Distribution of the FAR complex on the African continent, with *C. rosa* s.l. distributed the furthest south into South Africa (Virgilio *et al*. 2013).

Little is known about the ecology of the FAR species complex (Copeland *et al*. 2006). In a study conducted by Copeland *et al*. (2006) fruit were collected from different regions and altitudes in Kenya. *Ceratitis anonae* was reared from fruit from the western highlands and did not cross the xeric floor of the Gregory Rift Valley which acts as a natural barrier. *Ceratitis rosa* s.l. was naturally distributed toward the eastern regions of Kenya and towards the coast, with the Nyika (*Acasia-Commiphora* grassland) acting as a barrier for any spread to the western regions of the country (Copeland *et al*. 2006). However, from 2003 onwards, *C. rosa* s.l. was collected from the central highlands in Kenya, indicating its range expansion into higher altitude temperate areas (1533 to 1771 m), although this specific range expansion was due to anthropogenic activities (Copeland *et al*. 2006). *Ceratitis fasciventris* has never been reared from fruit collected in coastal areas or in the central highlands of Kenya. With *C. rosa* s.l. being the new invader in these areas, it is likely that it might become the superior

competitor which could have a negative effect on the numbers of *C. fasciventris* (Hancock 1984; Duyck *et al*. 2004).

Ceratitis fasciventris was collected at altitudes between 1670 and 2220 m a.s.l. but it was absent above 2284 m a.s.l. *Ceratitis rosa* s.l., on the other hand, were collected from sea-level to even higher altitudes, indicating that it can withstand cold winter temperatures (Ripley & Hepburn 1930; Copeland *et al*. 2006). In the western and central highlands of Kenya, host utilization, which includes feral stands of guava and bugweed as well as indigenous wild plants, is adequate all year round to maintain *C. fasciventris* and *C. anonae* populations (Copeland *et al.* 2006). *Ceratitis rosa* s.l. was not collected from any coastal fruit during December and January indicating that it might exist in various microhabitats until the next available ripe fruit is present (Copeland *et al*. 2006). However, it is important to understand that host partitioning was reported between the FAR species and *C. capitata* in two plant families: Sapotaceae and Annonaceae (Copeland *et al*. 2006). Certain tribes of these two plant families will only be infected by either *C. capitata* or the *Ceratitis* FAR species. An absence of a fly species in a certain tribe does not necessarily mean that the fly is not present in the area (Copeland *et al*. 2006).

2.5.5. Ecology of *C. rosa* s.s. and *C. quilicii*

A study conducted by Mwatawala *et al*. (2015) indicated that there was a difference in the distribution of *C. rosa* s.s. and *C. quilicii* along an altitudinal transect, with *C. rosa* s.s. preferring the warmer, lower altitudinal areas and *C. quilicii* the cooler, higher altitudinal areas, although the two species overlapped in most areas. A study conducted by Duyck *et al*. (2006b) in Réunion found that *C. rosa* s.l., which is possibly *C. quilicii*, preferred a cooler and more humid climate compared to *C. capitata* (Wiedemann). Tanga *et al*. (2015) also indicated physiological development preferences at different temperatures between the two species. Since the species split, very little information is available on the ecology of *C. rosa* s.s., as a new separate identity, and *C. quilicii*. However, Grové *et al*. (2019) distinguished between the males of the two species, rearing them sympatrically from guava from two places in the north of South Africa, namely Tzaneen and Nelspruit. Unfortunately, Grové *et al*. (2019) did not collect any citrus fruit during their study. The host list of both species have to be re-established and previous quarantine treatments on *C. rosa* s.l. have to be validated for the two species.

2.6. Problem of identification

The implications of the species split are that previously collected data on pre- and postharvest treatments for *C. rosa* might now be questionable. There also exist uncertainties on the distribution, relative abundance and host utilization of *C. rosa* and *C. quilicii*. *Ceratitis rosa* and

C. quilicii occur sympatrically in South Africa (Virgilio *et al*. 2013) and whenever two closely related species occur together sympatrically, differences such as responses to abiotic and biotic factors can be expected between the two species (Scriven *et al*. 2016; Darwell & Cook 2017). The questions posed and addressed in this project were: (1) Are there similarities and differences between *C. rosa* and *C. quilicii* in their distribution and relative abundance in citrus production regions where they occur in sympatry? (2) Are there similarities and differences in the way the two species respond to attractants? (3) Are there similarities and differences in the way the two species utilise citrus?

2.7. References

AUGUSTINOS, A.A., DROSOPOULOU, E., GARIOU-PAPALEXIOU, A., BOURTZIS, K., MAVRAGANI-TSIPIDOU, P. & ZACHAROPOULOU, A. 2014. The *Bactrocera dorsalis* species complex: comparative cytogenetic analysis in support of Sterile Insect Technique applications. *BMC Genetics* **15**: 1-10.

BADII, K.B., BILLAH, M.K., AFREH-NUAMAH, K., OBENG-OFORI, D. & NYARKO, G. 2015. Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae) in Africa. *African Journal of Agricultural Research* **10**: 1488-1498.

BAKER, A.C., STONE, W.E., PLUMMER, C. & MCPHAIL, M. 1944. A review of studies on the Mexican fruit fly and related Mexican species. *U.S. Department of Agriculture Miscellaneous Publication* **531**: 155 pp.

BARNES, B.N. 2000. Monitoring and control of fruit flies in South African fruit orchards. 147-152 pp. In: Price, N.S. & Seewooruthun, I. (Eds.). Proceedings of the Indian Ocean Commission. Regional Fruit Fly Symposium, 5-9 June 2000, Mauritius. Indian Ocean Commission, Mauritius, Flic en Flac.

BARR, N.B., COPELAND, R.S., DE MEYER, M., MASIGA, D., KIBOGO, H.G., BILLAH, M.K., OSIR, E., WHARTON, R.A. & MCPHERON, B.A. 2006. Molecular diagnostics of economically important *Ceratitis* fruit fly species (Diptera: Tephritidae) in Africa using PCR and RFLP analyses. *Bulletin of Entomological Research* **96**: 505-521.

BARRY, G.H. 1996. Citrus production areas of Southern Africa. *Proceedings of the International Society of Citriculture* **1**:145-149.

BATEMAN, M.A. 1968. Determinants of abundance in a population of the Queensland fruit fly. *Symposium Royal Entomological Society London* **4**: 119-131.

BATEMAN, M.A. 1972. The ecology of fruit flies. *Annual Review of Entomology* **17**: 493-518.

BEGON, M., TOWNSEND, C.R. & HARPER, J.L. 2006. *Ecology: From Individuals to Ecosystems.* 30-31. Blackwell Publishing, Oxford.

BERLOCHER, S.H., MCPHERON, B.A., FEDER, J.L. & BUSH, G.L. 1993. Genetic differentiation at allozyme loci in the *Rhagoletis pomonella* (Diptera: Tephritidae) species complex. *Annals of the Entomological Society of America* **86**: 716-727.

BEZZI, M. 1920. Notes on the Ethiopian fruit flies of the family Trypaneidae, other than *Dacus*. – III. *Bulletin of Entomological Research* **10**: 211–272.

BICKFORD, D., LOHMAN, D.J., SODHI, N.S., NG, P.K.L., MEIER, R. WINKER, K., INGRAM, K.K. & DAS, I. 2006. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**: 148–155.

BIJZET, Z. 2006. Climatic requirements. In: De Villiers, E.A. & Joubert, P.H (Eds.) *The Cultivation of Citrus*. 28-38. ARC-Institute for Tropical and Subtropical crops, Nelspruit, South Africa.

BOLLER, E.F., HAISCH, A. & PROKOPY, R.J. 1970. Ecological and behavioural studies preparing the application of the sterile-insect-release-method (SIRM) against *Rhagoletis cerasi.* Presented at International Atomic Energy Agency Symposium, Athens, 1970. *Sterility Principle for Insect Control or Eradication.*

BOYKIN, L.M., SCHUTZE, M.K., KROSCH, M.N., CHOMIČ, A., CHAPMAN, T.A., ENGLEZOU, A., ARMSTRONG, K.F., CLARKE, A.R., HAILSTONES, D. & CAMERON, S.L. 2014. Multi-gene phylogenetic analysis of the south-east Asian pest members of the *Bactrocera dorsalis* species complex (Diptera: Tephritidae) does not support current taxonomy. *Journal of Applied Entomology* **138**: 235–253.

BUSH, G.L. 1966. The taxonomy, cytology, and evolution of the genus *Rhagoletis* in North America (Diptera: Tephritidae). Museum of Comparative Zoology, Cambridge, MA.

BUSH, G.L. 1994. Sympatric speciation in animals: new wine in old bottles. *Trends Ecology Evolution* **9**: 285-288.

BYERS, J.E. 2000. Competition between two estuarine snails: implications for invasions of exotic species. *Ecology* **81**: 1225-1239.

CÁCERES, C., SEGURA, D.F., VERA, M.T., WORNOAYPORN, V., CLADERA, J.L., TEAL, P., SAPOUNTZIS, P., BOURTZIS, K., ZACHAROPOULOU, A. & ROBINSON, A.S. 2009. Incipient speciation revealed in *Anastrepha fraterculus* (Diptera; Tephritidae) by

studies on mating compatibility, sex pheromones, hybridisation and cytology. *Biological Journal of the Linnaean Society* **97**: 152–165.

CALECA, V., GIACALONE, C. & ALLSOPP, E. 2015. Olive fruit fly?: A threat to the South African olive industry. *Technology* **August/September**: 71-72.

CAREY, J.R. 1991. Establishment of the Mediterrean fruit fly in California. *Science* **253:** 1369-1373.

CARSTENS, B.C., PELLETIER, T.A., REID, N.M. & SATLER, J.D. 2013. How to fail at species delimitation. *Molecular Ecology* **22:** 4369-4383.

CGA. 2018. Annual Report 2018. *Citrus Growers Association of Southern Africa*. 45. KwaZulu-Natal, South Africa.

CGA. 2019. 2019 industry statistics 2018 export season, Edmonds, J. (Ed.) *Citrus Growers Association of Southern Africa.* 45. KwaZulu-Natal, South Africa.

CHINVINIJKUL, S., SRIKACHAR, S., KUMJING, P., KIMJONG, W., SUKAMNOUYPORN, W. & POLCHAIMAT, N. 2015. Inter-regional mating compatibility among *Bactrocera dorsalis* (Diptera, Tephritidae) populations in Thailand. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *ZooKeys* **540**: 299–311.

CHRISTENSON, L.D. & FOOTE, R.H. 1960. Biology of fruit flies. *Annual Review of Entomology* **5**: 171-192.

CLARKE, A.R., ARMSTRONG, K.F., CARMICHAEL, A.E., MILNE, J.R., RAGHU, S., RODERICK, G.K. & YEATES, D.K. 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology* **50**: 293-319.

CLARKE, A.R. 2017. Why so many polyphagous fruit flies (Diptera: Tephritidae)? A further contribution to the 'generalism' debate. *Biological Journal of the Linnaean Society* **120**: 245-257.

COGAN, B.H. & MUNRO, H.K. 1980. Family Tephritidae. In: Crosskey, R.W. (Ed.) *Catalogue of the Diptera of the Afrotropical Region.* 518-554. British Museum (Natural History), London.

COPELAND, R.S., WHARTON, R.A., LUKE, Q., DE MEYER, M., LUX, S., ZENZ, N., MACHERA, P. & OKUMU, M. 2006. Geographic Distribution, Host Fruit, and Parasitoids of African Fruit Fly Pests *Ceratitis anonae, Ceratitis cosyra, Ceratitis fasciventris,* and *Ceratitis rosa* (Diptera: Tephritidae) in Kenya. *Ecology and Population Biology*. **99**: 261-278.

COSTA, G.C. 1998. Olive production in South Africa: *A handbook for Olive Growers*. ARC-Infruitec, Stellenbosch, South Africa.

COYNE, J.A. & ORR, H.A. 1989. Patterns of evolution in *Drosophila*. *Evolution* **43**: 362–381.

CUNNINGHAM, R.T. 1989. Parapheromones. In*:* Robinson, A.S. & Hooper, G. (Eds.) *Fruit flies their biology, natural enemies and control*. 221-229. Elsevier, Amsterdam, Netherlands.

DAFF. 2010a. Marula Production Guideline. Department of Agriculture, Forestry and Fisheries. 1-2. Pretoria, South Africa.

DAFF. 2010b. Olives Production Guideline. Department of Agriculture, Forestry and Fisheries. 3. Pretoria, South Africa.

DAFF. 2017. Abstract of agricultural statistics. Department of Agriculture, Forestry and Fisheries. 50-53, 81. Pretoria, South Africa.

DARWELL, C.T. & COOK, J.M. 2017. Cryptic diversity in a fig wasp community-morphologically differentiated species are sympatric but cryptic species are parapatric. *Molecular Ecology* **26**: 937-950.

DAVIS, R.A. 1928. The citrus fruits. 405-440. In: *Fruit Growing in South Africa.* Central News Agency, South Africa, Johannesburg, South Africa.

DELATTE, H., VIRGILIO, M., SIMIAND, C., QUILICI, S. & DE MEYER, M. 2013. Isolation and characterisation of sixteen microsatellite markers cross-amplifying in a complex of three African agricultural pests (*Ceratitis rosa*, *C. anonae* and *C. fasciventris*, Diptera: Tephritidae). *Conservation Genetic Resources* **5**: 31–34.

DE MEYER, M.D. 2000. Systematic revision of the subgenus *Ceratitis* MacLeay ss. (Diptera, Tephritidae). *Zoological Journal of the Linnaean Society* **128**:439-467.

DE MEYER, M. 2001a. Distribution patterns and host-plant relationships within the genus *Ceratitis* MacLeay (Diptera: Tephritidae) in Africa. *Cimbebasia* **17**: 219-228.

DE MEYER, M. 2001b. On the identity of the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **71**: 55-62.

DE MEYER, M., COPELAND, R.S, LUX, S.A., MANSELL, M.W., QUILICI, S., WHARTON, R.A., WHITE, I.M. & ZENZ, N.J. 2002. Annotated check list of host plants for Afrotropical fruit flies (Diptera: Tephritidae) of the genus Ceratitis. *Documentation Zoologique* **27**: 91

DE MEYER, M., COPELAND, R.S. WHARTON, R.A. & McPHERON, B.A. 2002. On the geographic origin of the Medfly *Ceratitis capitata* (Wiedemann) (Diptera : Tephritidae). *Proceedings of 6th International Fruit Fly Symposium*, 6-10 May 2002, Stellenbosch, South Africa 45-53.

DE MEYER, M., CLARKE, A.R., VERA, M.T. & HENDRICHS, J. 2015a. Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international Trade. *Zookeys* **540**: 1-3.

DE MEYER, M., DELATTE, H., EKESI, S., JORDAENS, K., KALINOVÁ, B., MANRAKHAN, A., MWATAWALA, M., STECK, G., VAN CANN, J., VANÍČKOVÁ, L., BŘÍZOVÁ, R. & VIRGILIO, M. 2015b. An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 405-427.

DE MEYER, M., MWATAWALA, M., COPELAND, R.S. & VIRGILIO, M. 2016. Description of new *Ceratitis* species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy* **233**: 1-23.

DE QUEIROZ, K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**: 879-886.

DE VILLIERS, M., MANRAKHAN, A., ADDISON, P. & HATTINGH, V. 2013. The distribution, relative abundance, and seasonal phenology of *Ceratitis capitata*, *Ceratitis rosa* and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. *Environmental Entomology* **42**: 831-840.

DREW, R.A.I. 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanic regions. *Memoirs of the Queensland Museum* **26**: 1-521.

DUYCK, P.F., DAVID, P., JUNOD, G., BRUNEL, C., DUPONT, R. & QUILICI, S. 2006a. Importance of competition mechanisms in successive invasions by polyphagous Tephritids in La Réunion. *Ecology* **87**:1770-1780.)

DUYCK, P.F., DAVID, P., PAVOINE, S. & QUILICI, S. 2008. Can host-range allow niche differentiation of invasive polyphagous fruit flies (Diptera: Tephritidae) in La Réunion? *Ecological Entomology* **33(**4): 439-452.

DUYCK, P.F., DAVID, P. & QUILICI, S. 2004. A review of relationships between interspecific competition and invasions in fruit flies (Diptera: Tephritidae). *Ecological Entomology* **29**:511-520.

DUYCK, P.F., DAVID, P. & QUILICI, S. 2006b. Climatic niche partitioning following successive invasions by fruit flies in La Réunion. *Journal of Animal Ecology* **75**: 518-526.

EKESI, S. & BILLAH, M.K. 2006. *Field guide to the management of economically important tephritid fruit flies in Africa.* 166. ICIPE SciencePress, Nairobi, Kenya.

EKESI, S., BILLAH, M.K., NDERITU, P.W., LUX, S.A. & RWOMUSHANA, I. 2009. Evidence for competitive displacement of *Ceratitis cosyra* by the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) on mango, and mechanisms contributing to the displacement. *Journal of Economic Entomology* **102**: 981-991.

EPPO/CABI. 1997. In: Smith, I.M., McNamara, D.G., Scott, P.R. & Holderness, M. (Eds.) *Quarantine Pests for Europe*. 2nd edition. CAB International, Wallingford, UK.

EPSKY, N.D., KENDRA, P.E. & SCHNELL, E.Q. 2014. History and development of food-based attractants. In: Shelly, T.E., Epsky, N.D., Jang, E.B., Reyes-Flores, J. & Vargas R. (Eds.) *Trapping and detection, control and regulation of Tephritid fruit flies: Lures, area-wide programs and trade implications.* 15-75. Springer Science + Business, Dordrecht, The Netherlands.

FEDER, J.L. & BUSH, G.L. 1989. A field test of differential host-plant usage between two sibling species of *Rhagoletis pomonella* fruit flies (Diptera: Tephritidae) and its consequences for sympatric models of speciation. *Evolution* **43**: 1813-1819.

FEDER, J.L., POWELL, T.H., FILCHAK, K. & LEUNG, B. 2010. The diapause response of *Rhagoletis pomonella* to varying environmental conditions and its significance for geographic and host plant-related adaptation. *Entomologia Experimentalis et Applicata* **136**: 31-44.

GEORGALA, M.B. 1964. The response of the males of *Pterandrus rosa* (Karsch) and *Ceratitis capitata* (Wiedemann) to synthetic lures (Diptera: Trypetidae). *Journal of the Entomological Society of Southern Africa* **27**: 67-73.

GROUT, T.G., DANEEL J.-H., MOHAMED, S.A., EKESI, S., NDERITU, P.W., STEPHEN, P.R. & HATTINGH, V. 2011a. Cold susceptibility and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae). *Journal of Economic Entomology* **104**: 1180-1188.

GROUT, T.G., DANEEL, J.-H., WARE, A.B. & BECK, R.R. 2011b. A comparison of monitoring systems used for *Ceratitis* species (Diptera: Tephritidae) in South Africa. *Crop* Protection. **30**: 617-622.

GROUT, T.G. & MOORE, S.D. 2015. Citrus. In: Prinsloo, G.L. & Uys, V.M. (Eds.) *Insects of Cultivated Plants and Natural Pastures in Southern Africa.* 448–499 pp. Entomological Society of Southern Africa, Hatfield, Pretoria, South Africa.

GROUT, T.G., STEPHEN, P.R., DANEEL J.-H. & HATTINGH, V. 2011c. Cold treatment of *Ceratitis capitata* (Diptera: Tephritidae) in oranges using a larval endpoint. *Journal of Economic Entomology* **104**: 1174-1179.

GROUT, T.G. & STOLTZ, K.C. 2007. Developmental rates at constant temperatures of three economically important *Ceratitis* spp. (Diptera: Tephritidae) from Southern Africa. *Environmental Entomology* **36**: 1310-1317.

GROVÉ, T., DE JAGER, K. & DE BEER, M.S. 2017. Indigenous hosts of economically important fruit fly species (Diptera: Tephritidae) in South Africa. *Journal of Applied Entomology* **14**: 817–824.

GROVÉ, T., DE JAGER, K. & THELEDI, M.L. 2019. Fruit flies (Diptera: Tephritidae) and *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) associated with fruit of the family Myrtaceae Juss. in South Africa. *Crop Protection* **116**: 24-32.

HANCOCK, D.L. 1984. Ceratitinae (Diptera: Tephritidae) from the Malagasy subregion. *Journal of the Entomological Society of southern Africa* **47**: 277-301.

HANCOCK, D.L. 1987. Notes on some African Ceratitinae (Diptera: Tephritidae), with special reference to the Zimbabwean fauna. *Transactions of the Zimbabwe Scientific Association* **63**: 47-57.

HAUSDORF, B. 2011. Progress toward a general species concept. *Evolution* **65**: 923-931.

HENDRICHS, J., VERA, M.T., DE MEYER, M. & CLARKE, A.R. 2015. Resolving cryptic species complexes of major Tephritid pests. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 5-39.

HERING, E.M. 1935. Neue Bohrfliegen aus Afrika. (Dipt,) (5. Beitrag zur Kenntnis der Trypetidae). *Konowia* **14**: 154-158.

HERNÁNDEZ-ORTIZ, V., CANAL, N.A., SALAS, J.O.T., RUÍZ-HURTADO, F.M. & DZUL-CAUICH, J.F. 2015. Taxonomy and phenotypic relationships of the *Anastrepha fraterculus* complex in the Mesoamerican and Pacific Neotropical dominions (Diptera, Tephritidae). In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *ZooKeys* **540**: 95-124.

IAEA. 2003. Trapping guidelines for area-wide fruit fly programmes. Vienna, Austria. IAEA.

JULIANO, S.A. 1998. Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? *Ecology* **79**: 255-268.

KARSCH F. 1887. Dipterologisches von der Delagoabai. *Entomologischer Nachrichten* **13**: 22–26.

KARSTEN, M., ADDISON, P., JANSEN VAN VUUREN, B. & TERBLANCHE, J.S. 2016. Investigating population differentiation in a major African agricultural pest: evidence from geometric morphometrics and connectivity suggests high invasion potential. *Molecular Ecology* **25**: 3019-3032.

LATHROP, F.H. & DIRKS, C.O. 1945. Timing and seasonal cycles of insects: the emergence of *Rhagoletis pomonella. Journal of Economic Entomology* **38**: 330-334.

LATHROP, F.H. & NICKELS, C.B. 1932. Biology and control of the blueberry maggot in Washington County, ME. *United States of America, Department of Agriculture, Technical Bulletin* **275**:1-76.

LESKI, R. 1963. Studies on the biology and ecology of the cherry fruit fly, *Rhagoletis cerasi* flies. (Diptera: Tephritidae). *Polskie Pismo Entomologiczne Seria B* **31**: 153-240.

LIGHT, D.M. & YANG, E.B 1996. Plant volatiles evoke and modulate tephritid behaviour. 123-133. In: McPheron, B.A. & Steck, G.J. (Eds.) *Fruit fly pests: a world assessment of their biology and management.* St. Lucie Press, Delray Beach.

LIQUIDO, N.J., MCQUATE, G.T. & SUITER, K.A. 2015. MEDHOST: An encyclopedic bibliography of the host plants of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), version 3.0. *United States Department of Agriculture, Center for Plant Health Science and Technology Online Database*.

LUX, S.A., EKESI, S., DIMBI, S., MOHAMED, S. & BILLAH, M.K. 2003. Mango-infesting fruit flies in Africa: perspectives and limitations of biological approaches to their management. 227-294. In: Neuenschwander, P., Borgemeister, C. & Langewald, J. (Eds.) *Biological control in integrated IPM systems in Africa*. CABI Publishing, Wallingford, UK.

MALACRIDA, A.R., GOMULSKI, L.M., BONIZZONI, M., BERTIN, S., GASPERI, G. & GUGLIELMINO, G.R. 2007. Globalization and fruit fly invasion and expansion: the medfly paradigm. *Genetica* **131***:* 1-9.

