The correlation between fruit fly numbers trapped and damage levels in avocado orchards

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ABSTRACT
In South Africa, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), the Marula fruit fly, *Ceratitis cosyra* (Walker), the Natal fruit fly, *Ceratitis rosa* sensu lato Karsch, and the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), are of economic importance for subtropical fruit production. Studies indicated that the Natal fruit fly comprises a complex of two genotypes and morphotypes that should be considered as two different species. The two entities, R1 (lowland or hot rosa) and R2 (highland or cold rosa), can be distinguished based on morphological characteristics in males only, while females cannot be distinguished. The R2 type was described as a new species, *Ceratitis quilicii*, by De Meyer, Mwatawala and Virgilio and *C. quilicii* is now commonly referred to as the Cape fruit fly. The avocado is known to be a poor host for the development of fruit flies. However, fruit flies can develop in the fruit under certain conditions. Fruit flies were monitored in avocado orchards in the Tzaneen area, in the Limpopo Province. During the same time, fruit were inspected for the presence of fruit fly lesions. Fruit were sampled to determine if any fruit fly larvae were present. The data gathered is important for the development of economic threshold levels of trap catches. The information can also be used in the development of a systems approach to access new markets. Mediterranean fruit fly, Marula fruit fly and Oriental fruit fly catches remained below 1 fruit fly/trap/day in traps baited with Biolure® Fruit Fly. However, up to 10 Cape fruit flies/trap/day were captured. In traps baited with Invader-lure, the Oriental fruit fly reached up to 82 fruit flies/trap/day. Low numbers were captured with Sensus traps baited with Questlure. Fruit with lesions did not exceed more than 2%. No live larvae were found in the sampled fruit. Therefore, the results indicated that although some fruit fly species exceeded more than 1 fruit fly/trap/day, no live larvae were found in the fruit. It is important to continue with monitoring and fruit sampling for at least another season.

INTRODUCTION
Several fruit fly species (Diptera: Tephritidae) are known to attack different types of commercially-grown crops, causing considerable damage. The economic impact includes direct yield losses, increased costs of production, and fruit flies are often of quarantine importance depending on the recipient country. Few pests have a greater impact on world trade in agricultural products than fruit flies. *Ceratitis* MacLeay is predominantly an Afrotropical genus, and species within the genus attack a wide range of fruit, including cultivated crops (De Meyer, 2005). In South Africa, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), the Marula fruit fly, *Ceratitis cosyra* (Walker), and the Natal fruit fly, *Ceratitis rosa* sensu lato Karsch, are three fruit fly pests of importance for the horticultural industry (Prinsloo and Uys, 2015). Among these fruit flies, the Mediterranean fruit fly is the most polyphagous and widely distributed in South Africa (De Villiers *et al*., 2013). The Natal fruit fly is absent from, or only present in low numbers, in the drier regions of South Africa. Recent studies indicated that the Natal fruit fly comprises a complex of two genotypes and morphotypes. The two entities, R1 (lowland or hot rosa) and R2 (highland or cold rosa), can be distinguished based on morphological characteristics in males only, while females cannot be distinguished. These studies indicated that the two types should be considered as two different species (De Meyer *et al,.* 2015). De Meyer *et al.* (2016) described the R2 type as a new species, *Ceratitis quilicii* De Meyer, Mwatawala and Virgilio. *C. quilicii* is commonly referred to as the Cape fruit fly. The distribution of the Marula fruit fly is restricted to the northern and eastern regions of the country, following a similar pattern of distribution to the Marula tree, *Sclerocarrya birrea* (A.Rich.) Hochst., an important wild host. The Marula fruit fly is known to have a restricted host range and is especially an important pest of mango (Grové *et al*., 2009, 2015; Copeland *et al.,* 2006).

