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

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Markets importing citrus fruit including lemons, *Citrus limon* (L.) Burman f., from South Africa require that the fruit be free of fruit fly pests (Diptera: Tephritidae). Historically there has been no fruit fly infestation recorded on lemons destined for export from South Africa. In this study, we assessed the host status of commercial export grade Eureka lemons, *Citrus limon* (L.) Burman f. cv. Eureka, for four fruit fly pest species of economic importance in South Africa: *Ceratitis capitata* (Wiedemann), *Ceratitis rosa* Karsch, *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio, and *Bactrocera dorsalis* (Hendel). Trapping was conducted in 10 Eureka lemon orchards in two major citrus production regions over two citrus seasons between 2016 and 2017 to determine the level of fruit fly abundance in the sampled orchards. Lemons were collected at harvest over the two seasons in the same orchards where trapping was conducted. Fruit fly infestation of the sampled lemons was determined by dissection. Additionally, infestation of lemons was determined under forced exposure to mature mated females of *C. capitata* and *B. dorsalis*. Trapping data showed the presence of adults of all four fruit fly species in the sampled lemon orchards. No fruit fly infestation was detected in 43 222 Eureka lemons sampled at harvest. There was also no infestation of lemons under forced exposure conditions. The results of this study provide evidence with 99.99 % efficacy and a 99 % confidence level that South African commercial export grade Eureka lemon fruit is not a host for *C. capitata*, *C. rosa*, *C. quilicii* or *B. dorsalis*.

Key words: Eureka lemon, fruit sampling, forced exposure, host status, fruit flies.

INTRODUCTION

Fruit fly (Diptera: Tephritidae) pests are of general phytosanitary concern in international trade of fruits and vegetables. The fruit fly species potentially associated with some citrus fruit types produced in parts of South Africa are the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), Natal fly, *Ceratitis rosa s.l.* Karsch, and oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Grout & Moore 2015). *Ceratitis capitata* is widespread across South Africa whilst *C. rosa s.l.* has a more restricted distribution being absent from the drier inland areas (De Villiers *et al.* 2013). *Bactrocera dorsalis* is present in the northern and northeastern areas of South Africa (Manrakhan *et al.* 2015). In 2016, *C. rosa s.l.* was reclassified into two species based on molecular and morphological evidence: *C. rosa* Karsch and the Cape fly, *Ceratitis quilicii* De Meyer, Mwatawala & Virgilio (De Meyer *et al.* 2016). *Ceratitis quilicii* (previously known as *C. rosa* R2) is the more dominant of the two species across the country (Karsten *et al.* 2016).

Whilst prevention of citrus infestation by fruit flies in South Africa is mostly achieved by pre-harvest field control measures (Manrakhan 2016) and post-harvest measures such as grading at packhouses, some markets require additional assurances of fruit fly-free consignments. To provide these additional assurances, post-harvest disinfes-

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tation treatments may need to be applied. The disinfestation treatments mostly used for citrus exported from South Africa are cold treatments, which entail exposure of fruit to specified low temperatures for prescribed periods (Grout *et al.* 2011; Moore *et al.* 2016). However, not all citrus cultivars are suitable for export through the application of such cold treatments due to the chilling injury sensitivity of some citrus types including lemons, *Citrus limon* (L.) Burman f. (Underhill *et al.* 1995).

Lemon plantings in South Africa have increased rapidly in recent years (CGA 2013). Exports of lemons from South Africa have likewise increased strongly over the past decade (CGA 2013), but markets that require cold treatment for disinfestation of fruit flies have remained inaccessible for lemon fruit. However, there are historically no records of interception of fruit flies either during pre-export official inspections (Perishable Products Export Control Board, unpubl. data) or during on-arrival inspection in export markets that have not required post-harvest disinfestation treatment (Department of Agriculture, Forestry and Fisheries, South Africa, unpubl. data; EUROPHYT 2017). Additionally, in a survey to determine the host status of export grade Eureka lemons for the false codling moth, *Thaumatotibia leucotreta* (Meyrick) in South Africa between 2013 and 2014, no fruit fly larvae were incidentally found in over 30 000 Eureka lemon fruit examined (Moore *et al.* 2015). Moore *et al.* (2015) also reported that no fruit fly infestation was detected in examination of more than 7 million lemon fruit that were commercially produced for export in the Sundays River Valley, Eastern Cape Province, South Africa, over a 7-year period.

Other studies conducted on a number of citrus-infesting fruit fly species have shown that citrus cultivars generally differ in their susceptibility to fruit flies, with lemons being recognised as the most unfavourable (Back & Pemberton 1915; Greany *et al.* 1983; Lloyd *et al.* 2013; Papachristos & Papadopoulos 2009; Papachristos *et al.* 2008). Despite the existence of some reports of *C. capitata* and *B. dorsalis* on lemons, the condition (damaged or undamaged), cultivar, maturity stage (ripe or overripe) and source (collected from the ground or tree) of the sampled lemon fruit from which the fruit fly species were reared were not specified (Back & Pemberton 1915; De Meyer *et al.* 2002; Eskafi 1988; Goergen *et al.* 2011; Katsoyannos *et al.* 1998; Liquido *et al.* 1990; Rwomushana *et al.* 2008). Therefore, these reports do not provide evidence of commercial export grade lemons being a host for these fruit flies. In *B. dorsalis* surveys in Kenya, records of the pest in lemons varied according to location and again were without information on condition of the fruit from which the fruit fly species was reared (Rwomushana *et al.* 2008). Surveys in Tanzania produced no records of *B. dorsalis* in lemons (Mwatawala *et al.* 2006). In a recent survey in South Africa in Limpopo and Mpumalanga provinces where *B. dorsalis* is present, no *B. dorsalis* were reared from lemons sampled (17.6 kg) from the tree or on the ground in commercial orchards (Theron *et al.* 2017). There are no historical records of lemons being a host for *C. rosa s l.* (De Meyer *et al.* 2002).