MALLET, J. 2007. Species, concepts of. *Encyclopaedia of Biodiversity.* 15. University College, London, U.K.

MANRAKHAN, A. 2019. Fruit fly. In T.G. Grout (Ed.) *Integrated Production Guidelines for export citrus. Integrated pest and disease management.* 1-10. Nelspruit, South Africa: Citrus Research International.

MANRAKHAN, A., DANEEL, J.-H., BECK, R., THERON, C.D., WELDON, C.W., MOORE, S.D. & HATTINGH, V. 2018. Non-host status of commercial export grade lemon fruit (*Citrus limon* (L.) Burman f. cv. Eureka) for *Ceratitis capitata, Ceratitis rosa, Ceratitis quilicii* and *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. *African Entomology* **26**: 202-214.

MANRAKHAN, A., DANEEL, J.-H., BECK, R., VIRGILIO, M., MEGANCK, K. & DE MEYER, M. 2017. Efficacy of trapping systems for monitoring of Afrotropical fruit flies. *Journal of Applied Entomology* **141**: 825-840.

MANRAKHAN, A., VENTER, J.H. & HATTINGH, V. 2015. The progressive invasion of *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. *Biological Invasions* **17**: 2803-2809.

MARCHIORO, C.A. 2016. Global potential distribution of *Bactrocera carambolae* and the risks for fruit production in Brazil. *PloS ONE* **11**: e0166142. https://doi.org/10.1371/journal.pone.0166142

MCPHAIL, M. & BLISS, C.I. 1933. Observations on the Mexican fruit fly and some related species Cuernavaca, Mexico, in 1928 and 1929. *United States of America, Department of Agriculture, Technical Circular* **255**: 241.

MOHAMED, S.A., EKESI, S. & HANNA, R. 2010. Old and new host-parasitoid associations: parasitism of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) and five African fruit fly species by *Fopius arisanus*, an Asian opine parasitoid. *Biocontrol Science and Technology* **20**: 183-196.

MORAITI, C.A, NAKAS, C.T. & PAPADOPOULOS, N.T. 2014. Diapause termination of *Rhagoletis cerasi* pupae is regulated by local adaptation and phenotypic plasticity: escape in time through bet-hedging strategies. *Journal of Evolutionary Biology* **27**: 43-54.

MWATAWALA, M., DE MEYER, M., WHITE, I.M., MAERERE, A. & MAKUNDI, R.H. 2007. Detection of the solanum fruit fly, *Bactrocera latifrons* (Hendel) in Tanzania (Diptera: Tephritidae). *Journal of Applied Entomology* **131**: 441-504.

MWATAWALA, M.W., VIRGILIO, M., DOMINIC, M. & DE MEYER, M. 2013. Field evaluation of the relative attractiveness of EGOlure and trimedlure for African *Ceratitis* species (Diptera: Tephritidae). *Journal of Applied Entomology* **137**: 392-397.

MWATAWALA, M.W., VIRGILIO, M., JOSEPH, J. & DE MEYER, M. 2015. Niche partitioning among two *Ceratitis rosa* morphotypes and other *Ceratitis* pest species (Diptera, Tephritidae) along an altitudinal transect in Central Tanzania. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *Zookeys* **540**: 429-442.

NEILSON, W.T.A. 1964. Some effects of relative humidity on development of pupae of the apple maggot, *Rhagoletis pomonella*. *The Canadian Entomologist* **96**: 810-811.

NISHIDA, T. 1963. Zoogeographical and ecological studies of *Dacus cucurbitae* in India. *Hawaii Agricultural Experiment Station, Technical Bulletin* **54**: 28.

NISHIDA, T, & BESS, H.A. 1957. Studies on the ecology and control of the melon fly *Dacus (Strumeta) cucurbitae* Coquillett (Diptera: Tephritidae). *Hawaii Agricultural Experiment Station, University of Hawaii. Technical Bulletin* **34**: 44.

NYAMUKONDIWA, C. & TERBLANCHE, J.S. 2009. Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): Effects of age, gender and feedinding status. *Journal of Thermal Biology* **34**: 406-414.

OEPP/EPPO. 2005. Bulletin OEPP/EPPO Bulletin **35:** 371-373.

PAPADOPOULOS, N.T., KATSOYANNOS, B.I., KOULOUSSIS, N.A., HENDRICHS, J., CAREY, J.R. & HEATH, R.R. 2001. Early detection and population monitoring of *Ceratitis capitata* (Diptera: Tephritidae) in a mixed-fruit orchard in northern Greece. *Journal of Economic Entomology* **94**: 971-978.

PROKOPY, R.J. 1968a. Visual responses of apple maggot flies, *Rhagoletis pomonella:* Orchard studies. *Entomologia Experimentalis et Applicata* **11**: 403-422.

PROKOPY, R.J. 1968b. Sticky spheres for estimating apple maggot adult abundance. *Journal of Economic Entomology* **61**: 1082-1085.

PROKOPY, R.J., BENNETT, E.W. & BUSH, G.L. 1971. Mating behaviour in *Rhagoletis pomonella* (Diptera: Tephritidae): I. Site of assembly. *The Canadian Entomologist* **103**: 1405-1409.

RIPLEY, L.B. & HEPBURN, G.A. 1930. The wintering of the Natal fruit-fly. *Farming in South Africa* **5**: 439-441.

ROBACKER, D.C. 2007. Chemical ecology of bacterial relationships with fruit flies. *Integrated Protection of olive crops* **30**: 9-22.

ROITBERG, B.D. & PROKOPY, R.J. 1987. Insects that mark host plants. *Bioscience*, **37**: 400-406.

SCHUTZE, M.K., AKETARAWONG, N., AMORNSAK W., ARMSTRONG, K.F., AUGUSTINOS, A.A. & BARR, N., BO, W., BOURTZIS, K., BOYKIN, L.M., CÁCERES, C., CAMERON, S.L., CHAPMAN, T.A., CHINVINIJKUL, S., CHOMIČ, A., DE MEYER, M., DROSOPOULOU, E., ENGLEZOU, A., EKESI, S., GARIOU-PAPALEXIOU, A., GEIB, S.M., HAILSTONES, D., HASANUZZAMAN, M., HAYMER, D., HEE, A.K.W., HENDRICHS, J., JESSUP, A., JI, Q., KHAMIS, F.M., KROSCH, M.N., LE BLANC, L., MAHMOOD, K., MALACRIDA, A.R., MAVRAGANI-TSIPIDOU, P., MWATAWALA, M.,

NISHIDA, R., ONO, H., REYES, J., RUBINOFF, D., SANJOSE, M., SHELLY T.E., SRIKACHAR, S., TAN, K.H., THANAPHUM, S., HAQ, I., VIJAYSEGARAN, S., WEE, S.L., YESMIN, F., ZACHAROPOULOU, A. & CLARKE, A.R. 2015a. Synonymization of key pest species within the Bactrocera dorsalis species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. *Systematic Entomology* **40**: 456-71. doi: 10.1111/syen.12113. PubMed PMID: WOS: 000350982700010

SCHUTZE, M.K., KROSCH, M.N., ARMSTRONG, K.F., CHAPMAN, T.A., ENGLEZOU, A., CHOMIČ, A., CAMERON, S., HAILSTONES, D.L. & CLARKE, A.R. 2012. Population structure of *Bactrocera dorsalis* s.s., *B. papaya* and *B. philippinensis* (Diptera: Tephritidae) in southeast Asia: evidence for a single species hypothesis using mitochondrial DNA and wing-shape data. *BMC Evolutionary Biology* **12**: 130. (S 12,).

SCHUTZE, M.K., MAHMOOD, K., PAVASOVIC, A., BO, W., NEWMAN, J., CLARKE, A.R., KROSCH, M.N. & CAMERON, S.L. 2015b. One and the same: integrative taxonomic evidence that the African Invasive Fruit Fly *Bactrocera invadens* (Diptera: Tephritidae), is the same species as the Oriental Fruit Fly *Bactrocera dorsalis*. *Systematic Entomology* **40**: 472–486.

SCHUTZE, M.K., VIRGILIO, M., NORRBOM, A., & CLARKE, A.R. 2017. Tephritid Integrative taxonomy: Where we are now, with a focus on the resolution of three tropical fruit fly species complexes. *Annual Review of Entomology* **62**: 147-164.

SCRIVEN, J.J., WHITEHORN, P.R., GOULSON, D. & TINSLEY, M.C. 2016. Niche partitioning in a sympatric cryptic species complex. *Ecology and Evolution* **6**: 1328-1339.

SEGURA, D.F., VERA, M.T., RULL, J., WORNOAYPORN, V., ISLAM, A. & ROBINSON, A.S. 2011. Assortative mating among *Anastrepha fraterculus* (Diptera: Tephritidae) hybrids from two distinct populations as a possible route to radiation of the *fraterculus* cryptic species group. *Biological Journal of the Linnaean Society* **102**: 346–354.

SELIVON, D., PERONDINI, A.L.P. & MORGANTE, J.S. 1999. Haldane's rule and other aspects of reproductive isolation observed in the *Anastrepha fraterculus* Complex (Diptera: Tephritidae). *Genetics and Molecular Biology* **22**: 507–510.

SELIVON, D., PERONDINI, A.L.P. & MORGANTE, J.S. 2005. A genetic morphological characterization of two cryptic species of the *Anastrepha fraterculus* complex. (Diptera: Tephritidae). *Annals of the Entomological Society of America* **98**: 367–381.

SHELLY, T.E., EPSKY, N., JANG, E.B., REYES-FLORES, J. & VARGAS RI. 2014. (Eds.) *Trapping and detection, control and regulation of Tephritid fruit flies.* Springer, Dordrecht. doi: 10.1007/978-94-017-9193-9

SIVINSKI, J.M. & CALKINS, C. 1986. Pheromones and parapheromones in the control of Tephritids. *Florida Entomologist* **69**: 157-168.

SMYTH, E.G. 1960. A new Tephritid fly injurious to tomatoes in Peru. *California Agricultural Experimental Station Bulletin* **49**:16-22.

STECK, G.J. 1999. Taxonomic status of *Anastrepha fraterculus*. The South American fruit fly, *Anastrepha fraterculus* (Wied.): advances in artificial rearing, taxonomic status and biological studies. *International Atomic Energy Agency*, Vienna, 13–20.

TAN, K.H., NISHIDA, R., JANG, E.B. & SHELLY, T.E. 2014. Pheromones, male lures and trapping of Tephritid fruit flies. In: Shelly, T.E, Epsky, N., Jang, E.B., Reyes-Flores, J. & Vargas R.I. (Eds.) *Trapping and detection, control and regulation of tephritid fruit flies: Lures, area-wide programs and trade implications.* 15-75. Springer Science + Business, Dordrecht, the Netherlands.

TAN, K.H., TOKUSHIMA, I., ONO, H., & NISHIDA, R. 2011. Comparison of phenylpropanoid volatiles in male rectal pheromone gland after methyl eugenol consumption, and molecular phylogenetic relationship of four global pest fruit fly species: *Bactrocera invadens*, *B. dorsalis*, *B. correcta* and *B. zonata*. *Chemoecology* **21**: 25–33.

TAN, K.H., WEE, S.L., ONO, H. & NISHIDA, R. 2013. Comparison of methyl eugenol metabolites, mitochondrial COI, and rDNA sequences of *Bactrocera philippinensis* (Diptera: Tephritidae*)* with those of three other major pest species within the *dorsalis* complex. *Applied Entomology and Zoology* **48**: 275–282.

TANGA, C.M., MANRAKHAN, A., DANEEL, J.-H., MOHAMED, S.A., FATHIYA, K. & EKESI, S. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis rasa* Karsch (Diptera, Tephritidae) populations from Kenya and South Africa. In: DE MEYER, M., CLARKE, A.R., VERA, M.T. & HENDRICHS, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *Zookeys* **540**: 467-487.

THERON, C.D., MANRAKHAN, A. & WELDON, C.W. 2017. Host use of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), in South Africa. *Journal of Applied Entomology.***141**: 810–816.

VANÍČKOVÁ, L., BŘÍZOVÁ, R., POMPEIANO, A., EKESI, S. & DE MEYER, M. 2015. Cuticular hydrocarbons corroborate the distinction between lowland and highland Natal fruit fly (Tephritidae, *Ceratitis rosa*) populations. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *Zookeys* **540**: 507-524.

VAN SAUERS-MULLER, A.E. 2005. Host plants of the carambola fruit fly, *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae), in Suriname, South America. *Neotropical Entomology* **34**: 203-214.

VARGAS, R.I., MIYASHITA, D. & NISHIDA, T. 1984. Life-history and demographic parameters of three laboratory-reared Tephritids (Diptera: Tephritidae). *Annuals of the Entomological Society of America* **77**: 651-656.

VARGAS, R.I., WALSH, W.A., KANEHISA, D., STARK, J.D. & NISHIDA, T. 2000. Comparative demography of three Hawaiian fruit flies (Diptera: Tephritidae) at alternating temperatures. *Annuals of the Entomological society of America* **93**: 75-81.

VILA, M. & WEINER, J. 2004. Are invasive plant species better competitors than native plant species? Evidence from pair-wise experiments. *Oikos* **105**: 229-238.

VIRGILIO, M., BACKELJAU, T., BARR, N. & DE MEYER, M. 2008. Molecular evaluation of nominal species in the *Ceratitis fasciventris*, *C. anonae* and *C. rosa* complex (Diptera: Tephritidae). *Molecular Phylogenetics and Evolution* **48**: 270-280.

VIRGILIO, M., DELATTE, H., QUILICI, S., BACKELJAU, T. & DE MEYER, M. 2013. Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa, Ceratitis fasciventris* and *Ceratitis anonae* (Diptera, Tephritidae). *Molecular Ecology* **22**: 2526-2539.

WHITE, I.M. 2006. Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East. *African Entomology*, *Memoir No.* **2**: 1-156.

WHITE, I.M. & CLEMENT, S.L. 1987. Systematic notes on *Urophora* (Diptera, Tephritidae) species associated with *Centaurea solstitialis* (Asteraceae, Cardueae) and other Palaearctic weeds adventive in North America. *Proceedings of the Entomological Society of Washington* **89**: 571-580.

WHITE, I.M., DE MEYER, M. & STONEHOUSE, J. 2000. A review of the native and introduced fruit flies (Diptera, Tephritidae) in the Indian Ocean Islands of Mauritius, Réunion, Rodrigues and

Seychelles. In: Price, N.S. & Seewooruthun, I. (Eds.) Proceedings of the Indian Ocean Commission Regional Fruit Fly Symposium, Mauritius, 5–9th June 2000. Indian Ocean Commission, Mauritius, 15–21.

WHITE, I.M. & ELSON-HARRIS, M.M. 1992. *Fruit fly of economic significance: their identification and bionomics.* V-601. C.A.B. International, London, UK and Australian Centre for International Agricultural Research, Canberra, Australia.

CHAPTER 3: THE RELATIVE ABUNDANCE OF TWO CRYPTIC FRUIT FLY SPECIES, *CERATITIS QUILICII* AND *CERATITIS ROSA,* IN CITRUS ORCHARDS

3.1. Introduction

Fruit flies are one of the major pests of citrus in southern Africa (Grout and Moore 2015), threatening both production and export. Three fruit fly species threaten the South Africa citrus industry: the exotic fruit fly, *Bactrocera dorsalis* (Hendel), and two indigenous species, *Ceratitis capitata* (Wiedemann) and *C. rosa* Karsch (Manrakhan 2019).

Ceratitis rosa s.l. was recently divided into two species: *C. rosa* s.s. and *C. quilicii* De Meyer, Mwatawala & Virgilio (De Meyer *et al*. 2016) following genetic, physiological and morphological differences found between two population clusters. Since *C. rosa* and *C. quilicii* were considered as one species until 2016, any scientific literature published until recently did not distinguish between these species, therefore references to *C. rosa* s.l. cited below, unless otherwise stated, refer to mixed populations of these two species. References to *C. rosa* s.s. are kept as *C. rosa* from here on. In some studies on *C. rosa* biology, which led to, and confirmed the differences between biotypes, reference was made to morphotypes, strains or biotypes of *C. rosa*.

Ceratitis rosa (Natal fruit fly) is multivoltine (Orian & Moutia 1960; De Meyer 2001) and polyphagous (Etienne 1982; Quilici 1989; De Meyer *et al*. 2008; Virgilio *et al*. 2013; De Meyer *et al*. 2016). In Europe and America it is listed as a quarantine pest (Augustin *et al*. 2012), which necessitates the development of management strategies for the pest to protect South African citrus export markets.

On the African continent, *C. rosa* s.l. is present in the eastern and southern regions, with the most northern distribution in the Kenyan Highlands, and the most southern, the Western Cape province in South Africa (De Meyer *et al*. 2015). In South Africa, *C. rosa* s.l. is distributed in the northern and eastern parts of the country (Grout *et al*. 2011) and also along most of the coastal areas (including the south and western areas) but tends to be absent in the arid regions (De Meyer *et al*. 2008; De Villiers *et al*. 2013). *Ceratitis rosa* s.l. is more cold tolerant than *C. capitata* and adapted to a wide range of climatic conditions (Duyck & Quilici 2002; De Meyer *et al*. 2008). A study conducted in South Africa indicated that *C. rosa* s.l. occurs in higher numbers in natural areas than in commercial citrus orchards, whereas *C. quilicii* tends to be more abundant in the commercial orchards (Manrakhan *et al*. 2017a). De Villiers *et al*. (2013) found *C. rosa* s.l. to be more abundant in fruit grown in home gardens, than in orchards.

Laboratory studies conducted in Kenya and South Africa, indicated differences in thermal biology of the two *C. rosa* morphotypes (Tanga *et al*. 2015). The ecological differences and niche requirements, *C. quilicii* prefering cool higher altitudes and *C. rosa* prefering warm lower altitudes, were confirmed by Mwatawala *et al*. (2015) in field studies in Tanzania. Studies conducted on the distribution of *C. rosa* and *C. quilicii* in South Africa provided contradicting results. Virgilio *et al*. (2013) found that both species were present in South Africa, with *C. rosa* absent from the Highveld and Western Cape regions. Karstens *et al*. (2016) trapped *C. rosa* s.l. specimens in 22 different areas throughout South Africa and found all specimens to belong to *C. quilicii*. Manrakhan *et al*. (2017a) also found both *C. rosa* and *C. quilicii* in attractant based traps in the northern parts of South Africa. In the study by Manrakhan *et al*. (2017a), both *C. rosa* and *C. quilicii* responded to all attractants targeting *Ceratitis* species: male lures such as Capilure (trimedlure) and EGO Pherolure (enriched ginger root oil: EGO), hereafter refered to as Egolure, and food-based attractants such as protein hydrolysate and three-component Biolure. Based on these findings it was decided to use these three lures to establish the relative abundance and distribution of *C. rosa* and *C. quilicii* in citrus orchards, with emphasis on the affect of altitude and climatic region.

In the northern parts of South Africa, *C. quilicii* and *C. rosa* occur sympatrically (Virgilio *et a*l. 2013). This indicates the possibility of some niche partitioning. A better understanding of the ecology of fruit fly populations and their distribution is important for effective control (De Villiers 2013), and to address its management and phytosanitary concerns to stay competitive in export markets.

The objectives of this study were to determine the distribution and relative abundance in space and time of *C. rosa* and *C. quilicii* in commercial citrus orchards. Emphasis was placed on the effect of altitude, climate and time of the year on the relative abundance of the two species. The efficacy of different attactants for the two species was also compared. Citrus fruit were collected from orchards and from other fruit bearing plants to determine presence and abundance of the two species.

3.2. Material and Methods

3.2.1. Study sites

Since the Limpopo and Mpumalanga Provinces collectively make up 49% of the citrus production area of South Africa (38 073 ha) (CGA, 2018), this study was conducted in these areas. Nine citrus farms were selected as study sites (Figure 1). At each farm, traps were placed in a *Citrus sinensis* (L.) Osbeck orchard. Sites were chosen based on their altitude (m a.s.l.). The study sites were grouped into three categories: high altitude (900 – 1100 m a.s.l.),

medium altitude (400 – 600 m a.s.l.), and low altitude (150 – 300 m a.s.l.) (Table 1). Geographical data were taken with a Garmin GPSMAP 60 Cx. Registered pest and fruit fly control practices were applied on each farm, based on each farm's historic fruit fly experience and current method of spray application preference.

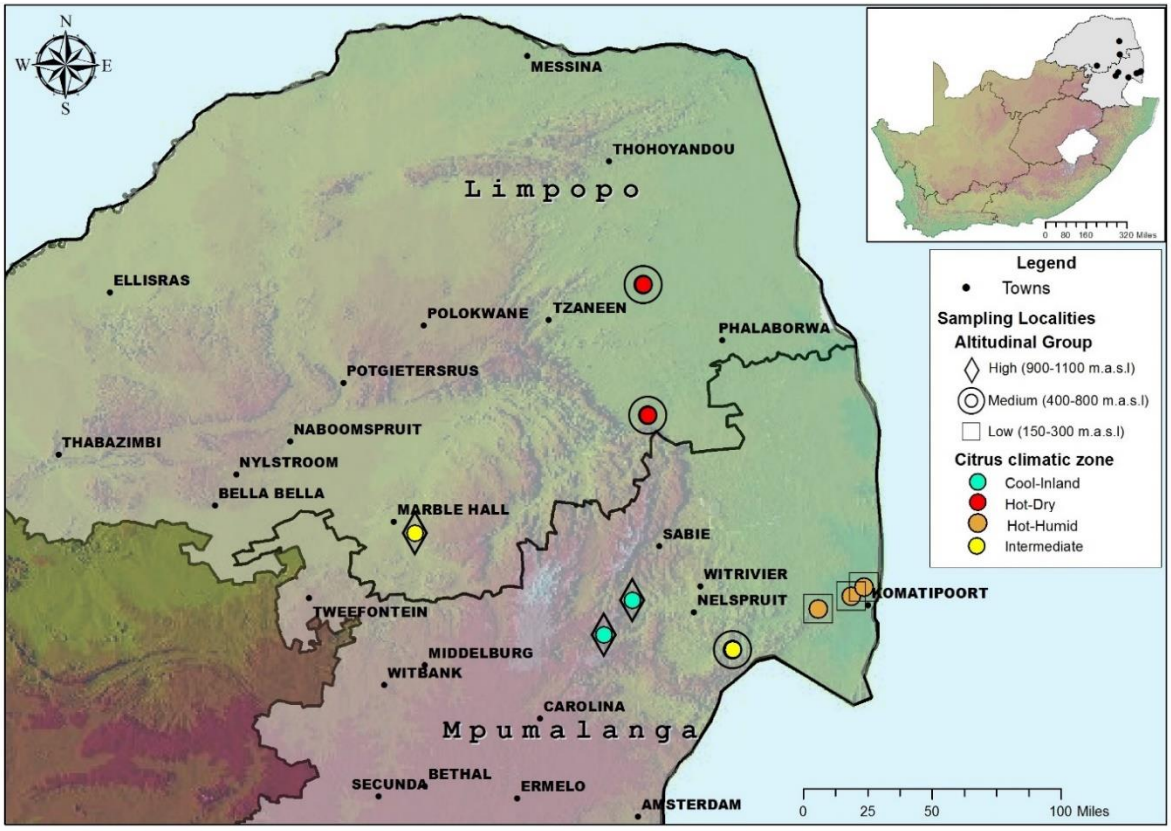

Figure 1. Locations of nine farms used in the study on distribution and relative abundance of *C. rosa* and *C. quilicii*. Three farms were located in the Limpopo province and six were located in the Mpumalanga province. The farms were distributed over three altitudes and four climatic zones, indicated by the outer circles, squares and diamond shapes, and four coloured circles on the map.