Species of the genus *Bactrocera* Macquart are predominantly of Indo-Australian origin (White, 2006). In Africa, 11 indigenous species are known. However, three Asian *Bactrocera* species have been introduced to Africa i.e. the Oriental fruit fly, *Bactrocera dorsalis* (Hendel), the Solanum fruit fly, *Bactrocera latifrons*

(Hendel), and the peach fruit fly, *Bactrocera zonata* (Saunders) (De Meyer *et al.*, 2014; Schutze *et al.,* 2014). Out of the three introduced species, the Oriental fruit fly is currently the most widespread on the African continent and poses the biggest threat to horticulture in Africa. The Oriental fruit fly was first found on the African continent in Kenya in 2003 (Lux *et al.,* 2003) and initially described as a new species, *Bactrocera invadens* Drew, Tsuruta and White (Drew *et al.*, 2005). *B. invadens* was synonymised with *B. dorsalis* based on similarities in morphological characters, molecular structure and pheromone composition as well as mating compatibility (Schutze *et al*., 2014). The Oriental fruit fly was reported in South Africa for the first time in 2010 in an area on the northern border of the country (International Plant Protection Convention [IPPC], 2010). Eradication attempts against the pest in those areas were unsuccessful and the pest was declared present but subjected to official control in the Vhembe District Municipality in the northern Limpopo province in South Africa (IPPC, 2013). The Oriental fruit fly was declared present but subjected to official control in certain district municipalities of Limpopo, Mpumalanga, North West, Gauteng and KwaZulu-Natal during 2015. Therefore, the Oriental fruit fly is present in the main avocado production areas of South Africa. The Oriental fruit fly is a highly polyphagous species (White and Elson-Harris, 1994). Before the arrival of the Oriental fruit fly in Kenya, the indigenous Marula fruit fly was the predominant fruit fly pest of mango. Within four years of invasion, the Oriental fruit fly displaced the Marula fruit fly. Therefore, in many of the African countries the Oriental fruit fly is now the dominant fruit fly species attacking mangoes and other commercial crops, and high damage levels were reported (Ekesi *et al.,* 2009; Mwatawala *et al.*, 2009; Rwomushana *et al.,* 2009; Massebo and Tefera, 2015).

Fruit fly species belonging the genera *Anastepha*, *Bactrocera* and *Ceratitis* are associated with avocado production in the world (White and Elson-Harris, 1994). Schwartz (1978) working in South Africa stated that fruit flies do not develop in avocado. 'Fuerte' avocado fruit at different stages of development were exposed to the Mediterranean- and Natal fruit fly in orchards in the Limpopo Province (Du Toit *et al.*, 1979). The results indicated that the Mediterranean fruit fly did not lay eggs in the fruit, while the Natal fruit fly did. Fruit-fly damage by the insect's ovipositor developed into a typical crack or star shaped lesion. Studies conducted in the same area found that the Natal fruit fly played an economically unimportant role in 'Fuerte' avocado fruit drop for the two-month period following petal drop, although high numbers of the fruit fly were present (Du Toit and Tuffin, 1980). Brink *et al.* (1997) artificially infested 100 fruit of five different cultivars ('Hass', 'Fuerte', 'Ryan', 'Edranol' and 'Rinton') with 250 Mediterranean fruit fly eggs per fruit. No survival occurred after cold storage between 5.5°C and 6.5°C for 28 days. Ten fruits from each cultivar were artificially infested with 250 eggs per fruit and left at room temperature. Live larvae were only found in the cultivar 'Ryan' and the survival rate from egg to larvae was 1.28%. De Villiers and Van Den Berg (1987) stated that under normal orchard practices no larval development takes place in the avocado fruit. According to Du Toit and De Villiers, (1990) fruit fly larvae do not develop in the fruit of commercial avocado cultivars. De Graaf (2009) conducted research on the susceptibility of 'Hass' avocados to the Mediterranean-, Marula- and Natal fruit fly in South Africa. 'Hass' was found to be a conditional non-host for the Mediterranean fruit fly and a potential host for the Marula- and Natal fruit fly, but that antixenosis and antibiosis mechanisms severely restricted development. No successful reproduction took place when exposing Marula- and Natal fruit fly to 'Hass' avocado while attached to the tree and not immediately following harvest. In conclusion, the study showed that the quarantine risk of fruit flies associated with 'Hass' avocado in South Africa is negligible under standard export conditions. Ware *et al.* (2016) working in Kenya and Tanzania exposed the cultivars 'Hass', 'Pinkerton' and 'Fuerte' to the Oriental fruit fly. Development took place in punctured fruit but not in uncompromised fruit. In the field studies, only fruit damaged by false codling moth were found to harbour Oriental fruit fly. In the light of these results, the risk imposed for 'Hass', 'Pinkerton' and 'Fuerte' by the Oriental fruit fly is negligible under standard export conditions.