Non-host status testing of a plant species or cultivar is an alternative approach to post-harvest disinfestation treatment in achieving quarantine security in international trade (Follett & Hennessey 2007; Follett & Neven 2006). There are many examples in the literature where a fruit type was recorded as a host in the absence of cultivar identification or information on fruit condition. However, following structured host testing and surveys of the fruit type, cultivar or specified maturity stage, the fruit type was categorised as a non-host for specified fruit fly species (Aluja *et al.* 2003; Cugala *et al.* 2014; Cugala *et al.* 2017; Villagrán *et al.* 2012). Due to fruit fly disinfestation treatments being required for the export of lemons from South Africa to some important export markets, despite the absence of reliable evidence of lemons being a host for fruit flies and the existence of evidence that commercially produced export grade lemons are not a host for fruit flies, it was deemed important to generate reliable host status data in accordance with international standards.

An international standard for determination of host status of fruit for fruit flies, International Standard for Phytosanitary Measures 37 (ISPM 37) was recently adopted (FAO 2016). In the standard, three categories of host status of fruit for fruit flies were described: natural host, conditional host and non-host (FAO 2016). A plant species or cultivar is considered to be a natural host when the latter is found to be infested under natural conditions and is able to sustain the development of the fly to viable adulthood (FAO 2016). A conditional host is a host which has been shown to be infested by the target fruit fly species in trials conducted under

semi-natural conditions (FAO 2016). A non-host is a plant species or cultivar that has not been found to be infested by the target fruit fly species under either natural conditions or semi-natural conditions (FAO 2016).

The aim of this study was to determine the host status of commercial export grade lemon fruit for fruit fly species of potential phytosanitary concern for citrus in South Africa, by following the decision-making procedures and testing methods described in ISPM 37. Following the procedural flow chart provided in ISPM 37, the first specific objective of the study was to determine natural infestation of commercial export grade lemons. The second objective was to determine infestation of lemons under semi-natural (forced exposure) conditions by *C. capitata* and *B. dorsalis*, as additional verification of the host status.

MATERIAL AND METHODS

Field surveys

Study sites

Surveys were conducted between February 2016 and May 2017 in 10 Eureka lemon orchards from 10 farms in the Mpumalanga and Limpopo provinces, South Africa (Table 1). The surveys covered two citrus production seasons: 2016 and 2017. Eureka was selected as the lemon cultivar in the surveys as it is the most important lemon cultivar grown in South Africa, constituting 75 % of South African commercial lemon plantings (CGA 2013). Study sites for both trapping and fruit sampling were selected in Mpumalanga and Limpopo provinces due to the presence of all four fruit fly pests in these regions and both being major citrus

Table 1. Locations and characteristics of study sites used in surveys of natural fruit fly infestation of Eureka lemons between February 2016 and May 2017. Presented weather data are from daily records during the Eureka lemon harvesting periods (between February and June of each study year). Weather data were from stations that were within 30 km of the study sites. Sites that were 40 km from each other shared data from one weather station. Weather stations were maintained by the Agrometeorology Programme of the Soil, Climate and Water Division of the Agricultural Research Council, Stellenbosch, South Africa.

Province	Farm name (Weather Station Identification Number)	GPS coordinates	Altitude (m a.s.l.)	Mean daily maximum temperature (°C)	Mean daily minimum temperature (°C)
Limpopo	Ambrosia Citrus Estate (30950)	S24°21′22.7″ E30°54′48.8″	505	29.3 ± 0.3	16.3 ± 0.2
	Schoeman Boerdery (30103)	S25°04′54.7″ E29°25′46.1″	896	31.6 ± 0.3	17.3 ± 0.2
	Schoonbee Boerdery	S25°03′48.3″ E29°24′23.9″	892	Same as Schoeman Boerdery	
	Unifrutti	S24°22′15.2″ E30°43′07.4″	495	Same as Ambrosia Citrus Estate	
	Van Veijeren Boerdery (30748)	S23°38′10.2″ E30°40′53.6″	433	30.6 ± 0.3	14.7 ± 0.3
Mpumalanga	Bakgat Boerdery (30785)	S25°22′51.7″ E30°32′00.5″	957	26.7 ± 0.3	14.3 ± 0.2
	Daarbo Boerdery (30420)	S25°32′57.1″ E31°14′36.7″	776	29.4 ± 0.3	14.2 ± 0.3
	Fountains	S25°18′53.2″ E31°02′15.8″	853	Same as Daarbo Boerdery	
	Ryton Estates (30903)	S25°36′41.2″ E30°29′37.2″	1086	24.9 ± 0.3	9.6 ± 0.3
	Siyalima Boerdery (30765)	S25°41′09.3″ E31°11′05.0″	555	28.0 ± 0.2	14.1 ± 0.2

production regions. Weather data were collected from stations located within 30 km of the study sites.