According to the Köppen-Geiger climate classification, these farms are all situated in a temperate climate, known to have dry winters and hot summers (Peel *et al*. 2007). In South Africa, citrus cultivation is categorised in six climatic zones: Hot-Humid (less than 200 km from the sea and below 300 m a.s.l.), Hot-Dry (less than 600 km from the sea and below 600 m a.s.l.), Intermediate (between the hot low areas and cool high areas, 600 to 900 m a.s.l.), Cold/Semi-Coastal (semi-coastal areas of the eastern, southern and western Cape), Cool-Inland (above 900 m a.s.l.), and Semi-Desert (hot summers and cold winters in the Northern Cape) (Barry 1996). The nine farms were distributed in four of these climatic regions with the Cold/Semi-Coastal and Semi-Desert not represented in this study (Table 1).

The weather data used in this study were supplied by Agroclimatology, Agricultural Research Council – Institute for Soil, Climate and Water (ARC - ISCW), Stellenbosch, South Africa. Weather stations were located between 5.2 and 24.4 km from each of the study sites (Table 1). Daily weather data were collected from May 2016 to May 2017. The weather data were averaged per month and monthly data were subsequently averaged for each site. The total rainfall for each farm was also recorded (Table 1). Due to the close proximity of two of the sites (Whisky and Vergenoeg), the same weather station was used to supply data for these farms.

Table 1. Characteristics of study sites where trapping and fruit sampling were carried out to determine distribution and relative abundance of *C. rosa* and *C. quilicii* in the northern citrus production areas of South Africa.

Province	Study sites (Weather station identification number)	GPS coordinates	Altitude (m a.s.l.)	Altitudinal group	Citrus climatic zone	Mean daily maximum temperature (°C)	Mean daily minimum temperature (°C)	Mean daily humidity (rH)	Total rainfall (mm)
Mpumalanga	Hectorspruit (30428)	S 25° 27' 32.3" E 31° 40' 16.1"	278	Low	Hot-Humid	29.9	15.7	62.1	842
	Joubert (30785)	S 25° 24' 37.5" E 30° 37' 31.1"	899	High	Cool-Inland	25.9	13.3	63.3	783
	Ryton (30903)	S 25° 36' 10.8" E 30° 27' 59.0"	1086	High	Cool-Inland	24.4	9.00	62.5	1050
	Siyalima (30765)	S 25° 41' 08.9" E 31° 11' 26.6"	550	Medium	Intermediate	27.9	13.7	63.1	1025
	Vergenoeg (30402)	S 25° 20' 13.9" E 31° 55' 33.5"	161	Low	Hot-Humid	30.6	16.6	65.1	829
	Whisky (30402)	S 25° 23' 09.7" E 31° 51' 18.3"	191	Low	Hot-Humid	30.6	16.6	65.1	829
Limpopo	Schoeman (30574)	S 25° 02' 02.6" E 29° 24' 17.9"	921	High	Intermediate	30.0	12.6	57.8	554
	Unifrutti (30950)	S 24° 22' 13.0" E 30° 42' 51.5"	473	Medium	Hot-Dry	28.5	15.8	57.5	736
	Van Veijeren (30748)	S 23° 38' 20.5" E 30° 41' 14.7"	426	Medium	Hot-Dry	30.5	14.9	59.3	437

3.2.2. Trapping survey

A trapping survey was carried out at each site from May 2016 to May 2017 to characterise the adult populations of *C. rosa* and *C. quilicii*.

Three attractants, known for their efficacy in attracting both *C. rosa* and *C. quilicii* (Manrakhan *et al*. 2017a) were used in this study. These were: Biolure (Chempac (Pty) Ltd., Suider Paarl, South Africa), consisting of three-components in a single sachet (single 9.5 cm square with a 3 cm opening on one side to assist with vapour release); Capilure (Green Trading (Pty) Ltd. Brits, South Africa) consisting of mainly trimedlure (tert-butyl 4 (or 5)–chloro-2-methylcyclohexane carboxylate) and proprietary extenders (Nakagawa *et al*. 1981); and Egolure (Insect Science (Pty) Ltd, Tzaneen, South Africa) consisting of enriched ginger root oil (EGO) which contains alpha-copaene (Mwatawala *et al*. 2013; Shelly & Pahio 2002). Biolure is a food-based attractant (FAO/IAEA 2018) while Capilure and Egolure are male attractants (FAO/IAEA 2018; Mwatawala *et al*. 2013).

Biolure was placed in a Chempac Bucket trap which consisted of two parts: a plastic opaque lid that screwed onto a yellow plastic base which had three entrance holes (2.0 cm diameter holes, with windows fitted) in the top part of the base section. A fourth entrance hole (2.5 cm diameter) was situated at the tip of an inverted cone moulded into the base of the trap. The trap with both sections fitted together was 16 cm in height, with a diameter of 13.5 cm at its widest point (Figure 2). Capilure was dispensed from a plastic capsule which contained 3 ml of attractant absorbed onto a cotton cloth. Egolure (2 ml) was contained in a small polyethylene bulb. Each of these two attractant types were placed individually and separately in a Sensus trap. The Sensus trap also consisted of two parts: a blue screw top lid, slightly elevated into a dome, which fitted onto a hyaline plastic bottom, with a total height of 13 cm and a diameter of 11.5 cm (Figure 2). The lid was 1 cm wider than the bottom section to protect any rain entering through the 12 entrance holes (0.6 x 0.7 cm), made around the top circumference of the bottom section.

Figure 2. Chempac bucket trap (left) and Sensus trap (right).

There were a total of nine traps per farm, three for each lure. Traps were placed at least 30 meters apart. Traps were set at a height of 1.4 m to 1.9 m inside the trees, on the south-eastern side. A thin, easily bendable wire was used for hanging each trap. Sticky ant barriers or petroleum jelly were added on each wire to protect the contents of the traps against potential crawling predators. A 1 cm^3 strip of 2,2-dichlororinyl dimethyl phosphate (DDVP)-block (Dichlorvos, 195 g/kg active ingredient) (River Bioscience, Port Elizabeth, South Africa) was placed in the bottom of each trap as a killing agent. Biolure and Capilure were replaced monthly, but Egolure was replaced only once after six months. DDVP blocks were replaced once a month in all traps. Every month, adult flies were collected from the traps, placed in numbered vials and transported to the laboratory at Citrus Research International (CRI), Nelspruit, South Africa for identification.

3.2.3. Specimen identification

Specimens collected were identified to species level and sexed using published keys which are based on morphological features (De Meyer 1996, 1998; De Meyer & Copeland 2005; De Meyer *et al*. 2015; De Meyer & Freidberg 2006; White 2006). A Zeiss Stemi 2000 – C dissecting microscope (Carl Zeiss (Pty) Ltd, Randburg, South Africa) with a magnification that

ranged from 6.5 to 50x was used during identification. The male flies of *C. rosa* and *C. quilicii* were differentiated based on the pattern of black colouration on the midtibia (De Meyer *et al*. 2016). In the midtibia of a *C. rosa* male, is a solid black area which extends to the dorsal and ventral margins whereas for a *C. quilicii* male, the black area on the midtibia does not extend to the dorsal and ventral margins (De Meyer *et al*. 2016).

All female flies of *C. rosa* s.l. and male flies with no midtibias were placed in 99% alcohol and shipped to the Royal Museum of Central Africa, Tervuren, Belgium (RMCA) for identification by means of molecular diagnostics, described in Virgilio *et al*. (2019). A reduced panel of six microsatellite markers (FAR4, FAR6, FAR7, FAR11, FAR16) was used to identify female specimens (Virgilio *et al*. 2019). In this study identification of the two species was confirmed using a threshold of 95% for the coefficient of the model used for clustering the species (Virgilio *et al*. 2019).

3.2.4. Fruit collection

Mature citrus fruit were collected from the ground or trees in citrus orchards. Fruit were placed in brown paper bags (42.5 cm x 30 cm) and brought back to the laboratory at CRI, Nelspruit. All fruit were dipped in a 0.1% Sporekill mixture (Didecyldimethylammoniun chloride, 120 g/L active ingredient) (ICA, Stellenbosch, South Africa). Fruit were counted, weighed and placed in rectangular plastic emergence containers with aerated lids, with volumes ranging from 13 L (35.5 x 24.5 x 18 cm) to 2.2 L (17.5 x 12.5 x 12 cm), and filled with a 2 cm layer of fine sand. The containers were kept in a room at ambient temperature for not less than 5 weeks and were inspected twice a week for adult emergence. Adult fruit flies emerging after eclosion were aspirated and placed in Petri dishes (using separate dishes for separate samples) allowing the colour of the flies to fully develop in order to facilitate identification of the flies as described above.

3.2.5 Statistical analysis

Trapping data were summarised as flies per trap per day (FTD) which is defined as the number of flies of a species collected in each trap and divided by the number of days between the service intervals. Trapping data were averaged for each attractant type, altitude, climatic region and time of the year. Relative abundance indexes (RAI) were calculated according to the methods used by Segura *et al*. (2006) in order to directly compare abundances of *C. rosa* and *C. quilicii*. RAI values were calculated from FTD values of each species. The RAI value was calculated as follows: RAI = FTD *C. quilicii/* (FTD *C. quilicii* + FTD *C. rosa*) (Segura *et al.* 2006). A value of 1 indicates the exclusive presence of *C. quilicii*, and zero, the exclusive presence of *C. rosa*. RAI values were averaged for each location, attractant type, altitude,

climatic region and time of the year. Effects of location, attractant type, altitude, climatic region and time of the year on RAI values were analysed using a hierarchical linear model (HLM) in SPSS Statistics Version 25. The dependence of data from the same site were taken into account when comparing means of the different variables. The correlations of weather data and trap catches were analysed by using PROC SURVEYREG in SAS, where the dependence of data from the same farm were taken into account.

Fruit infestation data were summarized for each fruit type sampled and were expressed as the total number of fruit collected in kilograms and the total number of adult flies reared from each citrus species.

3.3. Results

3.3.1. Abundance

Ceratitis quilicii was the dominant species and was caught at all nine sites (Figure 3). *Ceratitis rosa* was only collected from seven of the nine sites, and it never represented more than 5% of the total number of trapped flies except at two of the trial sites. The two sites where an abundance of *C. rosa* was equal or higher than *C. quilicii* were situated in the low altitude Hot-Humid climatic zone (Figure 3).

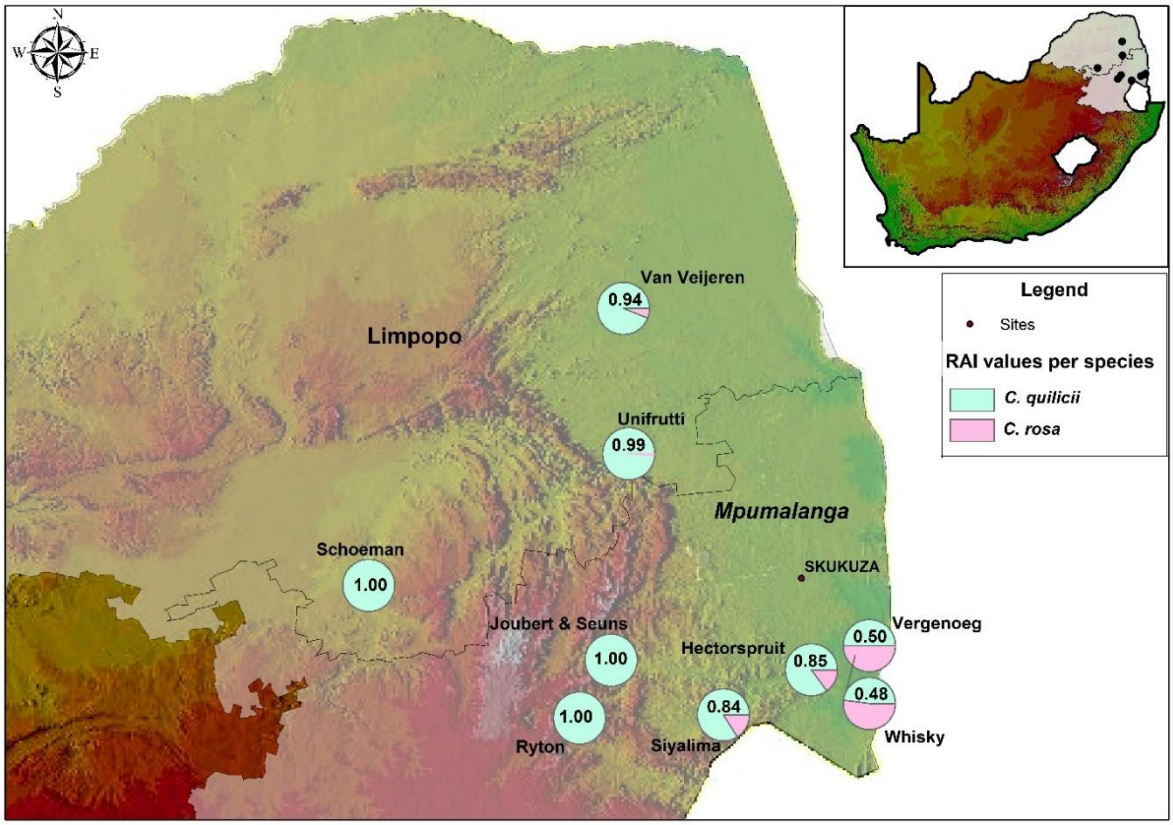

Figure 3. Relative Abundance Index (RAI) values of *C. quilicii* and *C. rosa* at the nine sampling sites in the Mpumalanga and Limpopo provinces. Total trap catches (males and females) of all sampling months and of all lures were combined.

The lures used in the study: Biolure, Capilure and Egolure confirmed the greater abundance of *C. quilicii* (Table 2).

Table 2. The relative abundance index (RAI) values of C*. quilicii* and *C. rosa* in the nine sampling sites using three different lures. A RAI value of 1 indicates total dominance of *C. quilicii* and a RAI value of 0, total dominance of *C. rosa*.

	Number of sites in each RAI category for each lure					
Lure	**Zero Catches**	**RAI = 0**	**0 - 0.33**	**0.34 - 0.66**	**0.67 - 1**	**RAI = 1**
Biolure	1	0	0	1	4	3
Capilure	1	1	1	1	1	4
Egolure	0	0	0	1	5	3

During the trial period, male flies were captured by all three lures (Table 3). Higher catches of *C. quilicii* and *C. rosa* were recorded in Egolure baited traps. Females were largely collected from Biolure traps with the exception of two flies caught in Egolure traps. The combined trap

catches from the nine locations were 1052 for *C. quilicii* and 89 for *C. rosa* males, and 494 and 17 females respectively of *C. quilicii* and *C. rosa.* The RAI average value for Biolure was similar to that of Egolure and both were significantly different from that of Capilure (Table 3) (P = 0.04).

Table 3. Total number of male and female flies caught in traps at nine localities over a 12-month period for *C. rosa* and *C. quilicii*.

Species	Biolure	Capilure	Egolure	Total
Average RAI value	0.89 a	0.76 b	0.88 a	0.86
***C. quilicii* males**	378	108	566	1052
***C. rosa* males**	21	19	49	89
***C. quilicii* females**	492	0	2	494
***C. rosa* females**	17	0	0	17
Total	921	127	617	1652

*Means within rows followed by the same letter do not differ significantly at P = 0.05

3.3.2. Comparison between male and female catches

Male and female catches of each species were compared in the different sites for each species. Although *C. quilicii* males were caught at all nine sites, females were only caught at eight sites (Figure 4). A ratio of 1.1:1 *C. quilicii* males in Egolure traps to *C. quilicii* females in Biolure traps was obtained when all nine sites were pooled together. For *C. rosa* males, the ratio of males in Egolure traps to females in Biolure traps was 1.3:1 when catches from all sites, excluding Vergenoeg, were pooled. In Vergenoeg, however, catches of males in Egolure traps were 8.5 times higher than catches of females in Biolure traps.

The female ratio of the two species in the Biolure traps and the male ratio of the two species in the Egolure traps were also compared at each site (Figure 4). In most sites, except the low altitude and Hot-Humid climate zone where *C. rosa* dominated, the ratio between the males and females of the two species were similar, irrespective of whether a male or a female trap was employed. At Vergenoeg, higher numbers of *C. rosa* males were caught in Egolure traps, whereas at Whisky, more *C. rosa* females were caught in Biolure traps (Figure 4).

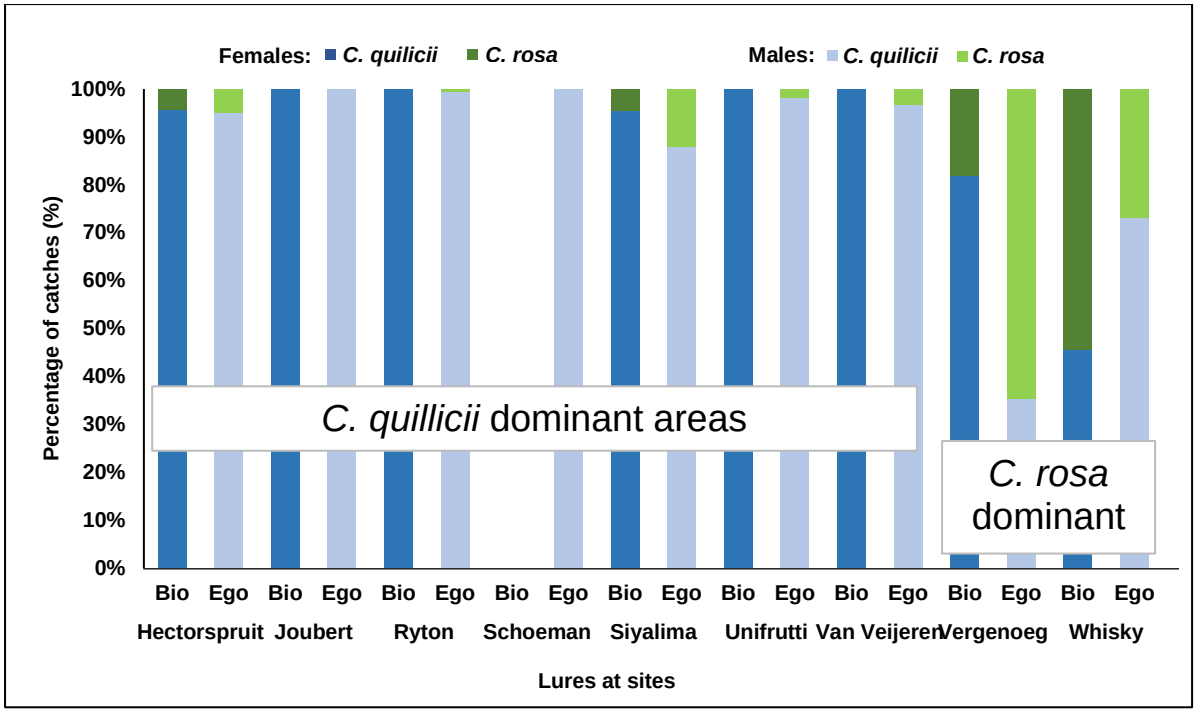

Figure 4. A comparison between the ratio of *C. quilicii* and *C. rosa*, female and male catches at nine sites.

3.3.3. The effect of altitude and the four climatic zones on abundance of *C. quilicii* and *C. rosa*

The abundance of *C. quilicii* was significantly higher for all lures (P = 0.04) at high-altitude sites, irrespective of which lure was used (Figure 5). At the lower altitudes, the RAI values were lower, indicating an increased presence of *C. rosa* (Figure 5). There was no interaction between altitude and lure (P = 0.154).

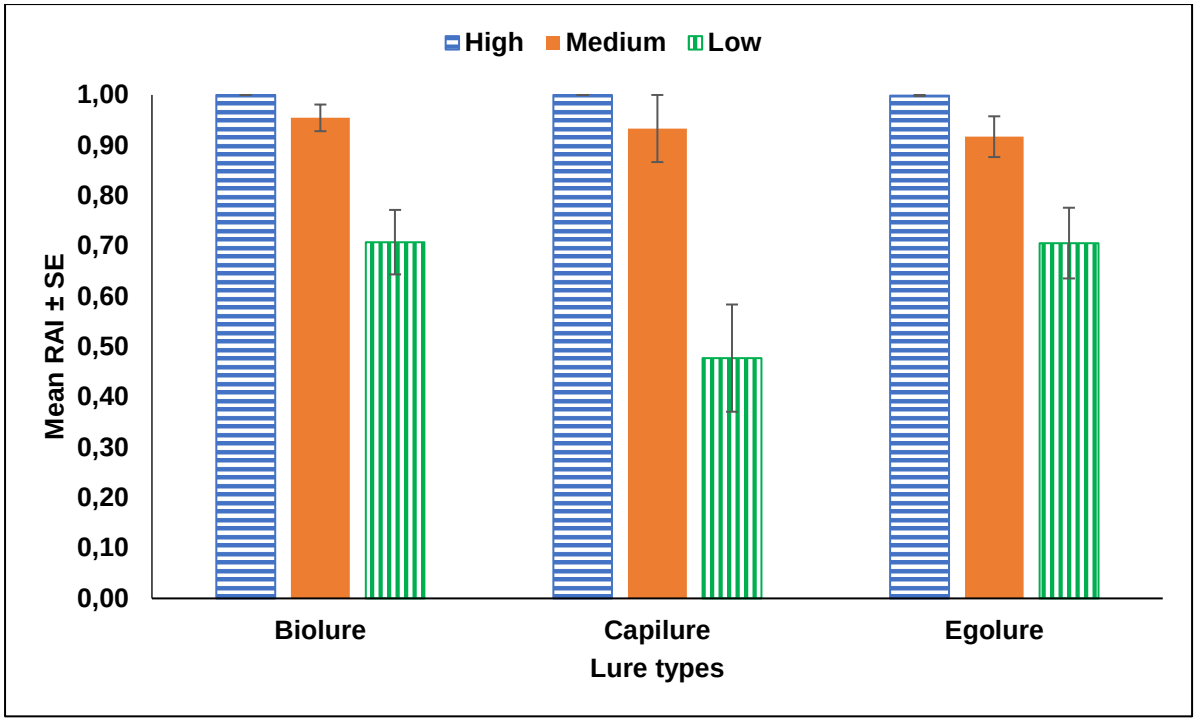

Figure 5. Average relative abundance index (RAI) values of fly captures by means of three lures at different altitudes. Bars indicate standard errors. The RAI value was calculated as follows: RAI = FTD *C. quilicii/* (FTD *C. quilicii* + FTD *C. rosa*).

Temperatures increased with a decrease in altitude, which resulted in a lower RAI value for *C. rosa* (Figure 6). The mean maximum temperature at the high altitude sites was 3.6 °C lower than at low-altitude sites (Figure 6). The mean minimum temperature was 4.7 °C lower at the high altitude sites than low altitude sites. With increases in maximum temperatures, the abundance of *C. rosa* also increased (lower RAI value). The average humidity recorded at the three different altitudes were similar while rainfall was highest in the high altitude region, followed by the low altitude region (Table 4).