Trapping systems for fruit flies are important components in integrated pest management programmes. Trapping systems give an indication of species present and their relative numbers. Trapping data can also be used to make decisions on the initiation or termination of suppression measures, as well as to assess efficacy of implemented suppression methods. Fruit flies were monitored in avocado orchards with three different monitoring systems. The objectives of the study were to determine:

1. the relative numbers of the different fruit fly species present in avocado orchards,
2. the presence of the Natal fruit fly and the Cape fruit fly,
3. the infestation levels by fruit sampling, and
4. establish the relationship between fruit fly numbers trapped and damage levels in avocado orchards.

MATERIALS AND METHODS

Fruit flies were monitored in avocado orchards in the vicinity of Tzaneen (Latitude: 23°49′59″S, Longitude: 30°09′48″E, Elevation above sea level: 719 m) (Mopani District Municipality, Limpopo Province). Monitoring was done in two 'Fuerte' orchards (referred to as 'Fuerte' 1 and 'Fuerte' 2), a 'Pinkerton' orchard and a 'Hass' orchard. All orchards used in the study were commercial, irrigated and well managed. Three different monitoring systems were used in each orchard in order to trap different fruit fly species of economic significance. Attractants used for trapping fruit flies can be mainly divided into two types: 1) male lures and 2) food baits (Cunningham, 1989).

Male lures are species-specific and are known to have a high efficacy in attracting flies from long distances. Male lures are mostly parapheromones. Food baits attract both male and female fruit flies. They are not species-specific and are known to have a lower efficacy compared to male lures. Food baits also attract high numbers of non-target organisms (Joubert *et al.*, 2015).

The three monitoring systems used were:

1. Sensus trap (River BioScience (Pty) Ltd, Port Elizabeth, South Africa) with Questlure (River BioScience (Pty) Ltd). Questlure contains protein hydrolysate and various plant extracts. This system is used for the monitoring of females and males of *Ceratitis* spp.
2. Chempac yellow bucket trap (Chempac (Pty) Ltd, Suider Paarl, South Africa) with Invader-Lure™ (River BioScience (Pty) Ltd). Invader-Lure is an attractant for the monitoring of the Oriental fruit fly males and the active ingredient is methyl eugenol 15 g (AI)/block.
3. Chempac yellow bucket trap with Biolure® Fruit Fly (Chempac (Pty) Ltd). Biolure Fruit Fly is a food bait that consists of three components i.e. ammonium acetate 211 g (AI)/kg, trimethylamine hydrochlorid 91 g (AI)/kg and 1,4-diaminobutane (putrescine) 3 g (AI)/kg. Both males and females of the four important *Ceratitis* spp. and the Oriental fruit fly are attracted to Biolure Fruit Fly.

Traps were deployed during January 2016. The traps were placed in diagonal line in the orchards. The bucket trap with Biolure Fuit Fly were placed in the centre of the orchard while the other two other traps were deployed near the edges of the orchards. Spacing between the traps were approximately 50 m. Traps were placed 1.5 m above the ground. A block of Vapona agricultural insecticide strip (Acorn Products (Pty) Ltd, Strubens Valley, South Africa) with the active ingredient dichlorvos @ 195 g (AI)/kg was used as killing agent. The blocks of Vapona and the lures used, were replaced every six weeks. Traps were serviced fortnightly to monthly. Fruit flies were identified to species level, and the sex of each recorded except in the case of the females of the Cape- and the Natal fruit fly. Multi-entry identification keys to African frugivorous fruit flies were used (Virgilio *et al.,* 2015). Population densities were expressed as the number of fruit flies/trap/day. Hundred fruit per orchard were inspected for the presence of any fruit fly lesions on a fortnightly to monthly basis. Fruit with suspected lesions were taken the laboratory. Lesions were cut and inspected with a stereo microscope. Hundred fruits were sampled twice in each orchard. One sample were taken when fruit were immature while the other sample were taken just prior to harvest. In the 'Hass' orchard fruit were sampled three times. Sampled fruit were left in the laboratory and inspected when soft for the presence of fruit fly larvae.

RESULTS

The cumulative number of the economic important species trapped are given in Table 1. Only males of the Cape fruit fly were trapped in the four orchards and no Natal fruit fly. The Cape fruit fly was the abundant species trap with Sensus traps with Questlure in the 'Hass' and 'Fuerte' 1 orchards, while the Marula fruit fly was the abundant species in the other orchards.