Fruit fly trapping

Five attractant-based traps were used for monitoring of adult female and male fruit fly pest populations in each Eureka lemon orchard. Two of these attractant-based traps were used for monitoring of adult female fruit flies. The first attractant was Questlure (Green Trading (Pty) Ltd, South Africa) which contains protein hydrolysate (2.0 g) and alpha-cypermethrin (0.006 g) in a sponge fitted inside a plastic capsule. Questlure was placed inside a Sensus trap (River Bioscience (Pty) Ltd, Port Elizabeth, South Africa), which is a clear bucket-type trap (8.6 cm height, a top diameter of 9.4 cm and a bottom diameter of 5.7 cm) with a transparent plastic bottom and a blue overhanging lid with 12 rectangular openings (0.7 cm × 0.8 cm), evenly spaced just underneath the lid (1.6 cm). The second attractant was three-component Biolure (Chempac (Pty) Ltd, Suider Paarl, South Africa) consisting of ammonium acetate, trimethylamine and putrescine. The three-component Biolure was placed inside a Chempac Bucket trap (Chempac (Pty) Ltd, Suider Paarl, South Africa), which is a McPhail type of trap, consisting of a yellow cylindrical base with an opaque lid (14.7 cm height and an external diameter of 13 cm). The trap has three openings (2.8 cm in diameter) on the top periphery of the yellow base.

Three of the attractant-based traps were used for monitoring of adult male fruit fly pests. Two of these traps targeted male *Ceratitis* species: (1) EGO Pherolure (Insect Science (Pty.) Ltd, Tzaneen, South Africa), which consisted of enriched ginger root oil, and (2) Capilure (Green Trading (Pty) Ltd, South Africa), which contains the male attractant trimedlure [tert-butyl 4 (and 5) - chloro-2-methylcyclohexane-1-carboxylate] (1.8 g active ingredient). EGO Pherolure and Capilure were each placed inside a Sensus trap. One yellow Lynfield trap (River Bioscience (Pty) Ltd, Port Elizabeth, South Africa) baited with methyl eugenol (ME) (Invader-Lure, River Bioscience (Pty) Ltd, Port Elizabeth, South Africa) was used to monitor *B. dorsalis* males.

Each attractant-based trap contained a 3 g dichlorvos (195 g/kg of active ingredient) strip to kill any attracted flies. In each lemon orchard, each trap was suspended on a tree in the shade at about 1.5 m above ground. The five attractant-based traps were placed along one selected row with a distance of approximately 30 m between the traps. Trapping was initiated from the beginning of February 2016 and traps were maintained and serviced monthly until May 2017. All traps were checked and emptied monthly. Dichlorvos and all attractants except EGO Pherolure were changed on a monthly basis. EGO Pherolure was changed every six months as per the recommendation of the manufacturer. Flies collected from the traps were identified and categorised according to species and sex.

Fruit sampling

At harvest between February and June of each citrus production season, Eureka lemons were picked from trees in the same orchards where the traps were placed. The lemons were taken to Citrus Research International (CRI) laboratories in Nelspruit. The collected fruit was first graded according to rind colour using Set Number 37 of the Colour Prints for Blemish and Appearance Standards (1997), published by CRI, endorsed by the Department of Agriculture, Forestry and Fisheries, South Africa, and reproduced in Moore *et al.* (2015). The colour grades in Set Number 37 range from completely yellow (colour grade 1) to completely green (colour grade 8), with grades 2 to 4 being predominantly yellow and grades 5 to 7 predominantly green.

The fruit were then dipped for 1 min in a fungicidal mixture to prevent fungal growth during storage before examination. The fungicide solution was a combination of Sporekill (120 g/l didecyldomethylammonium chloride, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa) at 1 ml/l and Citricure (210 g/l guazatine, ICA International Chemicals Pty. Ltd.) at 48 ml/l. The fungicidal mixture is regularly used in studies on post-harvest disinfestation treatments for fruit flies (Grout *et al.* 2011b). The fruit were then covered with a fine mesh material (3 mm mesh size) in order to prevent fruit fly infestation during storage and kept in a room at 25.4 °C ± 0.0 °C between 1 and 34 days before examination for fruit fly eggs and larvae. During fruit examination, the fruit were first peeled to determine presence of fruit fly eggs in the flavedo and albedo. Thereafter the fruit were cut to determine presence of larvae in the pulp. A headband magnifier (Opti Visor, Donegan Optical Co., U.S.A.) was used for detec-

tion of eggs and larvae in the different fruit regions.

Tests under semi-natural field conditions for infestation by C. capitata *and* B. dorsalis

Study sites

One of the previously described Eureka lemon orchards, Ryton Estates, Mpumalanga Province, was used for tests under semi-natural field conditions. Tests were carried out between 8 and 12 May 2017. During the tests the mean daily maximum temperature was 23 ± 0.5 °C and the mean daily minimum temperature was 6.6 ± 1.0 °C. There was no rain during the tests.