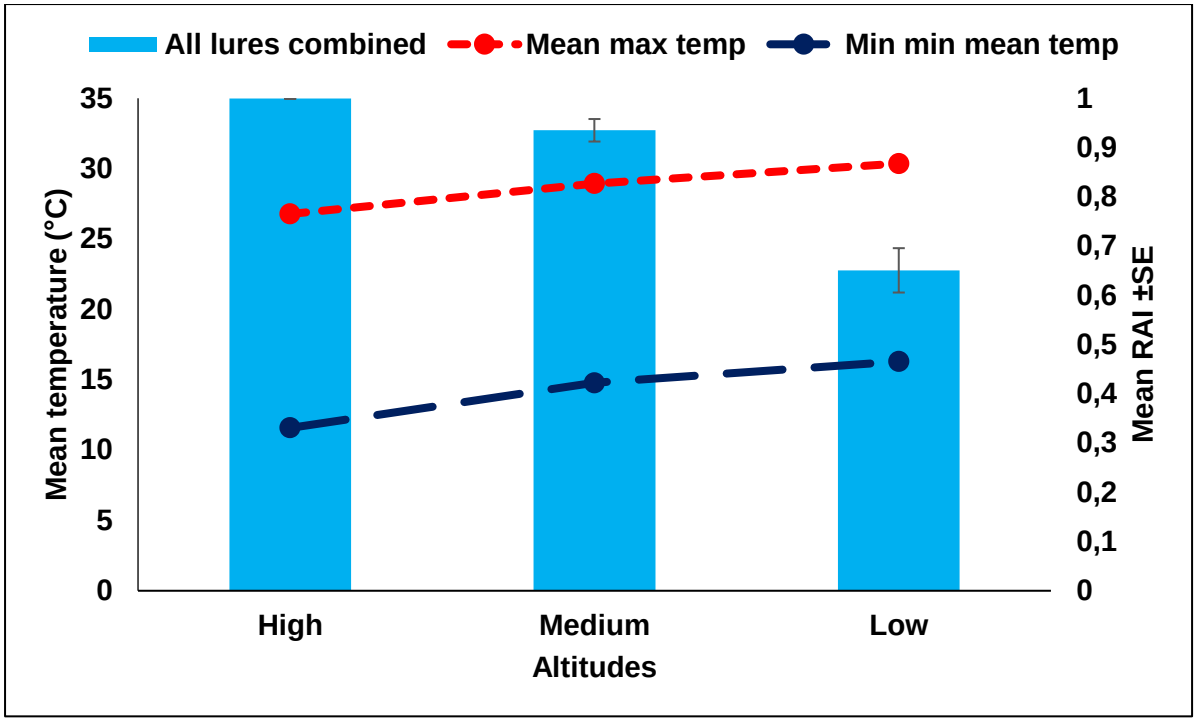

Figure 6. Mean relative abundance index (RAI) values at different altitudes compared to the average maximum and minimum temperature per altitudinal group. Bars represent standard error.

There were no significant differences (P = 0.10) in RAI values between the climatic zones when data where pooled for the three lure types. However, there was a statistically significant interaction between climatic region and lure (P = 0.05), with all lures combined, the abundance *C. quilicii* was low in the Hot-Humid zones, but with Capilure it was even lower in the Intermediate zone. In Capilure baited traps, catches in the Intermediate zone consisted of only *C. rosa* males (Figure 7). In the Hot-Humid zone, *C. rosa* numbers dominated in Capilure baited traps (RAI = 0.48) but not in Biolure or Egolure traps (Figure 7).

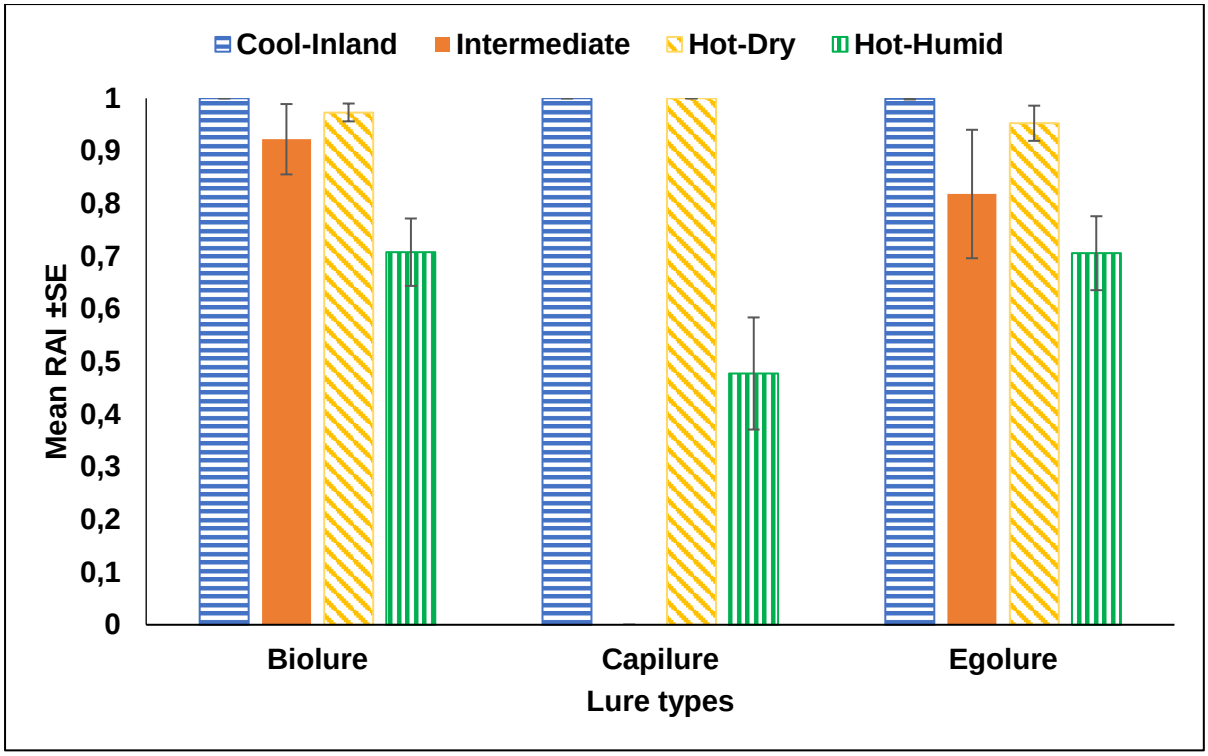

Figure 7. Mean relative abundance index (RAI) values distributed over four climatic zones according to numbers of flies caught by the different attractants. Bars represent standard errors.

Ceratitis quilicii was dominant in all four climatic zones (pooled data for different lures) (Figure 8). The average RAI value was at its highest in the Cool-Inland climatic zone. Lower numbers of *C. rosa* were captured in the zone with lower temperatures (Figure 8). Temperatures were the highest in the Hot-Humid and the Hot-Dry zones. The RAI was again lowest in the Hot-Humid zone (RAI =0.65) but high in the Hot-Dry area (RAI = 0.97). The difference between the maximum temperature in the Cool-Inland and Hot-Humid zone was 5.2 °C, while the minimum temperature at the latter was 5.2 °C lower. The average humidity was the highest in the Hot-Humid zone and lowest in the Hot-Dry zone (Table 4). The total rainfall recorded was the highest in the Cool-Inland zone followed by Hot-Humid zone, Intermediate and the Hot-Dry zone (Table 4).

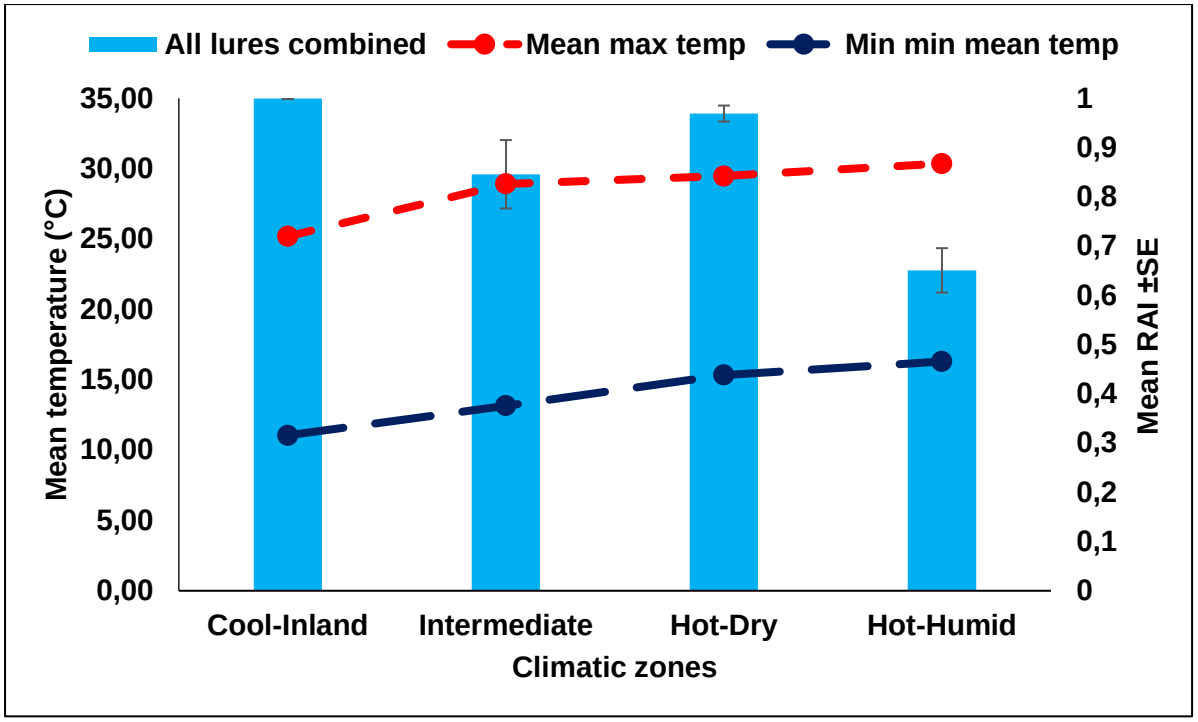

Figure 8. Mean temperatures and average relative abundance index (RAI) values pooled together over the four climatic zones. Bars represent standard errors.

Table 4. The mean daily relative abundance index (RAI) values, maximum and minimum average humidity and total rainfall for each altitude region and climatic zone.

		Mean RAI	Mean daily humidity (rH)	Mean total rainfall (mm)
Altitude	**High**	1.00	61.21	795.50
	Med	0.94	59.99	732.37
	Low	0.65	64.14	832.79
Climatic zone	**Cool-Inland**	1.00	62.92	916.30
	Intermediate	0.85	60.47	789.27
	Hot-Dry	0.97	58.42	586.24
	Hot-Humid	0.65	64.14	832.79

Ceratitis rosa catches were positively correlated with minimum temperature (Table 5). *Ceratitis rosa* catches were also negatively correlated with altitude (Table 5). This indicates a higher abundance of *C. rosa* with increasing minimum temperatures and a lower abundance of *C. rosa* at higher altitudes.

Table 5. Correlation between mean fruit fly numbers caught per day (all traps) and abiotic factors: altitude, maximum temperature, minimum temperature, maximum relative humidity and total relative humidity.

		Altitude (m a.s.l.)	Max Average temp. (°C)	Min Average temp. (°C)	Max Average rH (%)	Min Average rH. (%)	Total rainfall (mm)
C. quilicii	**Correlation Coefficient**	0.017	-0.402	-0.033	-0.017	-0.218	0.402
	***P*-value**	0.966	0.284	0.932	0.966	0.574	0.284
C. rosa	**Correlation Coefficient**	-0.869**	0.633	0.751*	0.380	0.278	0.270
	***P*-value**	0.002	0.067	0.020	0.313	0.468	0.482

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

3.3.4. Seasonal effect

In the high and medium altitude sites, *C. quilicii* was more abundant than *C. rosa* across the year (Figure 9). In May, *C. rosa* numbers increased in the medium altitude sites but *C. quilicii* still remained dominant in that month (Figure 9). In the low altitude sites, C. *quilicii* was only dominant during winter (July and August) and summer (January and February) months (Figure 9).

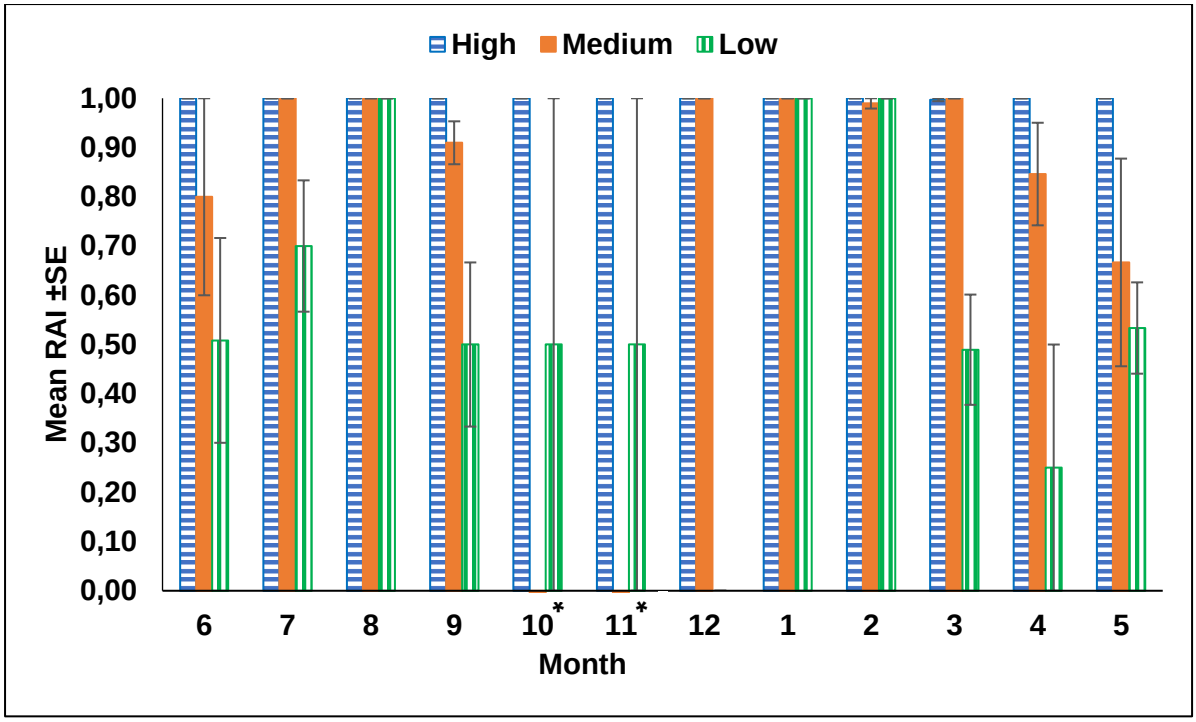

Figure 9. Mean relative abundance index (RAI) values for all lures combined for both males and females over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively) at three different altitudes. The two months (10 and 11) denoted by an asterisk indicate no catches at medium altitude.

In all climate zones except Hot-Humid, *C. quilicii* was more abundant than *C. rosa* for most parts of the year (Figure 10). In the Intermediate zone, *C. rosa* was more dominant during April and May (Figure 10). In the Hot-Humid zone, *C. rosa* was more abundant than *C. quilicii* across the year except in the winter (July and August) and summer (January and February) months (Figure 10).

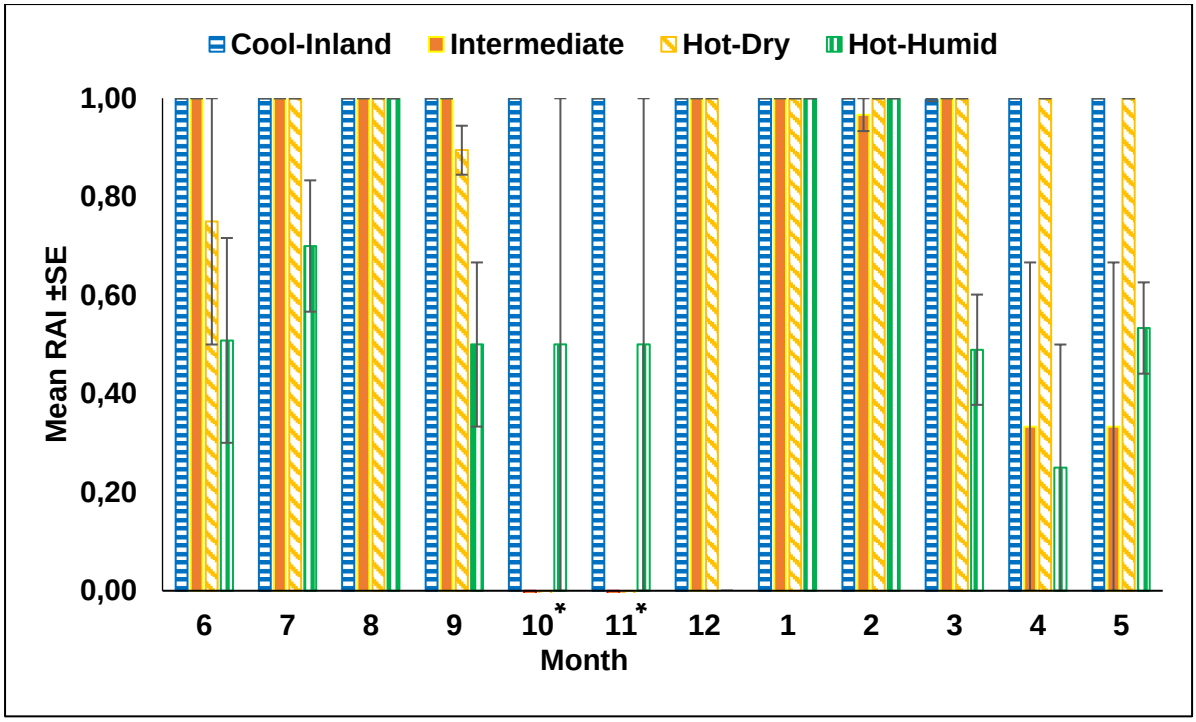

Figure 10. Mean relative abundance index (RAI) values, all lures combined for both males and females over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively), in four different climatic zones. The two months (10 and 11) denoted by an asterisk indicate no catches at medium altitude.

Ceratitis quilicii male and female catches showed similar peaks in September (spring) and February (late summer) (Figure 11). Catches of *Ceratitis rosa* males and females peaked between March and May (autumn) (Figure 12). High catches of *C. rosa* males also occurred in June (winter) and September (spring), (Figure 12).

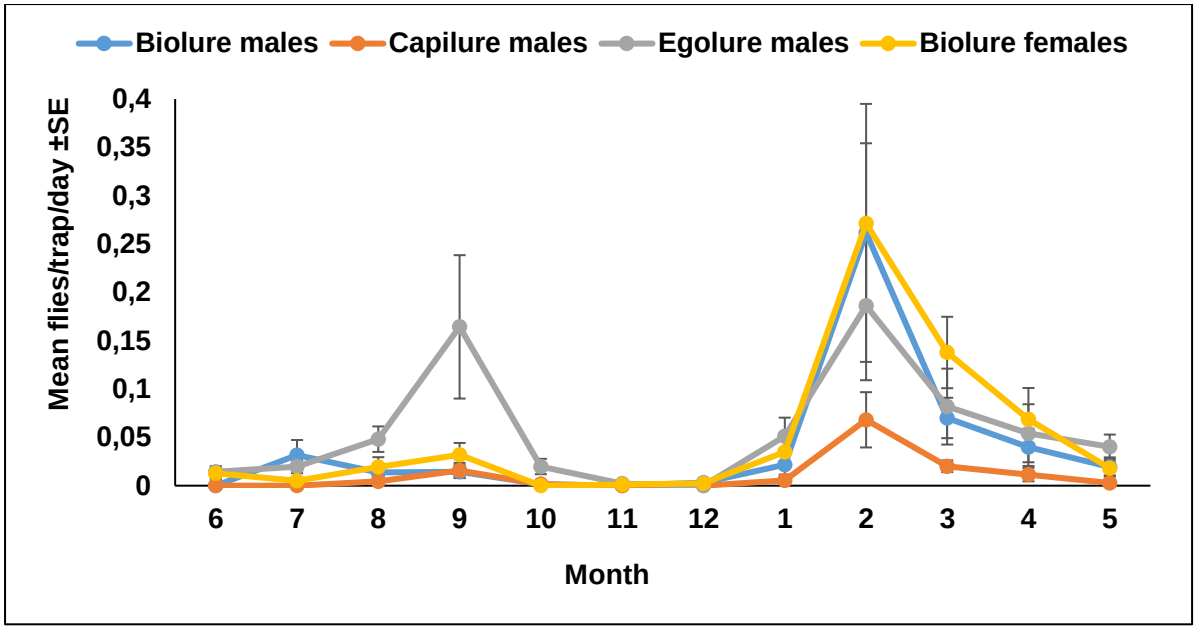

Figure 11. Mean numbers of male and female *C. quilicii* flies caught with different attractants over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively). Bars indicate standard error.

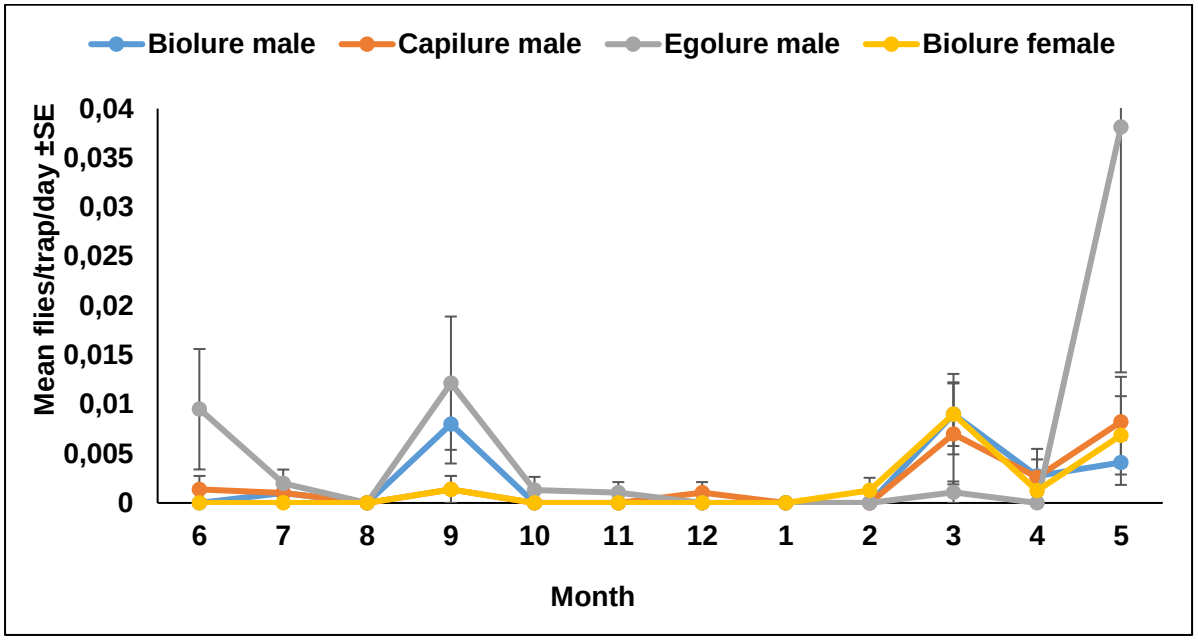

Figure 12. Mean numbers of male flies of *C. rosa* caught with different attractants over a twelve-month period (where month 1 equals the first month of the year i.e. January, with the rest of the months represented consecutively). Bars indicate standard error.

3.3.5. Host fruit

During the trial period, 188 kg of citrus, Clementine, grapefruit, lemon and oranges, were collected from the ground but neither *C. rosa* nor any *C. quilicii* adults were reared from the citrus collected from the commercial orchards (Table 6).

Table 6. The weight of fruit collected and number of flies reared from these.

Plant family	Citrus species	Weight (kg)	Number of *C. quilicii* males	Number of *C. rosa* males	Number of *C. rosa* s.l. females
Rutaceae	*Citrus limon*	147.48	0	0	0
Rutaceae	*Citrus paradisi*	4.74	0	0	0
Rutaceae	*Citrus reticulata*	2.65	0	0	0
Rutaceae	*Citrus sinensis*	33.03	0	0	0
Total		187.91	0	0	0

3.4. Discussion

Ceratitis quilicii was highly abundant and more widespread than *C. rosa* in the northern provinces of South Africa. Karsten *et al*. (2016) reported similar results, however, they did not collect any *C. rosa* from the sites where they sampled, even though some sites (Komatipoort and Nelspruit) were very close to the sites used in this study. A difference between their study and this study is that they used a different morphological method, geometric morphometric wing shape analyses, to confirm their molecular results, whereas this study used the shape and feathering pattern on the midtibia of the males to distinguish between the two species. However, the biggest difference between the two studies, as Karsten *et al.* (2016) mentions, is the importance to sample at the right time of the year when the fly numbers are at their highest due to the availability of ripening fruit, especially with species such as *C. rosa* that might occur in lower numbers, which was confirmed in this study. This study sampled over a twelve month period improving the chances of collecting *C. rosa* adults.