Table 1. Cumulative number of economic important fruit fly species trapped with three monitoring systems from January 2016 to November 2016.

	Cumulative number trapped with Sensus trap and Questlure			
Orchard	**Mediterranean fruit fly**	**Marula fruit fly**	**Cape fruit fly**	**Oriental fruit fly**
Hass	1	2	21	0
Fuerte 1	3	0	6	0
Fuerte 2	0	25	20	1
Pinkerton	1	14	3	3
	Cumulative number trapped with bucket trap and Invader-Lure			
Orchard	**Mediterranean fruit fly**	**Marula fruit fly**	**Cape fruit fly**	**Oriental fruit fly**
Hass	0	0	3	1483
Fuerte 1	0	0	0	4445
Fuerte 2	0	0	0	752
Pinkerton	0	0	0	3272
	Cumulative number trapped with bucket trap and Biolure Fruit Fly			
Orchard	**Mediterranean fruit fly**	**Marula fruit fly**	**Cape fruit fly**	**Oriental fruit fly**
Hass	6	9	957	28
Fuerte 1	11	5	39	25
Fuerte 2	7	15	301	28
Pinkerton	9	19	32	27

Only the Oriental fruit fly was trapped with bucket traps with Invader-Lure, except in the case of the 'Hass' orchard where three Cape fruit flies were trapped. The Cape fruit fly was the most abundant species trapped with the bucket traps with Biolure Fruit Fly in all four orchards. In Sensus traps with Questlure, all fruit fly species remained below 1 fruit fly/trap day. The number of Oriental fruit flies trapped is given in Figure 1. In the 'Fuerte' 2, the Oriental fruit fly reached 82 fruit flies/trap/day and high numbers were trapped in all orchards. Peak number of Oriental fruit fly were present during February and March. In bucket traps with Biolure Fruit Fly, all species were below 1 fruit fly/trap/day except for the Cape fruit fly. In the 'Hass' and the 'Fuerte' 2 orchards the Cape fruit fly were trapped in higher numbers than 1 fruit fly/trap/day and peak numbers were present during April (Fig. 2). The percentage of fruit with fruit fly lesions are given in Table 2. In most instances no lesions were found on the fruit and 2% was the highest level found. A total of 900 fruit were sampled and no live larvae were found in any fruit.

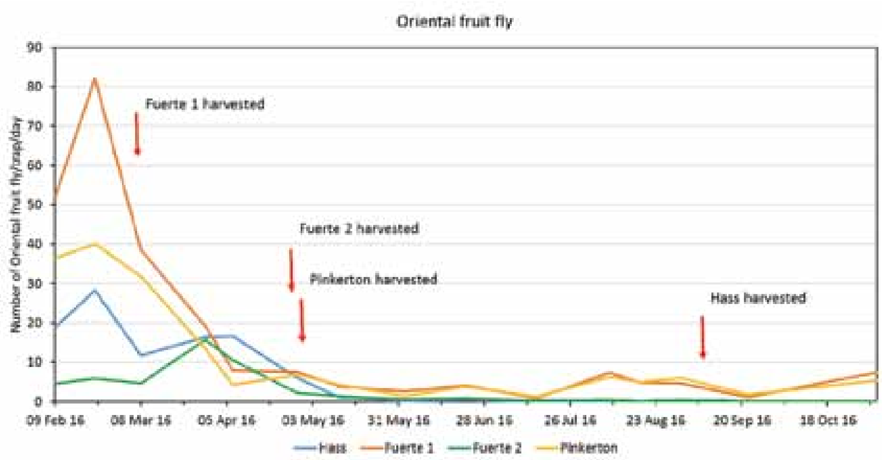

Figure 1. Number of Oriental fruit fly/trap/day in yellow bucket traps with Invader-Lure.