Insect materials

Ceratitis capitata and *B. dorsalis* originating from colonies maintained at CRI, Nelspruit, were used in the tests. Colonies of *C. capitata* were maintained for over 200 generations. Colonies of *B. dorsalis* were maintained for over 15 generations. Colonies were refreshed with wild males reared from fruit every two years. *Ceratitis capitata* was reared on a bran-based diet and *B. dorsalis* was reared on a carrot-based diet. For *C. capitata*, 9–12-day-old mated females were used. For *B. dorsalis*, 21–24-day-old mated females were used.

Before the test, males and females were kept together and fed with a mixture of sugar and yeast hydrolysate mixture in a ratio of 3:1 plus water *ad libitum*. Mating was observed during the day for *C. capitata* between 09:00 and 15:00 as from the third day after adult emergence. For *B. dorsalis*, mating was observed at dusk between 17:30 and 18:00 at the end of the second week after adult emergence. Mating couples found were gently captured in a vial and transferred to a different cage to ensure that all females used were mated. Flies were kept in the laboratory with natural light conditions at 20.4 ± 0.2 °C until use.

No choice semi-field tests

For each fruit fly species, five Eureka lemon trees bearing fruit at picking ripeness were selected in one row in the study orchard. On each selected tree, two separate branches, each with five unharvested and undamaged Eureka lemon, were selected on two opposite sides (northern and southern sides) of the tree. Each branch was covered with a cylindrical wire frame mesh cage of 43.5 cm in diameter and 52.5 cm in height. Two control cages for each fly species were also set up on each tree, on opposite sides. Each control cage consisted of a branch on which five harvested undamaged Golden Delicious apples, *Malus domestica* Borkh, were suspended. For each fruit fly species, there were therefore 10 cages with lemons and 10 cages with the control fruit, representing 10 replicates for each treatment. All cages were covered on top with a transparent plastic material to protect the cages from rain. The cages were secured from ants by smearing petroleum jelly on the branches just outside of the sleeve cages. Leaves from other branches touching the sleeve cages were trimmed.

For *C. capitata*, 25 mated females were released into each cage. For *B. dorsalis*, seven mated females were released into each cage. A lower number of *B. dorsalis* were used in the tests, due to fewer mated females being available. In each cage, flies were provided with water, granulated sugar and yeast hydrolysate *ad libitum*. Flies were left exposed to the fruit for 4 days. Mortality was recorded daily from each cage and dead mated females were replaced by mated females of the same ages in order to maintain a constant fruit fly pressure in each cage.

Four days after exposure, Eureka lemon fruit and control fruit from each branch were brought back to the laboratory at CRI, Nelspruit, weighed individually and incubated individually in aerated plastic containers over a layer of sterilised sand. A sample of 10 Golden Delicious apples used for the control cages were also incubated individually in the laboratory to determine natural infestation of the control fruit. The containers were maintained in a room at 25.9 ± 0.0 °C for 10 weeks to ensure larval development. Fruit samples were checked daily for pupal and adult emergence. Pupae found were placed in plastic Petri dishes on a moist filter paper. Emerged flies were kept for 3 days to allow for full colour development before being killed and identified. Fruit were dissected to determine presence of fruit fly larvae before being discarded.

During the trial, three samples of 12 Eureka lemon fruit from the test site were brought back to the laboratory at CRI, Nelspruit, on three separate dates (8 May 2017, 10 May 2017 and 12 May 2017) and were analysed for physical and chemical characteristics. Each fruit was weighed and the equatorial diameter was measured. The peel thickness was measured using a Vernier calliper. The

juice was extracted from each sample of 12 fruit. The Brix degrees, acidity and pH of the juice were measured by a refractometer, titration with NaOH and a pH meter.

Data analysis

Adult field population data for males and females of *B. dorsalis*, *C. capitata*, *C. rosa*, *C. quilicii* were summarised as flies per trap per day (FTD) and flies per trap per month (FTM). The FTD data were log $(x + 1)$ transformed. A log-linear model assuming a Poisson distribution of the response variable was used to analyse the effect of farm and sampling time on catches of the different fruit fly groups. Analysis of female fruit fly catches was only done for the three-component Biolure trapping system due to very low catches obtained with the Questlure traps. Analysis of male *C. quilicii* and *C. rosa* catches was only done for the EGO Pherolure trapping system due to very low catches of these fruit fly groups obtained in the Capilure trapping system.

Level of infestation in Eureka lemons sampled and examined at harvest was summarised as percentage fruit fly infestation. In tests under semi-natural conditions, the levels of infestation of lemon fruit and control fruit were determined as pupae per fruit and adults per fruit.

RESULTS

Adult fruit fly populations in lemon orchards

The presence of *B. dorsalis*, *C. capitata, C. rosa* and *C. quilicii* in Eureka lemon orchards was confirmed by trapping (Fig. 1 and Fig. 2). All target fruit fly species except males of *C. rosa* were trapped in all lemon orchards. *Ceratitis rosa* males were not captured in two of the lemon orchards in Limpopo Province (Schoeman Boerdery and Schoonbee Boerdery). The two fruit fly species which dominated the overall catches in most lemon orchards were *B. dorsalis* and *C. capitata*.