Ceratitis quilicii dominated at the two higher-altitude regions (426 – 1086 m a.s.l.). This is in agreement with Mwatawala *et al*. (2015) who reported that at low altitudes (< 550 m), *C. rosa* was more dominant, and at high altitudes (> 1170 m), *C. quilicii* was the dominant species. In this study, *C. rosa* was never recorded in two of the higher altitude sites. Mwatawala *et al*. (2015), on the other hand, found that the two species occurred together at all altitudes between 540 – 1650 m a.s.l. in Tanzania. In a study on the temperature related developmental times of *C. rosa* and *C. quilicii*, Tanga *et al*. (2015) estimated a lower developmental threshold for

C. quilicii than for *C. rosa* (lower threshold of Kenyan *C. rosa* population was 11.27 °C, and that of the *C. rosa* South African population was 8.99 °C). In the Cool-Inland zone in this study, average minimum air temperature was 11.05 °C which possibly limited the growth of *C. rosa* populations in these regions. According to Begon *et al*. (2006) there is a temperature drop of 1 °C for every 100 m gain in altitude in dry air, and 0.6 °C in moist air, with a corresponding decrease in species richness. In this study, an increase in *C. rosa* was observed with an increase in minimum air temperature and a decrease in altitude.

No significant relationships were observed in this study between species distribution and either humidity or total rainfall, leaving temperature as the main abiotic factor that could partition the distribution of the two species. Duyck *et al*. (2006) reported that *C. rosa* s.l. was more sensitive to low humidity than *C. capitata,* but that it was more tolerant to low temperatures than *C. capitata.* The optimum temperature for *C. rosa* s.l. is between 22 - 23 °C, with an annual rainfall of 3000 – 3500 mm (Duyck *et al.* 2006). De Villiers *et al*. (2013) confirmed that *C. rosa* s.l. was absent from the drier regions of South Africa and found a positive correlation between its distribution and precipitation. In Réunion, *C. rosa* s.l. occurs on the wetter side of the island (Normand *et al*. 2000). It is possible then that both *C. quilicii* and *C. rosa* will be affected with changes in humidity and rainfall.

A comparison between the two species did not show any change in ratio over twelve months at high altitude or the Cool-Inland zone, with total dominance of *C. quilicii* in the trap catches. This result was also similar for the medium altitude and Hot-Dry zone, except for May when more male C*. rosa* flies, trapped by Biolure and Capilure, were caught at medium-altitude sites. In the low-altitude and Hot-Humid zone, the dominance of the two species changed over time, with RAI-values of ≤ 0.5 in seven of the twelve months. A study conducted by Kounatidis *et al*. (2008) reported movement of *Bactrocera oleae* (Rossi) between higher and lower altitudes as seasons changed. During warm months there were more *B. oleae* flies in orchards at high and medium altitudes, while during winter, flies were more abundant in low-lying regions. Geurts *et al*. (2012) found that *C. rosa* s.l. was more abundant in high-altitude areas in Tanzania, during the rainy season (February and March), similar to the observations on *C. quilicii* in this study.

The effectiveness of trimedlure as a lure for *C. rosa* was demonstrated by Georgala (1964). Trimedlure has been reported to be more effective in attracting *C. capitata* than *C. rosa* s.l. (Grout *et al*. 2011) and is also considered an effective lure for monitoring the FAR complex (Virgilio *et al*. 2008). Grout *et al*. (2011) found that immature *C. rosa* males were less attracted to trimedlure. Egolure, which was recently introduced into the South African citrus industry,

contains α-copaene (Shelly & Pahio 2002) and, like trimedlure, gives a mating advantage to *C. rosa* (Quilici *et al.* 2013; Manrakhan *et al*. 2017b). One of the advantages of Egolure is that it attracts a wide range of *Ceratitis* species (Manrakhan *et al*. 2017b; Mwatawala *et al*. 2013, 2015). A study conducted by Manrakhan *et al*. (2017a) found that *C. rosa* and *C. quilicii* were attracted in similar numbers by Egolure and trimedlure. In this study most of the males of both species were caught using Egolure.

In this study, catches of *C. quilicii* males with Biolure, an attractant which is not species-specific and female biased, were lower compared to catches of conspecific females. Grové & De Beer (2019), trapped fruit flies in the Mbombela Local Municipality, Mpumalanga, using Biolure and reported higher catches of *C. rosa* s.l. females than males with this attractant. Results on catches of *C. rosa* with Biolure in this study were, however, different with catches of males being very similar to the female catches.

A practical problem for managing fruit flies in orchards is that only the males of *C. quilicii* and *C. rosa* can be differentiated morphologically, and this needs to be done by means of dissecting microscopes which are not available to all farmers (De Meyer *et al*. 2016; Virgilio *et al*. 2019). This causes some practical difficulties for growers who want to monitor for the presence of fruit flies in citrus orchards. Although fruit fly monitoring in the citrus industry is standardized, with specific attractants with recommended threshold values (Barnes 2000; Manrakhan 2019), growers are not using the same attractants to monitor for fruit fly and lately more trap types and attractants are becoming available on the market. Some growers prefer specific products such as male attractants, which they consider to be the most effective for use as an early warning method, while others prefer an attractant with a broader range, which not only catch different species, but also females.

In an integrated management approach, monitoring and control of fruit flies should be carried out. Growers are recommended to use male biased and female biased traps to track populations of males and females respectively. The male catches with these traps provide an early indication of the presence of a fruit fly pest in an area while the food-based attractants generally indicate a potential threat, especially during the period when fruit are maturing in orchards (Manrakhan *et al*. 2017a). There was no evidence in this study that citrus was utilized as a host for either *C. rosa* or *C. quilicii* although this result could have been affected by pesticide applications. However, since various citrus species are listed as hosts, the host status of citrus to these two species should be further investigated using semi field set ups as recommended by Aluja & Mangan (2008).

3.5. Conclusion

Ceratitis quilicii is more widely distributed and more abundant in the northern parts of South Africa than *C. rosa*. *Ceratitis quilicii* was present at all three altitudes and the dominant species at the two higher altitudes. Temperature and altitude were the main factors that influenced the distribution of *C. rosa*. All three lures employed in this study attracted both fruit fly species with most of the males caught in Egolure traps.

3.6. References

ALUJA, M. & MANGAN, R.L. 2008. Fruit Fly (Diptera: Tephritidae) host status determination: critical conceptual, methodological, and regulatory considerations. *Annual Review of Entomology* **53**: 473-502.

AUGUSTIN, S., BOONHAM, N., DE KOGEL, N., DONNER, P., FACCOLI, M., LEES, D.C., MARINI, L., MORI, N., TOFFOLO, E.P., QUILICI, S., ROQUES, A., YART, A. & BATTISTI, A. 2012. A review of pest surveillance techniques for detecting quarantine pests in Europe. *EPPO Bulletin* **42**: 515-551.

BARNES, B.N. 2000. Monitoring and control of fruit flies in South African fruit orchards. 147-152 pp. In: Price, N.S. & Seewooruthun, I. (Eds.). Proceedings of the Indian Ocean Commission. Regional Fruit Fly Symposium, 5-9 June 2000, Mauritius. Indian Ocean Commission, Mauritius, Flic en Flac.

BARRY, G.H. 1996. Citrus production areas of Southern Africa. *Proceedings of the International Society of Citriculture* **1**:145-149.

BEGON, M., TOWNSEND, C.R. & HARPER, J.L. 2006. *Ecology: From Individuals to Ecosystems.* 30-31. Blackwell Publishing, Oxford.

CGA. 2018. Annual Report 2018. *Citrus Growers Association of Southern Africa*. 45. KwaZulu-Natal, South Africa.

DE MEYER, M. 1996. Revision of the subgenus *Ceratitis* (*Pardalaspis*) Bezzi, 1918 (Diptera, Tephritidae, Ceratitini) *Systematic Entomology* **21**: 15-26.

DE MEYER, M. 1998. Revision of the subgenus *Ceratitis* (*Ceratalaspis*) Hancock (Diptera: Tephritidae). *Bulletin of Entomological Research* **88**: 257-290.

DE MEYER, M. 2001. Distribution patterns and host-plant relationships within the genus *Ceratitis* MacLeay (Diptera: Tephritidae) in Africa. *Cimbebasia* **17**: 219-228.

DE MEYER, M. & COPELAND, R.S. 2005. Description of new *Ceratitis* Macleay (Diptera: Tephritidae) species from Africa. *Journal of Natural History* **39**: 1283-1297.

DE MEYER, M., DELATTE, H., EKESI, S., JORDAENS, K., KALINOVÁ, B., MANRAKHAN, A., MWATAWALA, M., STECK, G., VAN CANN, J., VANÍČKOVÁ, L., BŘÍZOVÁ, R. & VIRGILIO, M. 2015. An integrative approach to unravel the *Ceratitis* FAR (Diptera, Tephritidae) cryptic species complex: a review. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 405-427.

DE MEYER, M. & FREIDBERG, A. 2006. Revision of the subgenus *Ceratitis (Pterandrus)* Bezzi (Diptera: Tephritidae). *Israel Journal of Entomology* **36**: 197-315.

DE MEYER, M., MWATAWALA, M., COPELAND, R.S. & VIRGILIO, M. 2016. Description of new *Ceratitis* species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy* **233**: 1-23.

DE MEYER, M., ROBERTSON, M.P., PETERSON, A.T. & MANSELL, M. 2008. Ecological niches and potential geographical distributions of Mediterranean fruit fly (*Ceratitis capitata*) and Natal fruit fly (*Ceratitis rosa*). *Journal of Biogeography* **35**: 270-281.

DE VILLIERS, M., MANRAKHAN, A., ADDISON, P. & HATTINGH, V. 2013. The distribution, relative abundance, and seasonal phenology of *Ceratitis capitata*, *Ceratitis rosa* and *Ceratitis cosyra* (Diptera: Tephritidae) in South Africa. *Environmental Entomology* **42**: 831-840.

DUYCK, P.F., DAVID, P. & QUILICI, S. 2006. Climatic niche partitioning following successive invasions by fruit flies in La Réunion. *Journal of Animal Ecology* **75**: 518-526.

DUYCK, P.F. & QUILICI, S. 2002. Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bulletin of Entomological Research* **92**: 461-469.

ETIENNE, J. 1982. Etude systématique, faunistique et écologique des Tephritidae de la Réunion. *Thèse de l'Ecole Pratique des Hautes Etudes*, Paris, 100 pp.

FAO/IAEA 2018. Trapping guidelines for area-wide fruit fly programmes. In: Enkerlin, W.R. & Reyes-Flores, J. (Eds.). *Food and Agriculture Organization of the United Nations International Atomic Energy Agency*. Second Edition. Rome, Italy, 65 pp.

GEORGALA, M.B. 1964. The response of the males of *Pterandrus rosa* (Karsch) and *Ceratitis capitata* (Wiedemann) to synthetic Lures (Diptera: Trypetidae). *Journal of the Entomological Society of Southern Africa* **27**: 67-73.

GEURTS, K., MWATAWALA, M. & DE MEYER, M. 2012. Indigenous and invasive fruit fly diversity along an altitudinal transect in Eastern Central Tanzania. *Journal of insect Science* **12**: 12.

GROUT, T.G., DANEEL, J.-H., WARE, A.B. & BECK, R.R. 2011. A comparison of monitoring systems used for *Ceratitis* species (Diptera: Tephritidae) in South Africa. *Crop* Protection. **30**: 617-622.

GROUT, T.G. & MOORE, S.D. 2015. Citrus. In: Prinsloo, G.L. & Uys, V.M. (Eds.) *Insects of Cultivated Plants and Natural Pastures in Southern Africa.* 448–499 pp. Entomological Society of Southern Africa, Hatfield, Pretoria, South Africa.

GROVÉ, T. & DE BEER, M.S. 2019. Species composition and abundance of fruit flies (Diptera: Tephritidae) in subtropical fruit orchards in the Mbombela local municipality, South Africa. *Fruits* **74**: 18-24.

KARSTEN, M., ADDISON, P., JANSEN VAN VUUREN, B. & TERBLANCHE, J.S. 2016. Investigating population differentiation in a major African agricultural pest: evidence from geometric morphometrics and connectivity suggests high invasion potential. *Molecular Ecology* **25**: 3019-3032.

KOUNATIDIS, I., PAPADOPOULOS, N.T., MAVRAGANI-TSIPIDOU, P., COHEN, Y., TERTIVANIDIS, K., NOMIKOU, M. & NESTEL, D. 2008. Effect of elevation on spatio-temporal patterns of olive fly (*Bactrocera oleae*) populations in northern Greece. *Journal of Applied Entomology* **132**: 722-733.

MANRAKHAN, A. 2019. Fruit fly. In: T.G. Grout (Ed.) *Integrated Production Guidelines for export citrus. Integrated pest and disease management.* 1-10. Citrus Research International, Nelspruit, South Africa.

MANRAKHAN, A., DANEEL, J.-H., BECK, R., VIRGILIO, M., MEGANCK, K. & DE MEYER, M. 2017a. Efficacy of trapping systems for monitoring of Afrotropical fruit flies. *Journal of Applied Entomology* **141**: 825-840

MANRAKHAN, A., DANEEL, J.-H., VIRGILIO, M. & DE MEYER, M. 2017b. Sensitivity of an enriched ginger oil based trapping system for *Ceratitis* fruit fly pests (Diptera: Tephritidae). *Crop Protection* **99**: 26-32.

MWATAWALA, M.W., VIRGILIO, M., DOMINIC, M. & DE MEYER, M. 2013. Field evaluation of the relative attractiveness of EGOlure and trimedlure for African *Ceratitis* species (Diptera: Tephritidae). *Journal of Applied Entomology* **137**: 392-397.

MWATAWALA, M.W., VIRGILIO, M., JOSEPH, J. & DE MEYER, M. 2015. Niche partitioning among two *Ceratitis rosa* morphotypes and other *Ceratitis* pest species (Diptera, Tephritidae) along an altitudinal transect in Central Tanzania. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *Zookeys* **540**: 429-442.

NAKAGAWA, S., HARRIS, E.J. & KEISER, I. 1981. Performance of Capilure® in capturing Mediterranean fruit flies Steiner plastic or cardboard sticky traps. *Journal of Economic Entomology* **74**: 244-245.

NORMAND, F., QUILICI, S. & SIMIAND, C. 2000. Seasonal occurance of fruit flies in strawberry guava (*Psidium cattleianum* Sabine) in Réunion Island: host phenology and fruit infestation. *Fruits* **55**: 271-281.

ORIAN, A. J. E. & MOUTIA, L. A. 1960. Fruit flies (Trypetidae) of economic importance in Mauritius. *Revue Agricole et Sucriere de l'Ile Maurice* **39**:142-150

PEEL, M.C., FINLAYSON, B.L & MCMAHON, T.A. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* **11**: 1633-1644.

QUILICI, S. 1989. Aménagement de la lutte chimique contres les mouches des fruits à la Réunion. In: Cavalloro, R. & Balkema, A.A. (Eds.) Fruit Flies of Economic Importance. *Proceedings of the CEC/ IOBC International Symposium*, 7-10 April 1987, Rome, Italy. Rotterdam, Netherlands. 515-524 pp.

QUILICI, S., SCHMITT, C., VIDAL, J., FRANCK, A. & DEGUINE, J.-P. 2013. Adult diet and exposure to semiochemicals influence male mating success in *Ceratitis rosa* (Diptera: Tephritidae). *Journal of Applied Entomology* **137**: 142-153.

SEGURA, F.D., VERA, M.T.,CAGNOTTI, C.L., VACCARO, N., DE COLL, O., OVRUSKI, S.M. & CLADERA, J.L. 2006. Relative abundance of *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) in diverse host species and localities of Argentina. *Annals of the Entomological Society of America* **99**: 70-83.

SHELLY, T.E. & PAHIO, E. 2002. Relative attractiveness of enriched ginger root oil and trimedlure to male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologists* **85**: 545-551.

TANGA, C.M., MANRAKHAN, A., DANEEL, J.-H., MOHAMED, S.A., FATHIYA, K. & EKESI, S. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis*

rasa Karsch (Diptera, Tephritidae) populations from Kenya and South Africa. In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of cryptic species complexes of Tephritid pests to enhance SIT application and facilitate international trade. *Zookeys* **540**: 467-487.

VIRGILIO, M., BACKELJAU, T., BARR, N. & DE MEYER, M. 2008. Molecular evaluation of nominal species in the *Ceratitis fasciventris*, *C. anonae* and *C. rosa* complex (Diptera: Tephritidae). *Molecular Phylogenetics and Evolution* **48**: 270-280.

VIRGILIO, M., DANEEL, J.-H., MANRAKHAN, A., DELATTE, H., MEGANCK, K. & DE MEYER, M. 2019. An integrated setup for the morphological and molecular identification of the *Ceratitis* FAR complex (*C. anonae, C. fasciventris, C. rosa, C. quilicii*, Diptera, Tephritidae). *Bulletin of Entomological Research* **109**: 376-382.

VIRGILIO, M., DELATTE, H., QUILICI, S., BACKELJAU, T. & DE MEYER, M. 2013. Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa, Ceratitis fasciventris* and *Ceratitis anonae* (Diptera, Tephritidae). *Molecular Ecology* **22**: 2526-2539.

WHITE, I.M. 2006. Taxonomy of the Dacina (Diptera: Tephritidae) of Africa and the Middle East. *African Entomology*, *Memoir No.* **2**: 1-156.

CHAPTER 4. DEVELOPMENT AND SURVIVAL OF *CERATITIS ROSA* AND *CERATITIS QUILICII* IN CITRUS FRUIT

4.1. Introduction

Ceratitis rosa Karsch, is an Afrotropical fruit fly with populations also occurring in Mauritius and Réunion (De Meyer 2001). *Ceratitis rosa* is highly polyphagous, attacking a wide range of plant families (Etienne 1982; Quilici 1989; Duyck & Quilici 2002; De Meyer *et al*. 2008; Virgilio *et al*. 2013; De Meyer *et al*. 2016; Hafsi *et al*. 2016) of commercial and non-commercial fruit (White and Elson-Harris 1992). This species is a pest of quarantine importance (Badii *et al.* 2015) infesting various citrus species (Rutaceae), such as *Citrus paradisi* Macfad, *Citrus reticulata* Blanco and *Citrus sinensis* (L.) Osbeck (De Meyer *et al.* 2002).

South Africa is the second largest exporter of citrus in the world, exporting 1.9 x 10^6 tons of citrus fruit in 2018 (CGA 2019). In South Africa, citrus contributes R19.1 billion to the total gross value of horticulture valued at R57.3 billion and, being labour intensive, employs more than a 100 thousand people (DAFF 2018a). Fruit flies (Diptera: Tephritidae) are a great threat to the citrus industry due to restrictions they pose on market access (Manrakhan 2019).

Quarantine procedures are designed specifically to reduce propagule pressure of undesirable species (De Meyer *et al*. 2008). Phytosanitary treatments are normally targeted at the larval stage, which is probably the least studied life stage of fruit flies, yet the most important when exporting fruit (Balmès & Mouttet 2017). One of the ways to ensure a pest free product to the export markets is by employing phytosanitary treatments such as cold, heat or irradiation (Aphis 2016; Dohino *et al*. 2016; Al-Behadili *et al*. 2019).

Non-chemical treatments such as cold treatments are becoming more favourable as postharvest treatments against Lepidoptera and Diptera pests because there are no chemical residues that remain on the fruit after treatment. Cold treatments can be applied at any time after packing and during transit and can be used in combination with other treatments (Follett & Snook 2013). Cold treatments target immature stages of fruit infesting pests. Studies in South Africa showed that cold treatments against *Ceratitis capitata* (Wiedemann), are also effective against *C. rosa* s.l. (Ware *et al*. 2004a,b). In citrus, a 16-day cold treatment at < 1.4 °C is effective for control of *C. capitata* (Grout *et al*. 2011b).

The thickness of citrus peels, together with the rind oils that it contains, acts as a barrier against invasions by fruit flies (Bateman 1972; Díaz-Fleischer & Aluja 2003; Manrakhan 2019). Manrakhan *et al*. (2018) reported that no fruit fly infestation was detected from 43 222 fruit that

were sampled and concluded that Eureka Lemon was a non-host for *C. capitata*, *C. rosa*, *C. quilicii* and *B. dorsalis* in South Africa, even though adults were present in the orchards at the time of the study. However, detached or damaged citrus would be susceptible to infestation (White & Elson-Harris 1992). In cases where a female fruit fly lays eggs inside broken peels, larvae may develop successfully. In such cases the possible result could be a difference in performance of the immature larvae in different citrus types, since citrus types differ in their host suitability (Papachristos *et al*. 2008).

Using molecular and morphological characters, Virgilio *et al*. (2013) confirmed two morphotypes of *C. rosa*: R1 and R2. This result together with other research results obtained led to the split of *C. rosa* into two species: *C. rosa* (R1) and *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio (R2) (De Meyer *et al.* 2016). The recent description of *C. quilicii* resulted in several new questions regarding its biology and ecology, as well as management and control methods. All previous research, pre-harvest results (such as monitoring, threshold values and control in the orchards), and postharvest results (various phytosanitary treatments), became questionable, due to the ambiguity of which species were used in those studies. Currently, no knowledge exists on which of the two species might be problematic to the citrus industry, if not both. Since Papachristos *et al*. (2008) indicated that the physiochemical characteristics of fruit influences the development and survival of pupae, and since there is evidence that the two species might have different thermal requirements (Duyck & Quilici 2002; Grout & Stoltz 2007; Tanga *et al*. 2015), it is important to determine the comparative development rates of these species in different citrus types at a constant temperature to determine which citrus type is the most suitable host to continue future cold sterilization trials with. Furthermore, it is also important to investigate whether *C. quilicii* and *C. rosa* have different developmental and survival rates in different fruit. Citrus is currently not listed as a host for *C. quilicii* (De Meyer *et al.* 2016) but this list may change as more information is gathered on this newly described species.

The objectives of this study were to determine the development and survival of *C. quilicii* and *C. rosa* in different citrus types. This study also forms the basis, Phase 1 as described by Grout *et al.* (2011a), for the development or validation of cold post harvest treatment for these two fly species.

4.2. Material and Methods

This study was carried out by inoculating fruit of four citrus fruit types with eggs of insectary reared *C. quilicii* and *C. rosa* in order to determine the developmental rate and survival of immatures (eggs, each larval instar, and pupae) of each fruit fly species.

4.2.1. Environment

The study was conducted at the Citrus Research International (CRI) premises in Nelspruit, Mpumalanga, South Africa, during July to October 2016. A temperature controlled room, with a photoperiod L12: D12 was used for incubation of fruit used in the study. Temperature in the room was monitored on an hourly basis using an ibutton, (Maxim ibutton, Fairbridge Technologies, (PTY) Ltd., Sandton, South Africa). Average air temperatures recorded during trials on different citrus types are provided in Table 1. An oscillating desk fan was used to create airflow over the fruit and to keep any unwanted secondary pests such as vinegar flies (Diptera: Drosophilidae) at bay.

Table 1. Mean temperatures recorded in the incubation room where fruit were kept during the trial for the control fruit and the dissection fruit.