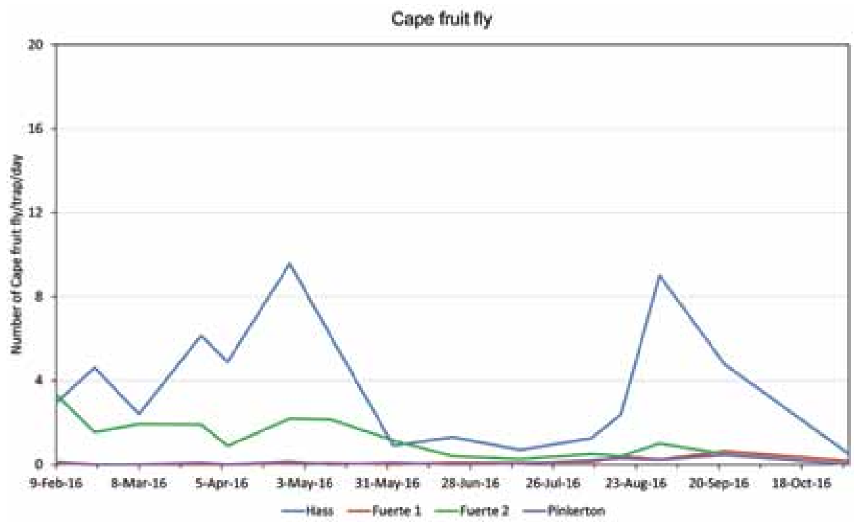

Figure 2. The Number of Cape fruit fly/trap/day in yellow bucket traps with Biolure Fruit Fly.

Table 2. Number of fruit with fruit fly lesions. Hundred fruits were inspected in each orchard from February until harvest on the dates given.

	Hass	Fuerte 1	Fuerte 2	Pinkerton
9 Feb 16	0	0	0	0
22 Feb 16	0	0	0	2
8 Mar 16	0	-	0	0
29 Mar 16	0	-	0	0
7 Apr 16	0	-	0	0
28 Apr 16	0	-	1	0
12 May 16	0	-	-	-
2 Jun 16	0	-	-	-
22 Jun 16	0	-	-	-
15 Jul 16	1	-	-	-
8 Aug 16	0	-	-	-
18 Aug 16	0	-	-	-
31 Aug 16	0	-	-	-
Total number of fruit inspected	1300	200	600	600
Total number of fruit with lesions	1	0	1	2

DISCUSSION

In Sensus traps with Questlure, low numbers of the different fruit fly species were trapped and numbers were below 1 fruit fly/trap/day. High numbers of Oriental fruit fly were tapped during February and March in traps baited with Invader-Lure. Invader-Lure is a male lure that attracts fruit flies from a faraway distance. Although high numbers were trapped, a low percentage of fruit with lesions were found and no live larvae were found in the fruit. This support the observation by Ware *et al.* (2016) that Oriental fruit flies did not lay eggs in uncompromised fruit. A multi-component systems approach was developed proposed to reduce the risk of Oriental fruit fly infestation in 'Sharwil' avocados exported from Hawaii into the United States to an acceptable level (Follett and Vargas, 2010). Traps with protein are used for monitoring Oriental fruit fly opposed to methyl eugenol. The food bait traps gave a better indication of population levels within orchards (Klungness *et al.*, 2009). In traps baited with Biolure Fruit Fly, the Cape fruit fly exceeded 1 fruit fly/trap/day in two orchards. Although high numbers were trapped a very low percentage of fruit with lesions were found in these orchards. Marula- and Natal fruit fly (as previously described) are known to be able to lay eggs inside avocado fruit. The avocado is known to be a poor host for the development of fruit flies and these studies support the observation.

Trapping is an indirect method of sampling and therefore producers are advised to use traps for monitoring fruit flies as well as fruit inspections to determine the presence of fruit fly lesions. Host plants near avocado orchards may influence trap catches. Producers are also advised to keep good records of fruit fly numbers trapped. These records can be used as a reference. Fruit flies can be suppressed in avocado orchards by using the bait application technique. Because high numbers of the Oriental fruit fly are trapped in avocado orchards, producers are advised to use the male annihilation technique as well as the bait application technique. Although not all species can lay eggs inside the avocado and due to the fact that avocado is not a good host for the development of fruit flies, it is still important to monitor fruit fly species and inspect fruit for lesions. Therefore, producers can make informed decisions on the management of fruit flies in avocado orchards. Fruit flies can cause lesions and eggs can be laid in fruit with surface lesions or defects. Therefore, there is some risk involved when exporting avocado fruit – although the risk is negligible (De Graaf, 2009; Ware *et al*., 2016). The different fruit fly species should be kept below 1/fruit/fly per trap per day in the food bait lures.

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