Peaks of *B. dorsalis* and *C. capitata* male catches occurred during the harvesting period of Eureka lemon (between February and June) (Fig. 1). Catches of *B. dorsalis* males in ME baited traps were not significantly different between lemon orchards ($\chi^2 = 12.00$, d.f. = 9, $P = 0.21$) but were significantly different across time ($\chi^2 = 53.17$, d.f. = 15, $P < 0.0001$) and peaked between February and April. Catches of *C. capitata* males in EGO Pherolure baited traps were significantly different between orchards ($\chi^2 = 19.83$, d.f. = 9, $P = 0.02$) and across time ($\chi^2 = 24.46$, d.f. =14, $P = 0.04$), peaking between April and August in the first study year. In Capilure baited traps, there were also significant differences in catches of *C. capitata* males between orchards ($\chi^2 = 19.79$, d.f. = 9, $P = 0.02$) but no significant differences in catches across time ($\chi^2 = 17.11$, d.f. = 15, $P = 0.31$). For *C. quilicii* and *C. rosa* males there were significant differences in catches between orchards (*C. quilicii*: $\chi^2 = 25.01$, d.f. = 9, $P = 0.00$; *C. rosa*: $\chi^2 = 51.99$, d.f. = 9, $P < 0.05$) but not across time (*C. quilicii*: $\chi^2 = 16.94$, d.f. = 14, $P = 0.26$; *C. rosa*: $\chi^2 = 9.76$, d.f. = 14, $P = 0.78$).

There were no significant differences in catches of female *B. dorsalis*, *C. capitata* or *C. rosa s.l.* in Biolure baited traps between orchards (*B. dorsalis*: $\chi^2 = 7.60$, d.f. = 9, $P = 0.58$; *C. capitata*: $\chi^2 = 7.36$, d.f. = 9, $P = 0.60$; *C. rosa s.l.*: $\chi^2 = 15.71$, d.f. = 9, $P = 0.07$). There were also no significant differences in catches of female fruit flies over time (Fig. 2) (*B. dorsalis*: $\chi^2 = 5.59$, d.f. = 15, $P = 0.99$; *C. capitata*: $\chi^2 = 6.81$, d.f. = 15, $P = 0.96$; *C. rosa s.l.*: $\chi^2 = 15.15$, d.f. = 15, $P = 0.44$).

Sampling of export grade lemons

No live or dead fruit fly larvae were found in 43 222 Eureka lemons inspected (Table 2), despite the presence, and in some cases high abundance of female fruit flies in the orchards during the harvesting period (February to May/June) of each year (Table 2). Eureka lemons obtained from the orchards at harvest in both years were mostly between colour grades 5 and 6 (predominantly green) (Table 3).

Tests under semi-natural field conditions for infestation by *C. capitata* and *B. dorsalis*

There was no infestation of Eureka lemons by *B. dorsalis* and *C. capitata* in the additional trials under semi-natural field conditions (Table 4). The Eureka lemons tested were between colour grades 4 and 6 (based on Set Number 37 of the Colour Prints for Blemish and Appearance Standards 1997 published by CRI and endorsed by the Department of Agriculture, Forestry and Fisheries, South Africa). Based on samples of Eureka lemons collected within the same colour grade range in the test orchard, the mean weight of Eureka lemon fruit at this ripeness condition was 121.51 ± 4.4 g. The mean equatorial diameter was 61.44 ± 0.80 mm. The peel thickness was