Mean temperature (°C ± SE) in the incubation room for the control fruit			
Eureka	**Star Ruby**	**Nadorcott**	**Late Valencia**
26.35 ± 0.05	26.50 ± 0.04	26.39 ± 0.05	26.18 ± 0.04
Mean temperature (°C ± SE) in the incubation room for the fruit kept to be dissected			
Eureka	**Star Ruby**	**Nadorcott**	**Late Valencia**
26.31 ± 0.07	26.36 ± 0.06	26.76 ± 0.06	26.06 ± 0.06

4.2.2. Fruit

Four citrus export grade fruit types were sourced from growers, depending on seasonal availability. These were lemon (*Citrus limon* (L.) Burman f. cv. Eureka) (hereafter referred to as Eureka), grapefruit (*Citrus paradisi* cv. Star Ruby) (hereafter referred to as Star Ruby), mandarin (*Citrus reticulata* cv. Nadorcott) (hereafter referred to as Nadorcott), and Valencia (*Citrus sinensis* cv. Late Valencia) (hereafter referred to as Late Valencia). The first available fruit, Eureka, was received on 17 June 2016 from Vutsela Iglobhu Investments (Pty) Ltd, (Ryton Estates), Star Ruby was received on 27 June 2016 from Golden Frontiers (Vergenoeg), Nadorcott, on 27 July 2016 from Indigo Fruit (Pty) Ltd, trading as Larten, and Late Valencia, received on 16 August 2016 from Crocodile Valley Estates.

The fruit used in these experiments were neither waxed nor exposed to any other packhouse treatments. Upon arrival at the CRI facilities in Nelspruit, all fruit were dipped into water baths that contained mixtures of fungicides and sanitizers: Citricure (210 g/ l guazatine, ICA International Chemicals Pty. Ltd.), Imazacure (imidazole 750 g/ kg, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa), Sporekill (120 g/ l didecyldimethylammonium chloride, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa), TBZ

(thiabendazole 500 g/ l, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa) and Protector (pyrimethanil 400 g/ l, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa). Different combinations were used for different citrus types. The combinations used were Citricure (4.8ml/ l) + Sporekill (1ml/ l), Citricure (4.8ml/ l) + Sporekill (1ml/ l) + Imazacure (0.67g/ l), Citricure (4.8ml/ l) + Protector (2.5 ml/ l) + TBZ (2ml/ l), Citricure (9.6ml/ l) + Sporekill (2ml/ l) + Imazacure (1.34g/ l), and Citricure (4.8ml/ l) + Sporekill (1ml/ l) + Protector (2.5 ml/ l). All treatments were done in 70 l plastic containers, big enough to facilitate the dipping of a lug box containing fruit. These treatments were done to protect fruit from fungal decay during storage and the experimental phase. All fruit were inspected to ensure that there were no signs of any previous infestations by dipterans, or any physical damage that would allow secondary infestations by pests such as vinegar flies. The dipped fruit were placed into the incubation room the day before the inoculation in order to allow the fruit to warm up to the target incubation temperature of 26°C ± 1 °C, to minimize the potential effect of colder or warmer fruit when the fruit were inoculated.

To determine the external and internal quality of the fruit used during the trial, twelve fruit were randomly selected for each citrus type and for every replicate (in total 36 fruit per citrus type). These fruit were not inoculated with fruit fly eggs. The fruit were graded according to their colour using Set Numbers 37 (Eureka), 35C (Star Ruby), 36 (Nadorcott), 34 (Late Valencia) of the Colour Prints for Blemish and Appearance Standards (1997), published by CRI, endorsed by the National Department of Agriculture, Directorate: Food safety and Quality Assurance described in CRI (1995). Each fruit were weighed on an analytical balance (Shimadzu ELB3000, Roodepoort, South Africa) and their equatorial diameter determined using a fruit size caliper. The fruit were then peeled and the thickness of the skin determined using a Vernier caliper. The fruit were then sent to the Citriculture Laboratory, Citrus Research International, Nelspruit, South Africa, for an internal quality test to establish the percentage extractable juice (%), Brix (°) and acidity (%) and to determine the pH content of the fruit (DAFF 2018b).

4.2.3. Insect rearing

Laboratory-reared *C. rosa* and *C. quilicii* were used in this study. Both species were reared in gauze cages at ambient temperatures, inside rooms with the walls consisting of either fine meshed screens or windows, so that the fruit fly cultures had exposure to natural light conditions (Figure 1), to allow for the adult flies' requirement for crepuscular conditions (natural dusk conditions) (Grout & Stoltz 2007). The *C. rosa* culture was renewed in September 2015 by adding flies reared from *Eriobotrya japonica* (Thunb.) Lindl. (Family: Rosaceae), collected in Nelspruit, South Africa. The *C. quilicii* culture was first renewed by flies reared from

Eriobotrya japonica collected in Pretoria during September 2015, and again from *Acca sellowiana* (O. Berg) Burret (Family: Myrtaceae), collected during February 2016 from Pretoria, South Africa. The adult flies were transferred to the insectaries where they were reared on a carrot-based diet.

Figure 1. Fruit fly cultures of *Ceratitis rosa* and *Ceratitis quilicii* were kept in rearing rooms

Fruit fly eggs for use in bioassays were obtained by placing apples (*Malus domestica* Borkh cv. Granny Smith) inside the mesh cages the day before the start of bioassays. The apples were removed from cages on the day of the inoculation, to ensure that the eggs were never older than 24 hours.

Each apple was punctured four times around its equator with a small pinning device (consisting of eight 6 mm x 0.8 mm diameter pins placed in a row, 2.5 mm apart), to create wounds for females to lay their eggs in and to facilitate easy recovery of eggs according to methods described by Grout & Stoltz (2007). Four males and four females from each of the two fly species were collected from the adult culture (the same culture from where the eggs were collected), and placed in 1.5 ml Boilproof tubes filled with absolute alcohol, to serve as voucher specimens of the test insects as recommended by PMRG (2019). Voucher specimens are kept at CRI.

4.2.4. Inoculation procedure

During inoculation, 20 aliquot samples (0.025 ml per aliquot) of eggs of each fruit fly species were placed on a moistened black cloth inside two separate 90 mm Petri dishes and each aliquot counted to determine how many eggs were placed inside each fruit. Similar aliquots were placed on five Petri dishes per species, to allow the eggs to hatch and to confirm the viability of the eggs after 72 hours.

The inoculation always commenced with *C. quilicii.* On the day of an inoculation a hole of 6 mm in diameter and 20 mm deep was made at the calyx end of each fruit, using a cork borer. After inoculation with eggs of *C. quilicii* was completed, fruit were stored and all equipment washed, before inoculation with *C. rosa* commenced to avoid contamination between the two species. It was estimated that 1 ml of eggs of each species contained approximately 15000 eggs. In order to inoculate 35 eggs into each fruit, 1 ml of eggs was placed into 10.71 ml of water. The water added was adjusted according to the available volume of eggs on the inoculation day. A 0.025 ml aliquot of this egg-water mixture was pipetted into the hole in each fruit. The mixture was always well agitated before an aliquot was drawn to ensure a homogenous mixture. Prior to placement of eggs, Torula yeast (Organic World, Randburg, South Africa), mixed with freshly boiled distilled water in a 1:2 ratio, was added (0.2 to 0.5 ml) into the hole to serve as a food source for the developing larvae (Grout *et al*. 2011b). The hole containing the eggs was filled with a piece of cotton wool and sealed with microwax (Sasolwax 7835, Sasol South Africa (PTY) Ltd., Sasolburg, South Africa) to prevent infestation by other arthropods or fungal infections.

For each fruit type and each fly species, 130 individual fruit were used. Of these, 105 were dissected and 25 served as a control treatment to determine adulthood development. Each fruit for dissection was enclosed individually in a brown paper bag to avoid any fungal growth from spreading to other fruit. Inoculated fruit of each citrus type with the different fly species were randomly selected and placed in crates according to day of dissection. The crates were then covered with shade cloth to further protect them from any secondary infections by other dipterans. The 25 fruit used as control were incubated in bulk on sterilised sand in 9 l aerated plastic containers to facilitate pupation of the jumping larvae. The control fruit were used to determine development period and success rate from eggs to adulthood. All inoculated fruit were kept in the incubation room. The above 130 fruit per citrus type was considered a replicate. For each citrus type, there were three replicates conducted with three separate batches of eggs.

4.2.5. Assessment of development and survival

Seven fruit inoculated with each species were dissected daily for 15 days, commencing one day after inoculation. During dissection, live and dead larvae were counted and recorded.

All live larvae from each fruit were collected and kept separate for determination of larval instar and measurement of length. In order to preserve larvae in a good state for subsequent analyses, larvae were killed in boiling hot water and placed in 70% ethanol. Larvae were kept in small microcentrifuge tubes (1.5 ml) with caps. Larvae collected each day, were placed on slides and inspected under a stereomicroscope (10 – 25x magnification) (Leica EZ 4 D, Leica Microsystems Ltd, Heerbrugg, Switzerland) to photograph each individual larva. Mouth part characteristics (presence or absence of pre-apical tooth), presence of a shed mouth hook, and body length were used to determine the larval stage (White & Elson-Harris 1992; Carroll 1998; Steck & Ekesi 2015).

For fruit incubated as control, sand was sieved each day from five days after inoculation onwards, until no more pupae were recovered. All pupae were weighed individually using a Mettler Toledo New Classic MS scale (Microsep (PTY) Ltd, Sandton, South Africa) after which they were placed on sand in Petri dishes. One half of the bottom of the Petri dish was covered with a thin layer of sand and the other half was covered by a piece of filter paper, cut in a semi-circle. The contents of each Petri dish was moistened daily with distilled water to avoid desiccation of the pupae. Emerging adults were allowed to age and to complete full colouring before sex was recorded.

4.2.6. Statistical analysis

The number of larvae collected per day were averaged for each species, fruit type and replicate. For each day after inoculation, the numbers of different instars was recorded to calculate development rate. For each fruit fly species in each citrus type, total gross larval survival rates were calculated by dividing the total number of larvae recovered from a fruit over the 15 days by the total number of eggs inoculated into the fruit. Total net larval survival rates were calculated by dividing the total number of larvae by total number of viable eggs (hatched eggs). When determining the larval survival rate of the two species, the 3rd replicate of the Late Valencia trial was not included in the analysis due to no larvae recorded for *C. quilicii* from day 10 onwards, and for *C. rosa* from day 8 onwards. The absence of larvae could have been due to high fungal growth. The percentage of the different instars per fruit per day was determined. For each instar and citrus type, mean body length was calculated for each fly species. For fruit that were set as control, the larval development was determined by recording the number of jumping larvae per day after inoculation until 27 days after inoculation. Pupal

mass was also recorded separately for each citrus type and the mean pupal mass was analysed as per species per cultivar.

The data were analysed using SPSS Statistics Version 25. The effect of citrus type on larval length was analysed by applying a two-way analysis of variance (ANOVA).

A three-way ANOVA was used to determine the effects of fruit fly species, citrus type and time on survival of all the larval stages, from day 2 until day 15. A three-way ANOVA was used to determine the effects of fruit fly species, citrus type and time on survival of only mature larvae recorded over the last 6-day period. Mean pupal mass was compared between the citrus types and fruit fly species by means of a two-way ANOVA.

4.3. Results

4.3.1. Fruit condition and internal quality

Different fruit conditions, internal quality of the fruit, or fruit that is too green or too ripe, may enhance or delay development and survival of the larvae in the fruit. Cold sterilization trials were conducted on export quality fruit and therefore all fruit used in this trial were selected with this as the standard. The internal quality of the fruit was evaluated against the Minimum Standards, Standards and Requirements for Citrus, No. 634 (DAFF 2018b) and found to be satisfactory.

Results on the external and internal characteristics of the four citrus types are provided in Table 2. Fruit of Star Ruby were the largest. Nadorcott fruit had the thinnest rind. Star Ruby, Nadorcott and Valencia fruit, had higher juice content and Brix-values. Acidity value for Eureka was much higher than that of other cultivars.

Table 2. External and internal characteristics of the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia) used in the trial. Fruit that were ready to be harvested, internally and externally mature, and of export quality were selected for determining these parameters. Colour grades were determined according to the scale of Grade 1 = fully coloured fruit to Grade 8 = totally green fruit.

Citrus types	Date tests were conducted	Site	Mean fruit size (mm)	Juice (%)	Brix (°)	Acid (%)	Ratio (Brix to Acid)	pH	Mean rind thickness (mm)	Mean weight (g)	External colour grade
Eureka	2016/07/06	Ryton	74.3	45.82	8.8	9.49	1.0	2.32	5.2	227.7	1
Star Ruby	2016/07/08	Vergenoeg	87.4	51.73	12.8	1.36	9.4	3.13	5.3	274.0	2
Nadorcott	2016/08/02	Larten	74.8	55.46	13.8	1.16	11.9	3.34	3.8	141.4	1
Late Valencia	2016/08/31	Croc Valley	78.2	55.56	11.6	1.27	9.2	3.61	5.0	212.7	1

4.3.2. Egg viability

Egg viability was tested outside the fruit by placing aliquots of an egg and water mixture on a black cloth in Petri dishes on the day of the inoculation. Mean egg hatch rates were lower for *C. quilicii* compared to *C. rosa* except for when trials were conducted with the Late Valencia fruit when *C. quilicii* was higher than *C. rosa* (Table 3).

Table 3. Inoculation dates, average number of eggs and average percentage egg hatch in the aliquots placed on the Petri dishes during each inoculation. All eggs used during the inoculations were less than 23 hours old.

		Ceratitis quilicii		***Ceratitis rosa***	
Citrus types	**Date of inoculation**	**Mean number of eggs**	**Mean % hatched**	**Mean number of eggs**	**Mean % hatched**
Eureka	2016/07/04 2016/07/06 2016/07/09	34.17 ± 2.32	54.64 ± 3.27	35.93 ± 1.94	87.47 ± 1.06
Star Ruby	2016/07/18 2016/07/20 2016/07/22	36.15 ± 1.83	60.95 ± 2.73	37.30 ± 2.25	79.28 ± 1.54
Nadorcott	2016/08/02 2016/08/08 2016/08/10	39.83 ± 2.05	60.73 ± 2.33	44.07 ± 2.68	74.97 ± 2.19
Late Valencia	2016/09/16 2016/09/21 2016/09/23	40.33 ± 2.19	61.87 ± 1.56	44.50 ± 1.91	60.74 ± 2.52

4.3.3. Larval development

Larval development of both species was influenced by citrus type. The 1st instar larvae of both fly species developed slower in Eureka compared to Star Ruby and Nadorcott (Figure 2). *Ceratitis rosa* was one day later than *C. quilicii* in Late Valencia (Figure 2D). On day 3, all larvae of both species were at the 1st instar stage in all citrus types (Figure 2). In Nadorcott, no 2nd instar larvae were collected for *C. quilicii* (Figure 3C). The number of 2nd instar larvae of *C. rosa* was lower in Nadorcott compared to other citrus types (Figure 3). For *C. rosa*, development of the 2nd instar larvae were quicker in Star Ruby and for *C. quilicii* development

were quicker in Late Valencia fruit compared to the other two citrus types (Figure 3). Development of larvae to 3rd instar for both species was the quickest in Star Ruby (Figure 4). Development of larvae to 3rd instar took one day longer for both species in Late Valencia compared to the other citrus types (Figure 4).

There were differences in larval developmental rates of the two species, depending on citrus type and stage of development. *Ceratitis quilicii* developed quicker to 2nd instar larvae than *C. rosa* in Eureka and Late Valencia but were similar to *C. rosa* in Star Ruby (Figure 3). Development of *C. rosa* to third instar was two days faster than *C. quilicii* in Eureka and two days faster than *C. quilicii* in Nadorcott (Figure 4A and C).

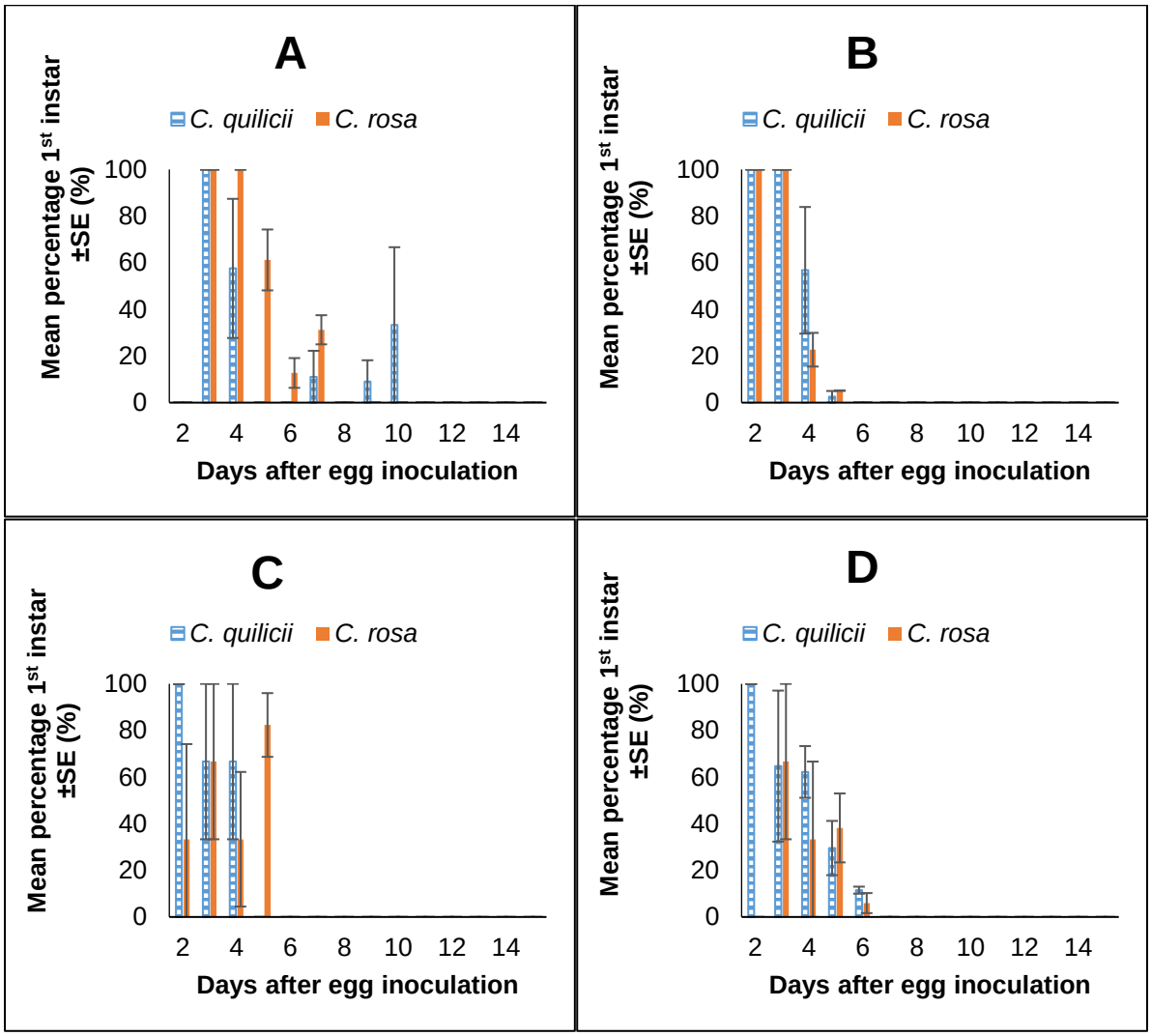

Figure 2. Daily occurrence of first-instar larvae of *Ceratitis quilicii* and *Ceratitis rosa* in fruit of Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) for 15 days after inoculation.

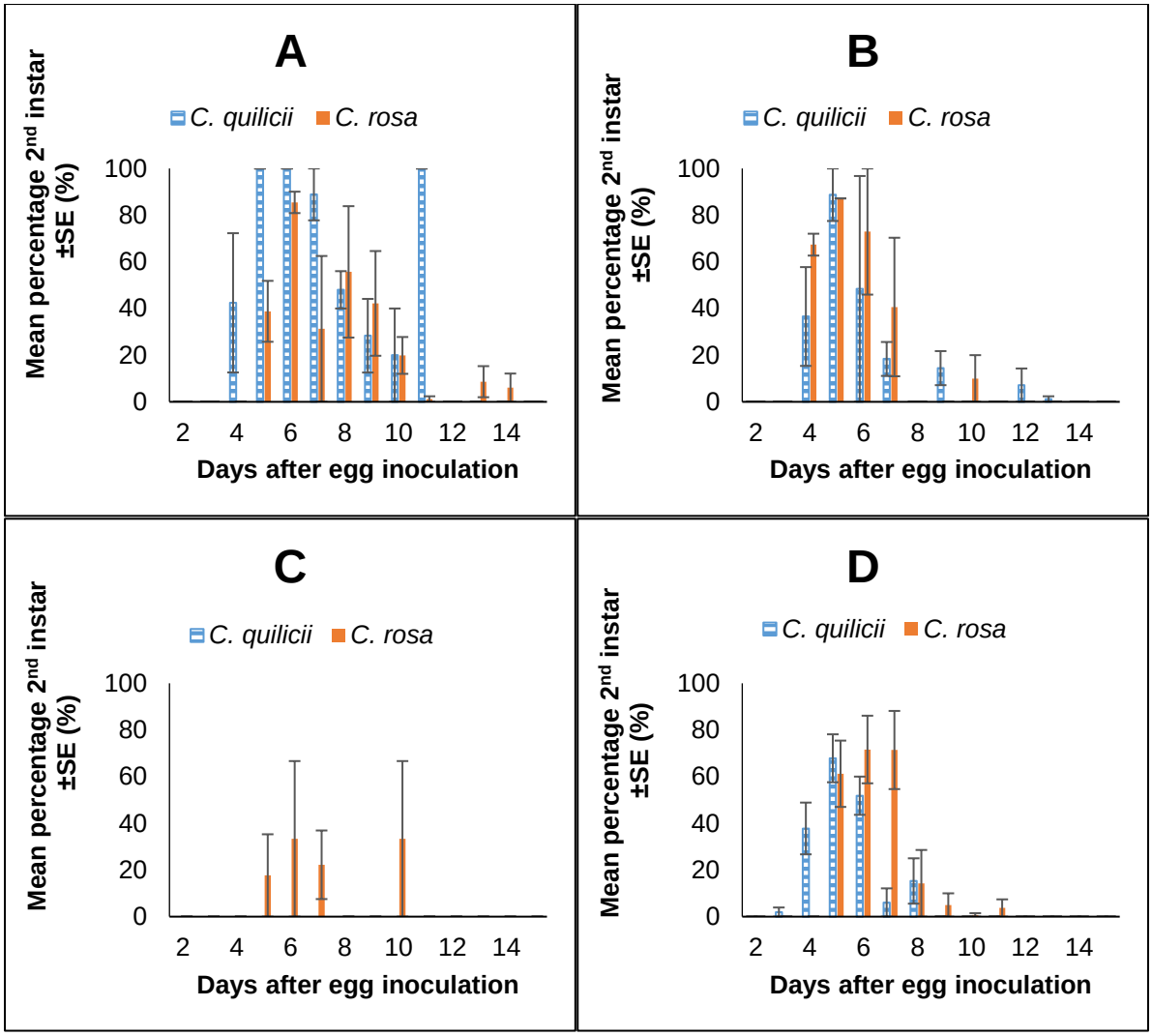

Figure 3. Daily occurrence of second-instar larvae of *Ceratitis quilicii* and *Ceratitis rosa* larvae collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit for 15 days after inoculation.