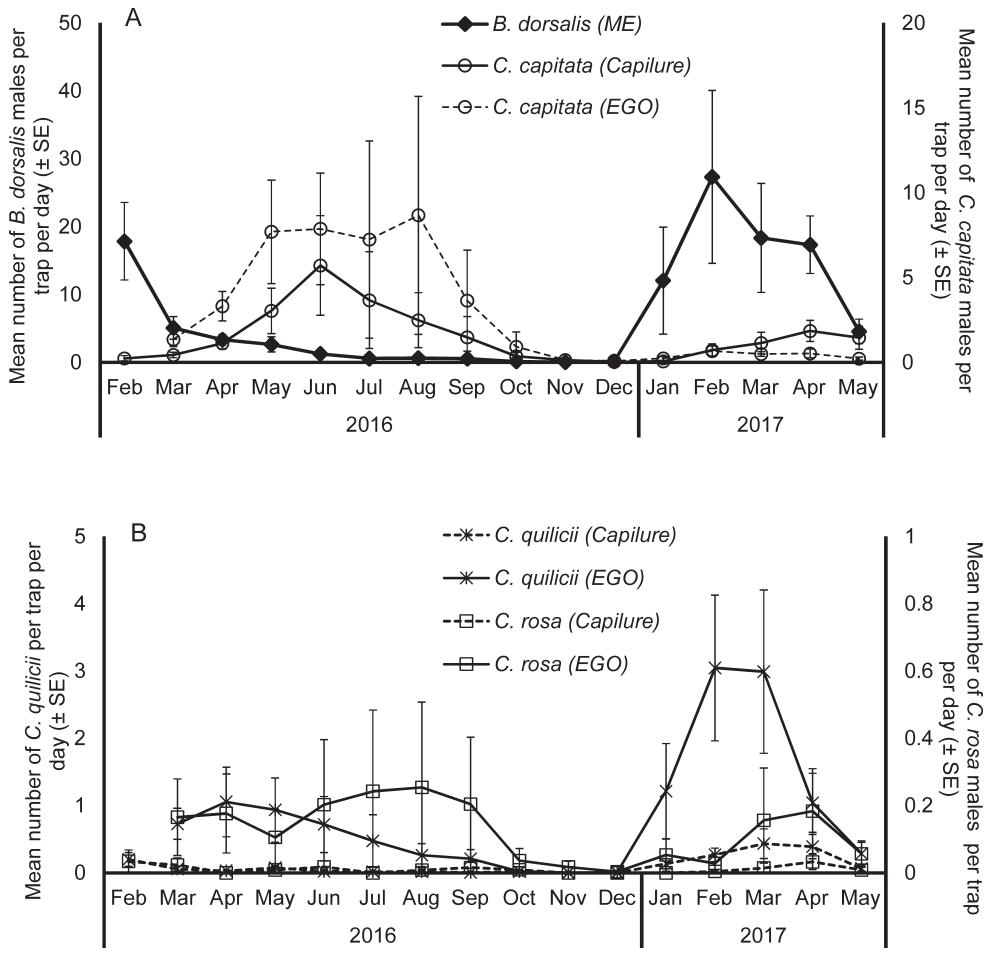

Fig. 1. Daily mean catches of male (**A**) *Bactrocera dorsalis* and *Ceratitis capitata*; and (**B**) *Ceratitis quilicii* and *C. rosa* in male attractant-based traps in Eureka lemon orchards in Limpopo and Mpumalanga provinces, South Africa, between February 2016 and May 2017. *Bactrocera dorsalis* males were targeted using methyl eugenol (ME) baited Lynfield traps. *Ceratitis capitata*, *C. quilicii* and *C. rosa* were targeted using Capilure baited and EGO Pherolure baited Sensus traps.

5.37 ± 0.13 mm. The mean Brix degree values, percentage acidity and pH were 8.07 ± 1.36, 6.76 ± 0.31 and 2.36 ± 0.02, respectively.

In the trial on *C. capitata*, the percentage infestation of the control fruit (Golden Delicious apples) was 42 % whilst in the trial on *B. dorsalis* the percentage infestation of the control fruit was only 8 %. The percentage infestation of a sample of Golden Delicious apples that was not exposed to fruit flies was 0 %. The mean pupal developmental time (days from incubation to first pupal formation) of *C. capitata* in the control fruit was 18.95 ± 0.87 days. For *B. dorsalis*, the mean pupal developmental time on the control fruit was 10.00 ± 0.00 days.

During the trial, zero or very low mortality was observed in cages with *C. capitata*. In cages with *B. dorsalis*, fly mortality was observed in five out of 10 cages with the control fruit on the second day of the trial. In each of these cages, except for one, there was one dead female which was then replaced. In one cage, there were three dead *B. dorsalis* females on the second day of the trial, which were then replaced.

DISCUSSION

The absence of fruit fly infestation in 43 222 Eureka lemons sampled in commercial orchards in this study clearly demonstrated that commercial,

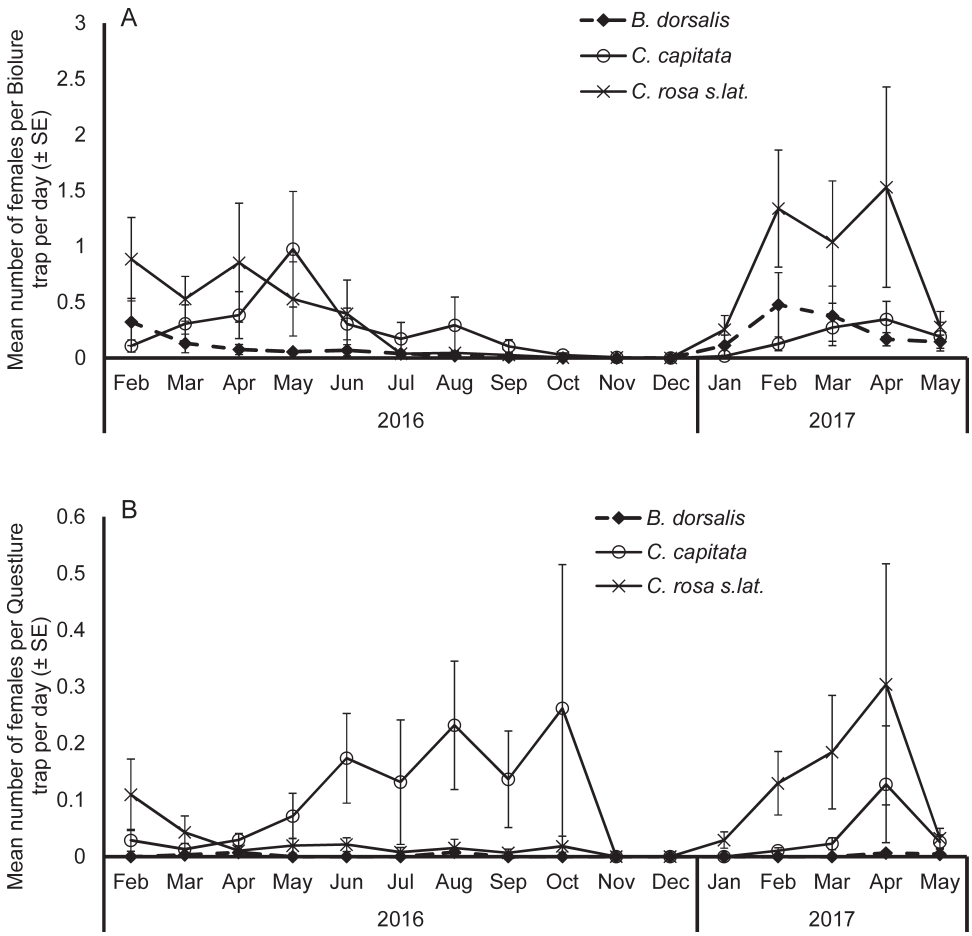

Fig. 2. Daily mean catches of female *Bactrocera dorsalis*, *Ceratitis capitata* and *C. rosa s.l.* in (**A**) three-component Biolure and (**B**) Queslure baited traps in Eureka lemon orchards in Limpopo and Mpumalanga provinces of South Africa between February 2016 and May 2017.

export grade, Eureka lemon is not a host for *B. dorsalis*, *C. capitata*, *C. quilicii* or *C. rosa*. In order to demonstrate a 99.99 % non-host level with 95 % confidence, 30 000 fruit should be tested (Follett & Hennessey 2007). Based on the number of Eureka lemon tested in this study, we demonstrated a 99.99 % non-host status of Eureka lemon to fruit fly pests in South Africa at a 99 % confidence level (Couey & Chew 1986; Follett & Hennessey 2007). The non-host status of many other crop pest combinations have similarly been established on the basis of such data and the analysis thereof (Armstrong 1991; Hennessey *et al.* 1992; Moore *et al.* 2015; Pringle *et al.* 2015).

The resistance of Eureka lemon to fruit fly infestation occurred despite the presence of *B. dorsalis*, *C. capitata*, *C. quilicii* and *C. rosa* in the area as demonstrated by catches of female and male flies of these species in traps placed in the same Eureka lemon orchards. The occurrence of such catches of fruit flies in non-host Eureka lemon orchards can be attributed to adult fruit flies using habitats to not only search for egg laying sites but to also forage for food, water and mates (Prokopy & Roitberg 1984). In studies on *C. capitata* in a heterogeneous region in central Israel, Israely *et al*. (1997) trapped significant numbers of the pest in the non-host English walnut, *Juglans regia* L. The trapping of *C. capitata* in English walnut was related to the presence of honeydew deposits on these trees by aphids (Israely *et al.* 1997), with honeydew representing a natural protein and sugar source for adult flies (Neilson & Wood 1966). It is likely that the Eureka lemon orchards were used as

Table 2. Fruit fly infestation in export-grade Eureka lemons at harvest and trap catches of female fruit flies during the harvesting period (February to May/June) in 10 commercial orchards on 10 farms in Mpumalanga and Limpopo provinces, South Africa, in 2016 and 2017.

Farm name	Sampling date	Number of Eureka lemon fruit examined	% fruit fly infestation	Mean catch of female fruit flies per trap per month (combined Biolure and Questlure catches) during Eureka lemon harvesting period (February–May/June)		
				B. dorsalis	*C. capitata*	*C. rosa s.l.*
Ambrosia	30 March 2016	1732	0	0.1 ± 0.1	2.4 ± 1.5	0.5 ± 0.3
Citrus Estate	7 February 2017	3419	0	0.5 ± 0.2	0.4 ± 0.4	0.0 ± 0.0
Schoeman Boerdery	16 May 2016	1636	0	0.3 ± 0.2	2.6 ± 0.8	0.3 ± 0.2
	19 April 2017	2185	0	0.3 ± 0.3	9.7 ± 3.7	0.6 ± 0.4
Schoonbee Boerdery	5 April 2016	1750	0	0.5 ± 0.3	1.1 ± 0.6	0.0 ± 0.0
	19 April 2017	1996	0	0.3 ± 0.2	1.9 ± 0.9	0.2 ± 0.1
Unifrutti	3 April 2016	1497	0	0.1 ± 0.1	0.9 ± 0.5	0.0 ± 0.0
	6 February 2017	2636	0	0.3 ± 0.3	0.6 ± 0.4	0.0 ± 0.0
Van Veijeren Boerdery	30 March 2016	2210	0	0.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0
	27 March 2017	2119	0	0.1 ± 0.1	1.5 ± 0.6	0.3 ± 0.2
Bakgat Boerdery	29 April 2016	1588	0	3.0 ± 1.4	20.9 ± 14.0	3.5 ± 2.3
	8 March 2017	2647	0	2.7 ± 2.1	7.8 ± 4.5	4.4 ± 3.8
Daarbo Boerdery	11 April 2016	2192	0	8.8 ± 4.1	19.2 ± 5.9	2.4 ± 0.8
	2 May 2017	2601	0	23.0 ± 9.6	11.6 ± 3.9	0.9 ± 0.4
Fountains	30 March 2016	1741	0	0.7 ± 0.4	3.0 ± 1.9	0.5 ± 0.5
	1 March 2017	2674	0	4.5 ± 0.5	0.5 ± 0.3	0.2 ± 0.2
Ryton Estates	7 June 2016	1625	0	0.9 ± 0.4	14.0 ± 7.0	1.2 ± 0.8
	18 April 2017	2466	0	2.2 ± 1.6	0.2 ± 0.2	0.0 ± 0.0
Siyalima Boerdery	8 March 2016	2135	0	2.8 ± 1.7	0.4 ± 0.1	1.1 ± 0.9
	8 March 2017	2373	0	10.1 ± 5.4	0.8 ± 0.5	0.4 ± 0.1