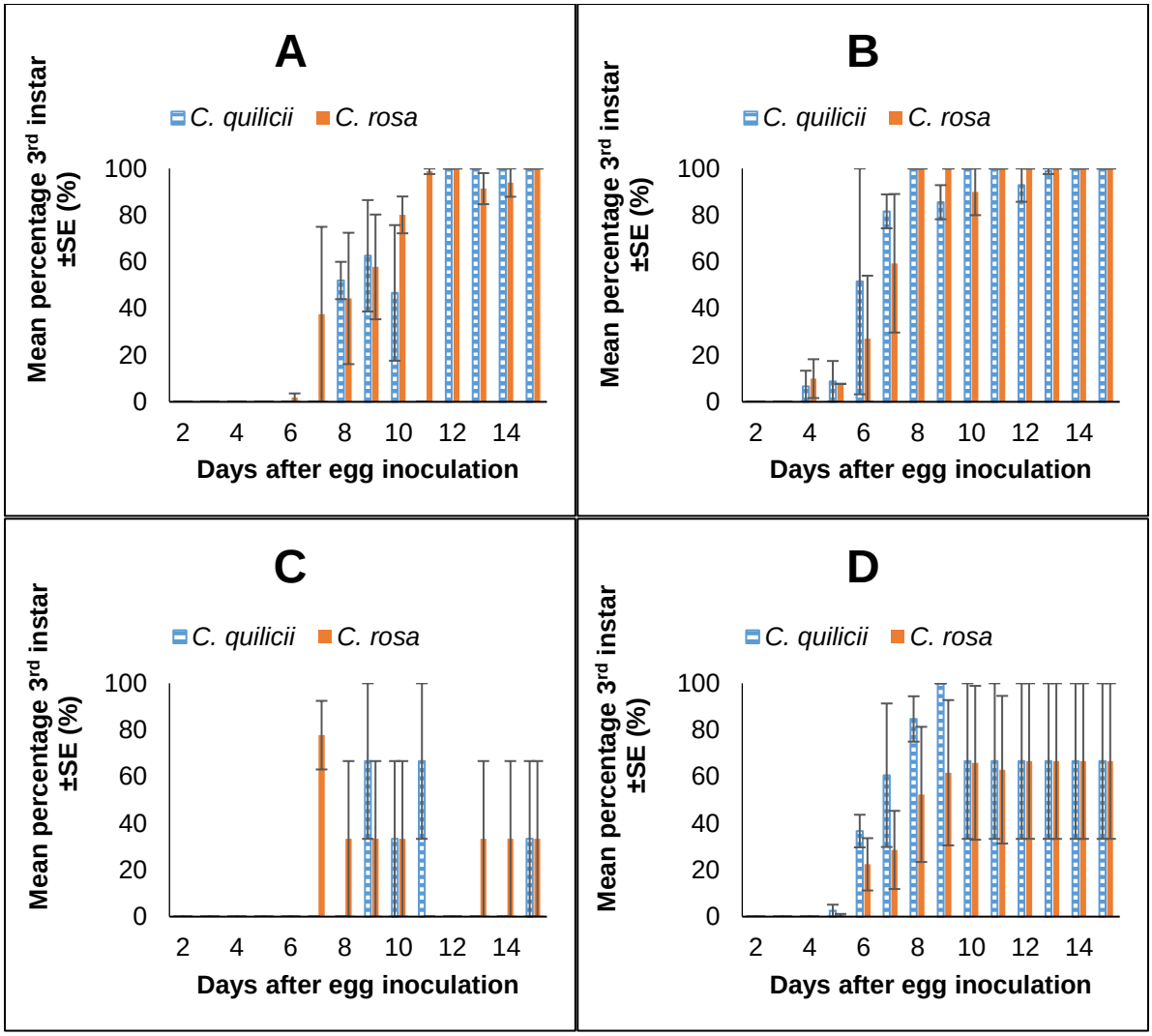

Figure 4. Daily occurrence of third-instar larvae of *Ceratitis quilicii* and *Ceratitis rosa* larvae collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit for 15 days after inoculation.

Larvae collected from the dissected fruit were measured to compare the length of the different instars of the two species from each citrus type. The number of larvae measured are represented in Table 4.

Table 4. Total number of larvae of *Ceratitis quilicii* and *Ceratitis rosa* that were measured after they were reared from different citrus types.

	Citrus types			
Species	**Eureka**	**Star Ruby**	**Nadorcott**	**Late Valencia**
C. quilicii	306	378	60	405
C. rosa	664	430	96	639
Total	970	808	156	1044

There were no differences between the lengths of all stages of the larvae of the two fly species ($P < 0.596$) (Figure 5) but larval sizes of both species differed significantly between citrus cultivars ($P < 0.001$) (Figure 5). Longer 1st instar larvae for both species were collected from Star Ruby (Figure 5). Second instar larvae of *C. quilicii* were also longer in Star Ruby (Figure 5) while 2nd instar larvae of *C. rosa* were longer in Eureka (Figure 5). At the third instar stage, larvae of *C. quilicii* were longer when reared in Nadorcott (Figure 5C). Third instar larvae of *C. quilicii* were, on the other hand, shorter in Star Ruby (Figure 5B). Third instar larvae of *C. rosa* were longer in Late Valencia (Figure 5D) and shorter in Eureka (Figure 5A).

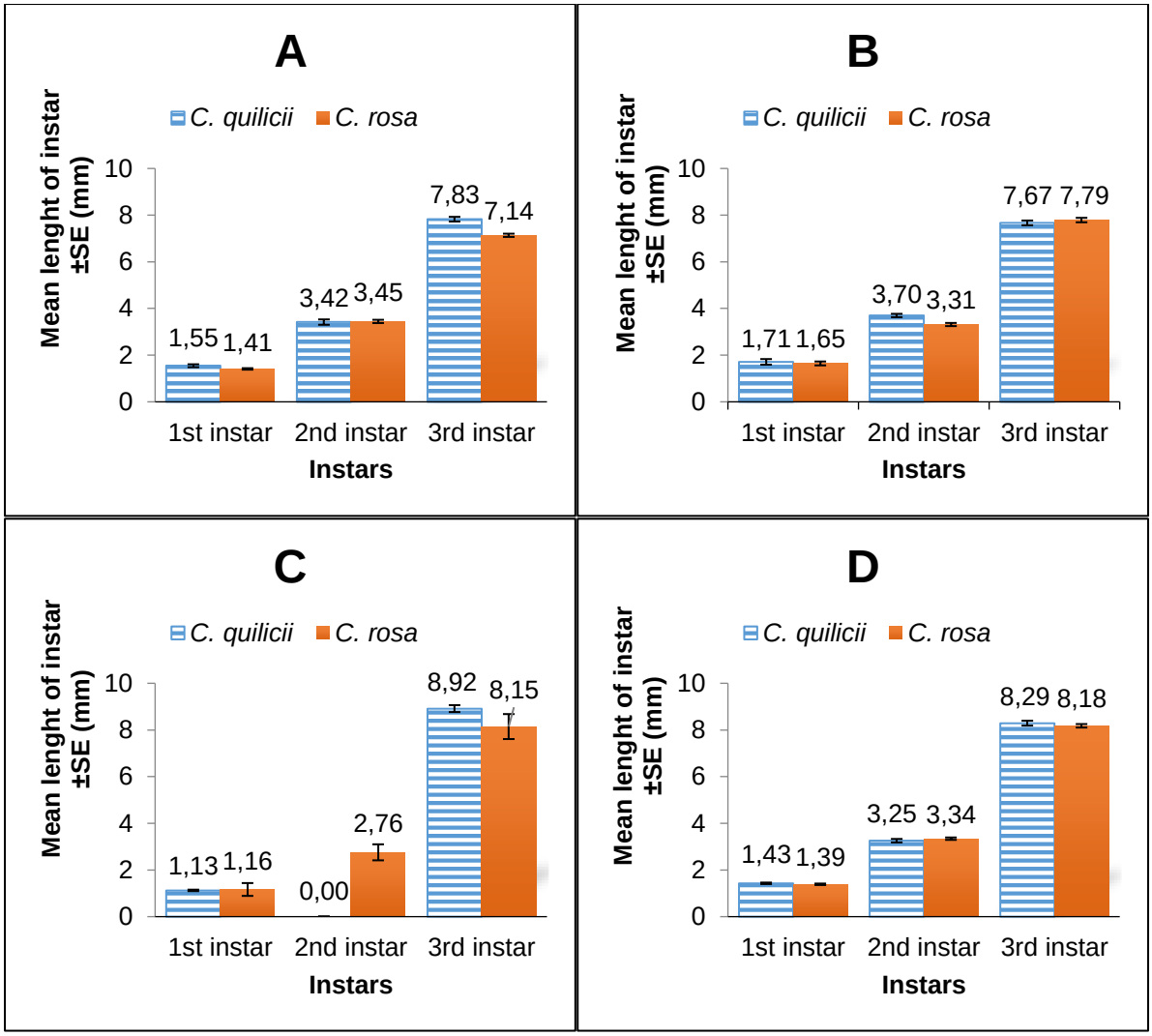

Figure 5. Mean length (mm), indicated above the bars, of *Ceratitis quilicii* and *Ceratitis rosa* larvae at the three development stages collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit over 15 days following inoculation.

4.3.4. Larval survival

There were significant differences in survival rates between days after inoculation ($P = 0.042$), between fly species ($P = 0.004$) and between citrus types ($P < 0.001$) but no significant interaction between fruit fly species and citrus types ($P = 0.052$) and between days, species and citrus type ($P = 0.988$). Larval survival was lower in Nadorcott for both species (Table 5 and Figure 6). The overall larval survival rates were higher for *C. quilicii* and for *C. rosa* in Late Valencia compared to the other three citrus types (Figure 6). In Eureka and Late Valencia, *C. rosa* performed better than *C. quilicii*.

Table 5. A comparison between the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia) indicating the total number of *Ceratitis quilicii* and *Ceratitis rosa* larvae that were collected over time

	Total number of larvae collected							
	Eureka		**Star ruby**		**Nadorcott**		**Late Valencia**	
Day after inoculation	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***	***C. quilicii***	***C. rosa***
1	0	0	0	0	0	0	0	0
2	0	0	1	3	16	2	30	0
3	21	48	6	11	8	24	42	60
4	18	10	28	75	12	13	53	11
5	12	37	41	39	0	22	61	92
6	10	78	32	43	0	1	38	78
7	6	12	66	54	0	9	17	70
8	30	42	12	29	0	1	19	16
9	45	96	60	28	2	9	22	71
10	28	43	36	35	5	10	22	68
11	1	43	14	31	15	0	17	48
12	29	39	15	30	0	0	22	58
13	40	81	51	43	0	2	30	39
14	27	70	1	4	0	1	16	3
15	39	65	15	5	2	2	16	25
Total gross survival	0.028	0.059	0.033	0.037	0.005	0.007	0.032	0.046
Total net survival	0.052	0.067	0.055	0.046	0.008	0.009	0.051	0.073

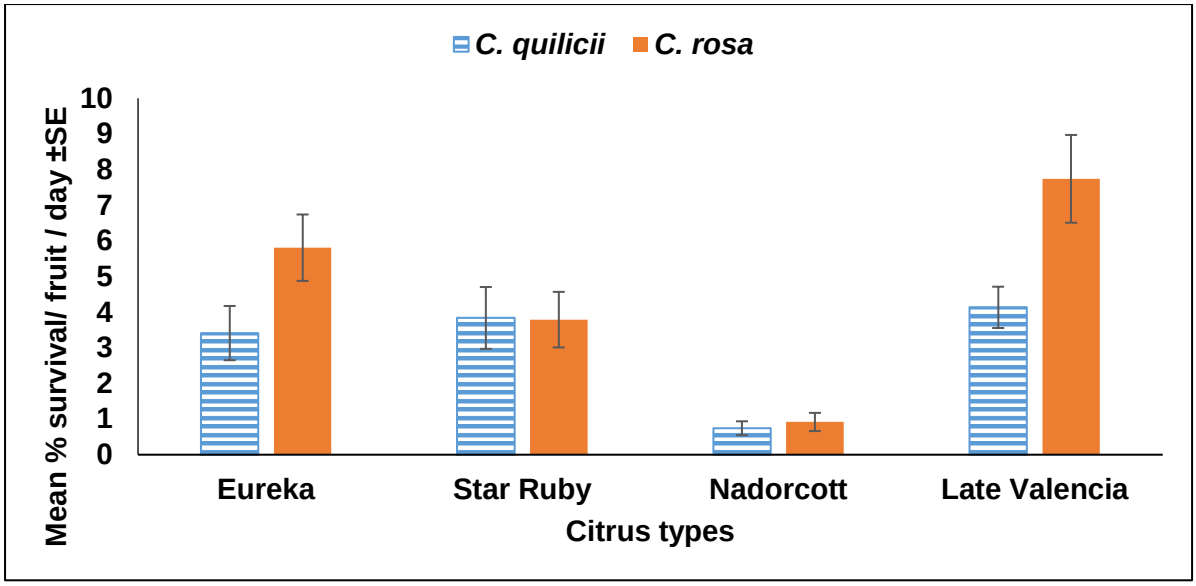

Figure 6. Mean daily larval survival rates of *Ceratitis quilicii* and *Ceratitis rosa* in four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia).

4.3.5. Pupal and adult development

The highest number of jumping larvae and pupae were collected from the Late Valencia fruit, followed by Star Ruby, for both fruit fly species (Table 6). For *C. quilicii* the highest number of adults were reared from Eureka and no *C. quilicii* adults were reared from Nadorcott. The highest number of *C. rosa* adults were reared from Late Valencia, however, adult eclosion was the lowest in the same citrus types. Severe fungal growth was observed in the control fruit and might have influenced the number of flies reared. The fruit were infected by green mould (*Penicillium digitatum* (Pers.: Fr.) Sacc.), sour rot (*Galactomyces citri-aurantii* E.E. Butler) and *Aspergillus* spp. (CRI Diagnostic Centre). *Ceratitis rosa* generally had higher pupal survival rates than *C. quilicii*.

Table 6. Total number of jumping larvae, pupae, adults and percentage eclosion recorded from fruit in the control treatments.

Citrus types	Fly Species	No. of jumping larvae	No. of pupae	No. of Males	No. of Females	Total no. adults	% Adult emergence
Eureka	*C. quilicii*	18	14	2	3	5	35.71
	C. rosa	17	16	4	4	8	50.00
Star Ruby	*C. quilicii*	21	15	1	2	3	20.00
	C. rosa	78	72	3	5	8	11.11
Nadorcott	*C. quilicii*	2	2	0	0	0	0.00
	C. rosa	38	37	2	2	4	10.81
Late Valencia	*C. quilicii*	58	44	1	1	2	4.55
	C. rosa	148	145	1	8	9	6.21

In contrast to results on the development of larvae to the 3rd instar stage in fruit, the period from inoculation to larval jumping was the shortest in Late Valencia for both fruit fly species followed by Star Ruby (Figure 7). With the control fruit, *Ceratitis quilicii* had a faster larval developmental rate than *C. rosa* in Eureka (Figure 7A) and Star Ruby (Figure 7B), but the two fly species had similar developmental times from inoculation to larval jumping in Late Valencia (Figure 7D). Too few *C. quilicii* larvae were collected from Nadorcott to determine any development rate (Figure 7C).

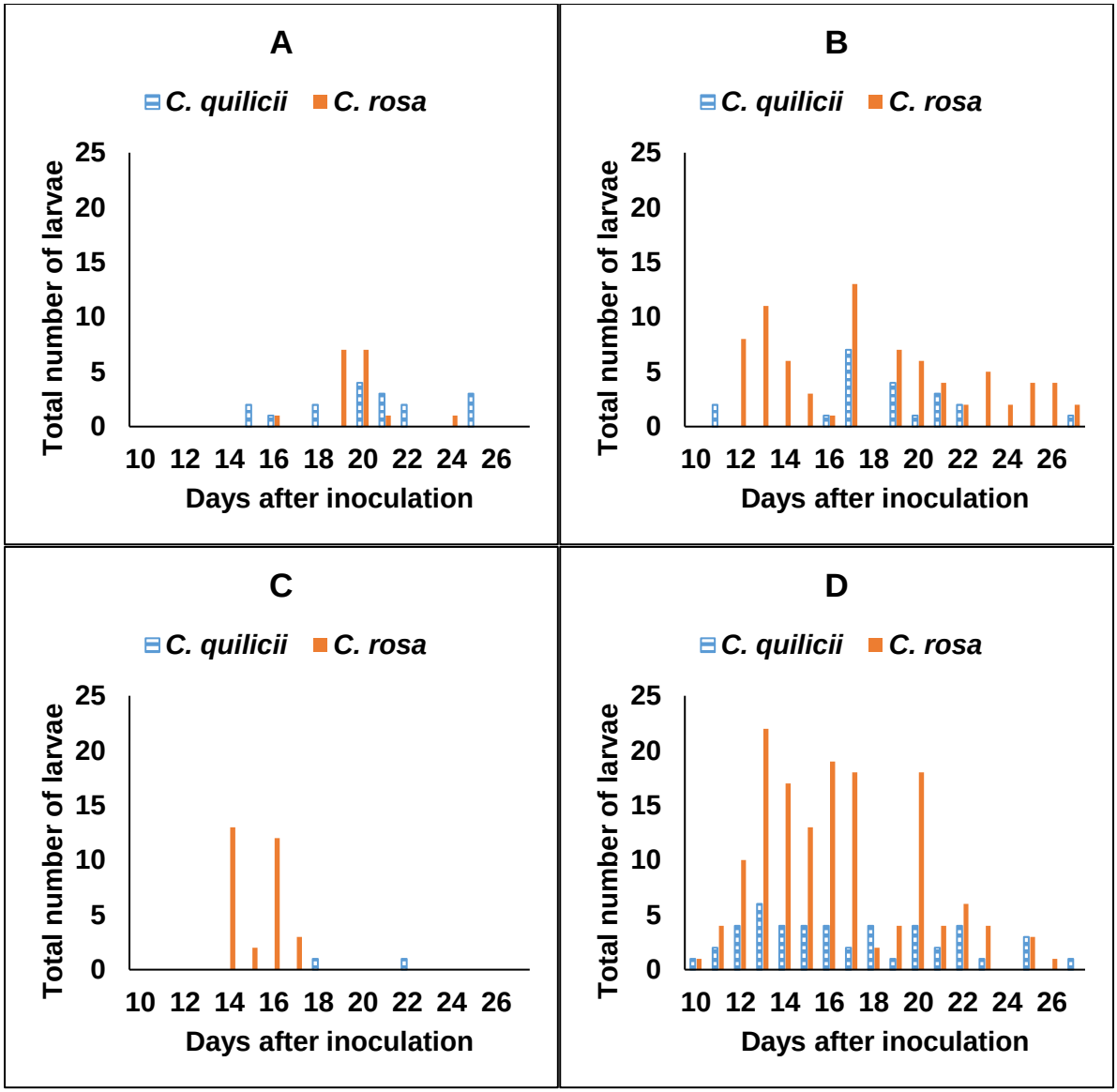

Figure 7. Total number of jumping larvae of *Ceratitis quilicii* and *Ceratitis rosa* collected from Eureka (A), Star Ruby (B), Nadorcott (C), and Late Valencia (D) fruit, each day after inoculation.

There was a significant difference between pupal mass and the different citrus types (P = 0.032) but not between the two fruit fly species (P = 0.118) or between citrus types and species (P = 0.174). While *C. rosa* larvae collected from Nadorcott were the heaviest, *C. quilicii* larvae from Nadorcott fruit were the smallest (Figure 8).

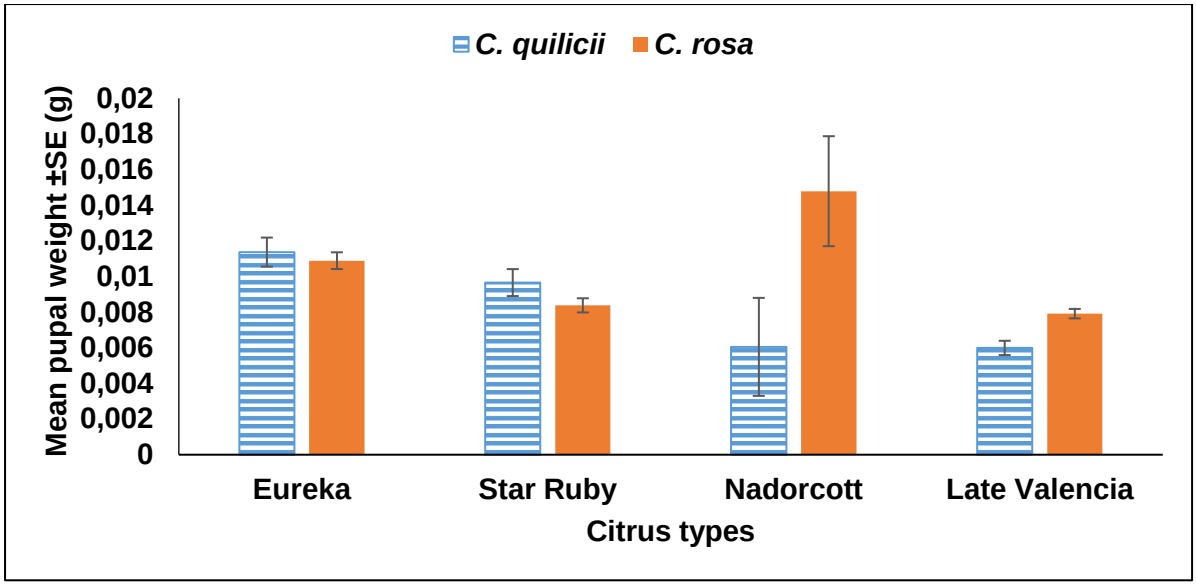

Figure 8. Mean pupal weight of *Ceratitis quilicii* and *Ceratitis rosa* flies collected from the four citrus types (Eureka, Star Ruby, Nadorcott, and Late Valencia).

4.4. Discussion

In this study, larval development and survival of both species were influenced by citrus type. Survival of *C. quilicii* and *C. rosa* was poor in Nadorcott fruit and no *C. quilicii* adults were reared from this citrus type. Larval performance of polyphagous fruit fly species such as *C. rosa* and *C. quilicii* depend on the host species, fruit size and nutrients in the fruit (Hafsi *et al*. 2016). Hafsi *et al*. (2016) reported that *C. rosa* s.l. larval survival was positively correlated with carbohydrate, lipid, and fibre contents of fruit, but negatively correlated to water content. In this study, Nadorcott had a high juice content together with the highest Brix and ratio. The high sugar content of Nadorcott could have been detrimental to larval development. Hafsi *et al*. (2016) also reported poor larval survival of *C. rosa* s.l. in mandarin, similar to both *C. rosa* and *C. quilicii* in this study. In this study the largest *C. rosa* pupae were recorded from Nadorcott. This heavier weight might be due to the survival of only a few individuals, which had more pulp available, and thus were less exposed to intraspecific competition (Begon *et al.* 2006).

De Lima *et al*. (2007) compared the development of *C. capitata* and *Bactrocera tryoni* (Froggatt) in five citrus types and reported that fewer *C. capitata* survived in lemon fruit. In this study, overall larval survival rates of *C. quilicii* were higher in Late Valencia and *C. rosa* were higher in Eureka and Late Valencia. Lemons have been declared a non-host for fruit flies (Manrakhan *et al*. 2018) and the only reason for the high level of larval survival observed in this study is ascribed to the artificial inoculation of fruit with fly eggs. The mean thickness of

lemon rind in this study was 5 mm, which is too thick a barrier for a fruit fly female to penetrate, and similarly a hard peel or pericarp will be difficult to penetrate (Bateman 1972; Díaz-Fleischer & Aluja 2003). Larvae of these species would therefore most likely also survive if they can gain secondary access to fruit pulp, via physical wounds. This emphasized the importance of the conditions/status of collected fruit (physical damage to the peel or splitting of a citrus fruit etc.) when hosts studies are conducted. This will prevent incorrect reporting of host status of certain plant species for these flies. The initial development of larvae of both *C. rosa* and *C. quilicii* were however slower in Eureka lemon which had the highest acidity and lowest pH among the citrus types tested. It is likely that acidity and pH are limiting factors for larval development of both species. Other studies (Vargas *et al*. 1984; Papadopoulos & Katsoyannos 2002; Papachristos *et al*. 2008) found a similar pattern in that the higher the pH (less acidity) the shorter the development rate of fruit fly larvae. However, for the congeneric species, *Ceratitis capitata*, Papachristos *et al*. (2008) found that acidity, pH and SSC had no influence on survival of the species.

In this study, we found that the development rates of *C. rosa* and *C. quilicii* were more or less similar in all citrus types. However, there were differences in the survival rates of the two species. In citrus types such as Eureka and Late Valencia, *C. rosa* had higher larval and pupal survival rates than *C. quilicii*. Virgilio *et al.* (2013) reported that *C. rosa* and *C. quilicii* occurred sympatrically in South Africa. The results described in Chapter 3 of this dissertation, confirmed that the distribution of these two species overlap, and that whenever two closely related species occur sympatrically, a difference, such as a temperature adaptation, or morphology adaptation or host utilization, is expected between the two species (Scriven *et al*. 2016; Darwell & Cook 2017). Results on the survival of the two species on four citrus types in this study suggest that the two species may differ in the utilization of fruit depending on certain fruit species. It is important to note that artificial procedures were employed in this study and the results obtained do not confirm the four citrus types as hosts of any of the two fruit fly species.

4.5. Practical implications

Since both fly species developed at more or less similar rates inside the different citrus types, both fly species can be exposed to cold sterilization treatments on similar days, depending on which life stage is the most tolerant against a cold treatment. Results from this study can be used for development of cold treatment trials for *C. rosa* and *C. quilicii* in citrus. The citrus types most suited for use in cold disinfestation trials is Late Valencia or any similar oranges.

According to Grout *et al*. (2011b) Valencias are available for a longer period during a season and are less affected by postharvest decay. Oranges are easier to handle, pack in crates due to their smaller size, and easier to later dissect in the laboratory than large grapefruit.

Grout *et al*. (2011b) and Ware *et al*. (2006) indicated that the most cold tolerant stage of *C. capitata* is the 2nd instar, whereas Hallman *et al*. (2011) and Ware & Du Toit (2017) came to the conclusion that the 3rd instar was the most cold tolerant. Al-Behadili *et al*. (2019) however, indicated that the 1st and 3rd instars are the most cold tolerant. If determination of cold tolerances of different instars in citrus are required for the development of a cold treatment, day 3 can be used for the 1st instar of both species, day 6 can be used for the 2nd instar of both species, and day 9 can be used for the 3rd instar of both species.

4.6. References

AL-BEHADILI, F.J.M., BILGI, V., LI J., WANG, P., TANIGUCHI, M., AGARWAL, M., REN, Y. & XU W. 2019. Cold response of the Mediterranean fruit fly (*Ceratitis capitata*) on a lab diet. *Insects* **10**: 1-11.

APHIS. 2016. *United States Animal and Plant health Inspection Services, Quarantine Treatment Manual*. Second Edition. Accessed August 2019: https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf. 1-958.

BADII, K.B., BILLAH, M.K., AFREH-NUAMAH, K., OBENG-OFORI, D. & NYARKO, G. 2015. Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae) in Africa. *African Journal of Agricultural Research* **10**: 1488-1498.

BALMÈS, V. & MOUTTET, R. 2017. Development and validation of a simplified morphological identification key for larvae of tephritid species most commonly intercepted at import in Europe. *EPPO Bulletin* **47**: 1-9.

BATEMAN, M.A. 1972. The ecology of fruit flies. *Annual Review of Entomology* **17**: 493-518.

BEGON, M., TOWNSEND, C.R. & HARPER, J.L. 2006. *Ecology: From Individuals to Ecosystems*. Blackwell Publishing, Oxford.

CARROLL, L.E. 1998. Description of the third instar larva of *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Proceedings of the Entomological Society of Washington* **100**: 88-94.

CGA. 2019. Industry Statistics. 2019. Citrus Growers Association of Southern Africa, KwaZulu-Natal, South Africa 5-46 pp.

CRI. 1995. Colour Prints for Blemish and Appearance Standards. Set No. 34 – 37. Citrus Research International, Nelspruit, South Africa.

DAFF. 2018a. A profile of the South African citrus market value chain. Department of Agriculture, Forestry and Fisheries. Pretoria, South Africa. 107 pp.

DAFF. 2018b. Standards and requirements regarding control of the export of citrus fruit as stipulated by government notice No. 1983 of 23 August 1991, No. 634, 7 May 1999. Revised 4 May 2018. No. 468. Accessed August 2019: www.nda.agric.za>Citrus(PART 1-General REG). reg(2018)1.doc.

DARWELL, C.T. & COOK, J.M. 2017. Cryptic diversity in a fig wasp community-morphologically differentiated species are sympatric but cryptic species are parapatric. *Molecular Ecology* **26**: 937-950.

DE LIMA, C.P.F., JESSUP, A.J., CRUICKSHANK, L. WALSH, C.J. & MANSFIELD, E.R. 2007. Cold disinfestation of citrus (*Citrus* spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science* **35**: 39-50.

DE MEYER, M. 2001. On the identity of the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **71**: 55-62.

DE MEYER, M., COPELAND, R.S., LUX, S.A., MANSELL, M.W., QUILICI, S., WHARTON, R.A., WHITE, I.M. & ZENZ, N.J. 2002. Annotated check list of host plants for Afrotropical fruit flies (Diptera: Tephritidae) of the genus *Ceratitis*. *Documentation Zoologique* **27**: 91

DE MEYER, M., MWATAWALA, M., COPELAND, R.S., VIRGILIO, M. 2016. Description of new *Ceratitis* species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy* **233**: 1-23.

DE MEYER, M., ROBERTSON, M.P., PETERSON, A.T. & MANSELL, M. 2008. Ecological niches and potential geographical distributions of Mediterranean fruit fly (*Ceratitis capitata*) and Natal fruit fly (*Ceratitis rosa*). *Journal of Biogeography* **35**: 270-281.

DÍAZ-FLEISCHER, F. & ALUJA, M. 2003. Clutch size in frugivorous insects as a function of host firmness: the case of the tephritid fly *Anastrepha ludens. Ecological entomology* **28**: 268-277.

DOHINO, T., HALLMAN, G.J., GROUT, T.G., CLARKE, A.R., FOLLETT, P.A., CUGALA, D.R., TU, D.M., MURDITA, W., HERNANDEZ, E., PEREIRA, R. & MYERS, S.W. 2016. Phytosanitary treatments against *Bactrocera dorsalis* (Diptera: Tephritidae): Current situation and future prospects. *Journal of Economic Entomology* **110**: 67-79.

DUYCK, P.F. & QUILICI, S. 2002. Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bulletin of Entomological Research* **92**: 461-469.

ETIENNE, J. 1982. Etude systématique, faunistique et écologique des Tephritidae de la Réunion. *Thèse de l'Ecole Pratique des Hautes Etudes*, Paris, 100 pp.

FOLLETT, P.A. & SNOOK, K. 2013. Cold storage enhances the efficacy and margin of security in postharvest irradiation treatments against fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* **106**: 2035-2042.

GROUT, T.G., DANEEL J.-H., MOHAMED, S.A., EKESI, S., NDERITU, P.W., STEPHEN, P.R. & HATTINGH, V. 2011a. Cold susceptibility and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae). *Journal of Economic Entomology* **104**: 1180-1188.

GROUT, T.G., STEPHEN, P.R., DANEEL J.-H. & HATTINGH, V. 2011b. Cold treatment of *Ceratitis capitata* (Diptera: Tephritidae) in oranges using a larval endpoint. *Journal of Economic Entomology* **104**: 1174-1179.

GROUT, T.G. & STOLTZ, K.C. 2007. Developmental rates at constant temperatures of three economically important *Ceratitis* spp. (Diptera: Tephritidae) from southern Africa. *Environmental Entomology* **36**: 1310-1317.

HAFSI, A., FACON, B., RAVIGNÉ, V., CHIROLEU, F., QUILICI, S., CHERMITI, B. & DUYCK, P.-F. 2016. Host plant range of a fruit fly community (Diptera: Tephritidae): does fruit composition influence larval performance? *BMC Ecology* **16**: 1-12.

HALLMAN, G.J., MYERS, S.W., JESSUP, S.W. & ISLAM, A. 2011. Comparison of in vitro heat and cold tolerances of the new invasive species *Bactrocera invadens* (Diptera: Tephritidae) with three known tephritids. *Journal of Economic Entomology* **104**: 21-25.

MANRAKHAN, A. 2019. Fruit fly. In T.G. Grout (Ed.) *Integrated Production Guidelines for export citrus. Integrated pest and disease management.* 1-10. Nelspruit, South Africa: Citrus Research International.

MANRAKHAN, A., DANEEL, J.-H., BECK, R., THERON, C.D., WELDON, C.W., MOORE, S.D. & HATTINGH, V. 2018. Non-host status of commercial export grade lemon fruit (*Citrus limon* (L.) f. cv. Eureka) for *Ceratitis capitata, Ceratitis rosa, Ceratitis quilicii* and *Bactrocera dorsalis* (Diptera: Tephritidae) in South Africa. *African Entomology.* **26**: 202-214.

PAPACHRISTOS, D.P., PAPADOPOULOS, N.T. & NANOS, G.D. 2008. Survival and development of immature stages of the Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruit. *Horticultural Entomology* **101**: 866-872.

PAPADOPOULOS, N.T. & KATSOYANNOS, B.I. 2002. Development of *Ceratitis capitata* (Diptera: Tephritidae) in three apple varieties in the laboratory. In: B. Barnes (Ed.), Proceedings of 6th International Fruit Fly Symposium, 6-10 May, 19-22. Stellenbosch, South Africa. Isteg Scientific Publication, Irene, South Africa.

PMRG. 2019. PMGR research guideline: Guidelines for the development of cold disinfestation treatments for fruit fly host commodities. 1 - 22. Online at: https://www.ippc.int/en/external-cooperation/organizations-page-in-ipp/phytosanitarymeasuresresearchgroup/

QUILICI, S. 1989. Aménagement de la lutte chimique contres les mouches des fruits à la Réunion. In: Fruit Flies of Economic Importance 87. *Proceedings of the CEC/ IOBC International Symposium*, 7-10 April 1987, Rome, Italy. Cavalloro, R. & Balkema, A.A. (Eds.) Rotterdam, Netherlands. 515-524 pp.

SCRIVEN, J.J., WHITEHORN, P.R., GOULSON, D. & TINSLEY, M.C. 2016. Niche partitioning in a sympatric cryptic species complex. *Ecology and Evolution* **6**: 1328-1339.

STECK, G.J. & EKESI, S. 2015. Description of third instar larvae of *Ceratitis fasciventris*, *C. anonae*, *C. rosa* (FAR complex) and *C. capitata* (Diptera: Tephritidae). In: De Meyer, M., Clarke, A.R., Vera, M.T. & Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 443-466.

TANGA, C.M., MANRAKHAN, A., DANEEL, J.-H., MOHAMED, S.A., FATHIYA, K. & EKESI, S. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis rasa* Karsch (Diptera, Tephritidae) populations from Kenya and South Africa. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 467-487.

VARGAS, R.I., WILLIAMSON, D.L., CHANG, H. & KOMURA, M. 1984. Effects of larval-diet pH on worker comfort and insect quality during mass-production of Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. *Journal of Environmental Science and Health, Part A* **19**: 621-630.

VIRGILIO, M., DELATTE, H., QUILICI, S., BACKELJAU, T. & DE MEYER, M. 2013. Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa, Ceratitis fasciventris* and *Ceratitis anonae* (Diptera, Tephritidae). *Molecular Ecology* **22**: 2526-2539.

WARE, A.B. & DU TOIT, C.L.N. 2017. Cold disinfestation of 'Hass' Avocado (*Persia americana*) of three species of fruit fly (Diptera: Tephritidae) - *Ceratitis capitata, Ceratitis rosa,* and *Ceratitis cosyra. Journal of Economic Entomology* **110**: 954-960.

WARE, A.B., TATE, B.A., DANEEL, J.-H. & BECK, R. 2006. Sensitivity of Mediterranean fruit fly eggs and larvae in lemons, grapefruit and oranges to cold treatment of 1 °C. In: CRI Group Annual Research Report for 2005. 100-106. Citrus Research International, Nelspruit, South Africa.

WARE, A.B., TATE, B.A., STEPHEN, P.R. & DANEEL, J.-H. 2004a. Cold disinfestation of Medfly- and Natalfly-infested persimmons (Phase 1 and 2). In: CRI Group Annual Research Report for 2004. 172-177. Citrus Research International, Nelspruit, South Africa.

WARE, A.B., TATE, B.A., STEPHEN, P.R., DANEEL, J.-H. & BECK, R.R. 2004b. Cold disinfestation of Mediterranean (*Ceratitis capitata* [Wiedemann]) and Natal (*Ceratitis rosa* Karsch) fruit fly-infested litchis (*Litchi chinensis* Sonn.). *SA Litchi Growers' Association Yearbook* **16**: 47-51.

WHITE, I.M. & ELSON-HARRIS, M-M. 1992. *Fruit fly of economic significance: their identification and bionomics.* C.A.B. International, London, UK and Australian Centre for International Agricultural Research, Canberra, Australia. xii + 601 pp.

CHAPTER 5. CONCLUSION AND RECOMMENDATIONS

5.1. Chapter 3: Relative abundance of *C. rosa* and *C. quilicii* in citrus orchards

5.1.1. Conclusions

The split of a fruit fly pest, *C. rosa* into two species, *C. rosa* and *C. quilicii* (De Meyer *et al*. 2016), impacts management strategies for these pests and holds implications for export of fruit since both species are of quarantine importance. This sudden “appearance” of an indigenous species as a pest immediately questioned all previously generated data on *C. rosa*, since uncertainty exists on which of the two species the data were based on, if the adult flies were not kept on record.

This compelled the current study which was carried out to determine the distribution and abundance of the two species in citrus orchards, and to investigate if and how these two species react to the different attractants used to monitor fruit flies in citrus orchards. Since the two species occur sympatrically (Virgilio *et al*. 2013), differences in their ecology were expected.

Tanga *et al*. (2015) found that *C. rosa* and *C. quilicii* had different thermal preferences for their development in laboratory studies. This was confirmed in this field study. Temperature had a greater influence on the distribution of *C. rosa*, and a negative correlation with altitude, and positive correlation with minimum temperature, was observed. The lower the minimum temperature, the more negatively it effects the abundance and distribution of *C. rosa*. Within the northern areas of South Africa, the abundance and distribution of *Ceratitis quilicii* was not limited by altitude, temperature, relative humidity or rainfall. *Ceratitis quilicii* was the more widely distributed of the two species in the northern parts of the Mpumalanga and Limpopo provinces. *Ceratitis rosa* became more prominent in the hot, low lying regions of the country.

This study also indicated that *C. quilicii* was dominant at all three altitudes and in all four climatic zones where surveys were conducted. Rainfall and relative humidity were not observed to influence the distribution of the two species. However, since irrigation in the South African citrus industry is an established practice, this may have created suitable and unique microhabitats for these flies, which could have masked the possible effects of humidity on the distribution of the flies recorded in this study.

In this study, both *C. rosa* and *C. quilicii* responded to three attractants used: Capilure, Egolure and Biolure. Any of these attractants can be used to monitor both these species, but most male flies (both species) were caught with Egolure. Both Biolure and Egolure are important monitoring tools for the two species. In areas where *C. quilicii* dominated, the ratio of *C. rosa* and *C. quilicii* males in Egolure was similar to the ratio of *C. rosa* and *C. quilicii* females in Biolure traps. There were however no clear patterns in trap catches in areas where *C. rosa* dominated. Since the females of the two species cannot be distinguished using morphological methods (De Meyer *et al*. 2016), these findings would provide a basis to assume the composition of females of the two species in Biolure traps are similar to Egolure, if Egolure traps are also placed for monitoring of males.

The results obtained in the *C. quilicii* dominant areas has important management implications since it showed that whether a male attractant or a female attractant is used, the ratio between the two species can still be estimated. It is also comforting to know that the accuracy of the morphological identifications was confirmed by molecular analysis, implying that it is possible to differentiate between males of the two species by means of a dissecting microscope. This enables male fly identification at grower level if such facilities are available.

Since this study was conducted in only two provinces it leaves questions on the distribution of the two species in other citrus producing areas. The southernmost distribution of *C. rosa*, along the Indian ocean coast line, as well as its inland distribution along major rivers such as the Limpopo or Orange Rivers are unknown. *Ceratitis quilicii* is the biggest range expansion threat of the two species to fruit production regions, being able to tolerate a wide range of temperatures, and especially its ability to tolerate very cold temperatures.

5.1.2. Practical implications and recommendations

Based on the findings of this study, the following recommendations can be made:

1. Growers should use Egolure in the orchards to monitor for the presence of *C. quilicii* and *C. rosa* as it is the best lure to use when monitoring for males of both fruit fly species in commercial citrus orchards. Adult male flies of the two species can be correctly identified by growers, provided that they have access to correctly trained scouts and a suitable microscope in their laboratories.
2. If a food-based attractant is preferred, which are normally female biased, the use of Biolure is recommended to monitor for female and male flies of both species in commercial orchards. The results of female catches from Biolure can give a good

indication of the male flies present in *C. quilicii* dominant areas that have a cooler climate and a higher altitudinal location.

3. It is recommended that further research should be conducted in the *C. rosa* dominant areas to better understand the ratio between the two fruit fly species, and whether that ratio remains the same throughout different seasons and if it is comparable to the *C. quilicii* dominant areas.
4. Both Biolure and Egolure attract non-target species so care should be taken when investigating the contents of these traps, so as not to make the wrong conclusions based on the presence of unimportant tephritids for citrus production, such as *C. pedestris* (Bezzi) or *C. rubivora* Coquillett, and therefore implement incorrect management decisions.
5. Future research must focus on establishing threshold values for both Biolure and Egolure in citrus orchards, not just for *C. rosa* and *C. quilicii* but for other commercially important fruit fly species regularly encountered in citrus orchards. This is to ensure that fruit fly numbers do not reach high numbers before fruit ripening, as well as to minimize the use of pesticides when fruit fly numbers are below threshold in the orchards.
6. It is important to clarify the distribution of the two species in South Africa, since *C. rosa* might be absent from the majority of citrus orchards in South Africa, which means that certain areas might be *C. rosa* pest free.
7. More research is required to establish a better, more accurate, host status record of these two fruit fly species in South Africa, to assist in establishing a better picture of the distribution of the two fruit fly species in South Africa.
8. Further research is needed to understand competition interaction between the two fruit fly species, and other commercially important fruit fly species, in order to understand which species might exclude which, and which will have a higher competitive ranking, in an attempt to better understand and interpret host utilization results.

5.2. Chapter 4: Development of *C. rosa* and *C. quilicii* in citrus

5.2.1. Conclusions

The description of the new species also has consequences regarding previously acquired results on cold disinfestation requirements and whether they exhibit differential preferences for specific citrus fruit cultivars as hosts. The first phase in any cold sterilization program is to determine larval development rate in fruit after inoculation, so that the most cold tolerant larval

stages can be assessed and thereafter the exposure periods needed for the most cold tolerant stage during cold treatments can be determined (Grout *et al*. 2011).

The postharvest results indicated that there were no differences between the development of the larvae of the two fruit fly species, when artificially inoculated into four citrus types (Eureka, Star Ruby, Nadorcott and Late Valencia). The different citrus types allowed similar development of larvae, however, Nadorcott was less suitable since it allowed poor larval development and survival and on multiple occasions no larvae were collected. Although the largest pupae of *C. rosa,* and largest 3rd instar of *C. quilicii* was collected from Nadorcott fruit, it is not a suitable host and further cold sterilization research should not include this citrus type due to the low larvae numbers recovered, causing an incorrect positive biased result for any cold treatment. Larval survival in Late Valencia was higher than in the other citrus types for *C. rosa* and *C. quilicii*, however, only when comparing the mature larvae.

Late Valencia fruit are the most suitable for further cold disinfestation trials due to the following reasons: 1) this type of fruit is available for a longer period during the season, 2) is easier to procure, 3) it is smaller, which facilitates easier packing into crates for a cold treatment, than Star Ruby, 4) it is easier to dissect under a microscope, 5) the peel is not as hard as in Eureka, or thick as in Star Ruby, and 6) it is longer lasting during storage. Both fruit fly species can be exposed to a cold treatment on the same day, for the 2nd cold disinfestation phase, to determine which life stage is the most cold tolerant.

5.2.2. Practical implications and recommendations

Based on the findings of this study, the following recommendations can be made:

1. It is recommended that more citrus fruit from different commercial orchards should be collected to determine which citrus types are natural hosts to the two fruit fly species.
2. There was no difference between the two fruit fly species in their reactions to different citrus types. Therefore, for future studies any cultivars chosen could be used on both fruit fly species simultaneously without influencing the results.
3. It is recommended that for any cold sterilization trials, Nadorcott should not be used to conduct any cold sterilization trials, because it might give a biased result to the effectiveness of the cold treatment, since larval development and survival were so negatively different compared to the other three citrus types.
4. In addition, although any of the other three citrus types tested are suitable hosts for trial work, Eureka was proven not to be a host to fruit flies in published literature, so it

is also not suitable. Similarly, it can be inferred that Star Ruby is also not a natural host due to its thick peel, which would probably never be exposed to a primary invasion by fruit flies. Therefore, out of the four citrus cultivars tested, Late Valencia is recommended as the best host to continue with any further cold treatment.

5. It is also a practical recommendation that Late Valencias, or any other orange, should be used, due to its durability when stored in cold rooms and its smaller size compared to Star Ruby, making it easier to pack, handle, inoculate and dissect.
6. It is recommended that the two fruit fly species can be exposed on the same days for each instar, to determine which instar is the most cold tolerant, and whether this result is going to be the same for both species?
7. For future cold treatment trials, the best days to expose the larvae, of both species, to a cold treatment, in order to establish which instar is the most cold tolerant, is day 3 for the 1st instar, day 6 for the 2nd instar, and day 9 for the 3rd instar.
8. Due to the greater temperature tolerance of *C. quilicii*, and thus greater invasion potential than *C. rosa*, it is recommended that the cold treatment consisting of all four phases, are conducted.

5.3. References

DE MEYER, M., MWATAWALA, M., COPELAND, R.S. & VIRGILIO, M. 2016. Description of new *Ceratitis* species (Diptera: Tephritidae) from Africa, or how morphological and DNA data are complementary in discovering unknown species and matching sexes. *European Journal of Taxonomy* **233**: 1-23.

GROUT, T.G., STEPHEN, P.R., DANEEL J.-H. & HATTINGH, V. 2011. Cold treatment of *Ceratitis capitata* (Diptera: Tephritidae) in oranges using a larval endpoint. *Journal of Economic Entomology* **104**: 1174-1179.

TANGA, C.M., MANRAKHAN, A., DANEEL, J.-H., MOHAMED, S.A., FATHIYA, K. & EKESI, S. 2015. Comparative analysis of development and survival of two Natal fruit fly *Ceratitis rasa* Karsch (Diptera, Tephritidae) populations from Kenya and South Africa. In: De Meyer, M., Clarke, A.R., Vera, M.T., Hendrichs, J. (Eds.) Resolution of Cryptic Species Complexes of Tephritid Pests to Enhance SIT Application and Facilitate International Trade. *Zookeys* **540**: 467-487.

VIRGILIO, M., DELATTE, H., QUILICI, S., BACKELJAU, T. & DE MEYER, M. 2013. Cryptic diversity and gene flow among three African agricultural pests: *Ceratitis rosa, Ceratitis*

fasciventris and *Ceratitis anonae* (Diptera, Tephritidae). *Molecular Ecology* **22**: 2526-2539.