habitats for other resources, like food and shelter, by these fruit fly species.

Following the steps in determination of host status of fruit to fruit flies in ISPM 37 (FAO 2016), for *C. rosa* and *C. quilicii* there was no need to conduct field surveys of fruit infestation given that there are no existing records of these pests on lemons, and as such lemons would immediately qualify as a non-host for these two species. The results from this study nonetheless reinforce the non-host status of lemon for *C. rosa* and *C. quilicii*.

For *C. capitata* and *B. dorsalis*, species where there are some historical records of having been reared from lemons, albeit without evidence of any naturally occurring infestation of commercial export grade lemons, the absence of infestation of commercially produced export grade Eureka lemons in the larval field survey establishes the non-host status of this cultivar for these pests,

Table 3. Colour grading of Eureka lemons examined for fruit fly infestation (based on Set Number 37 of the Colour Prints for Blemish and Appearance Standards 1997 published by CRI and endorsed by the Department of Agriculture Forestry and Fisheries, South Africa). The colour ranged from 2 (Yellow) to 7 (Green), with colours closer to 2 being more yellow than green and colours closer to 7 being more green than yellow.

Colour grade	Number of Eureka lemon examined for fruit fly infestation/sampling year	
	2016	2017
2	0	1996
3	4	0
4	1676	0
5	5093	11239
6	7836	11830
7	3497	0
Unspecified	0	51

Table 4. Mean number ± S.E. of pupae and adults per fruit in attached undamaged Eureka lemons and detached undamaged Golden Delicious apples exposed separately to 25 mated *Ceratitis capitata* or seven mated *Bactrocera dorsalis* females over four days under semi-natural field conditions.

Fruit fly species	Mean number of pupae per fruit		Mean number of adults per fruit	
	Eureka lemon	Apple	Eureka lemon	Apple
C. capitata	0.00 ± 0.00	8.00 ± 2.51	0.00 ± 0.00	6.04 ± 1.81
B. dorsalis	0.00 ± 0.00	0.94 ± 0.49	0.00 ± 0.00	0.46 ± 0.31

based on ISPM 37 (FAO 2016). Nonetheless, trials under semi-natural conditions for *B. dorsalis* and *C. capitata* were conducted as confirmation of the non-host status of Eureka lemon for these two species. Absence of infestation of Eureka lemon by *C. capitata* and *B. dorsalis* was confirmed in these semi-natural tests. The control fruit used in these tests, Golden Delicious apple, has been recorded as a host for both *B. dorsalis* and *C. capitata* (Clarke *et al.* 2005; De Meyer *et al.* 2002), and was one of the few host fruit common to these two species which was in season at the time of the study (in May). Infestation of the control fruit by the two species was recorded in the semi-natural trials, albeit at a low level. The rate of infestation of *C. capitata* in the control fruit compared well to natural infestation rates of the same cultivar by *C. capitata* in Greece (between 4.2 and 18.8 pupae per fruit) (Papadopoulos *et al.* 2002). Apple was reported to be a less preferred host for *B. dorsalis* in southwestern China (Ye & Liu 2005), which could explain the low infestation rates of apple by *B. dorsalis* in this study.

The resistance of lemons to fruit fly infestation has been attributed to chemicals in the peel (Back & Pemberton 1915; Greany *et al.* 1983; Salvatore *et al.* 2004). Oil from the rind of oranges and lemons was found to cause high mortality of *C. capitata* eggs (Back & Pemberton 1915). Citral, coumarins and linalool found in lemon peel extracts were found to have significant larvicidal activity when tested on *C. capitata* (Salvatore *et al.* 2004). Concentrations of citral and coumarins were, however, found to decrease in the peel after harvest (Salvatore *et al.* 2004). The flavedo of 'Eureka' and 'Lisbon' lemons were found to be thicker than those of other citrus varieties and lemons were found to have more oil per unit area of peel than other citrus varieties (Greany *et al.* 1983). The latter authors suggested that the flavedo thickness and amount of oil in the peel could render lemons resistant to fruit fly infestation.

The categorisation of lemons as a non-host for fruit flies is relevant to international phytosanitary trade regulation. In 2008, the United States Department of Agriculture (USDA) convened an expert panel to assess the host status of lemons for fruit flies and the panel concluded that green lemons were not a host for *C. capitata* (USDA 2008). This conclusion was based on an evaluation of over 90 publications and reports with regard to host status of lemons for *C. capitata* (USDA 2008). The panel considered the potential susceptibility of lemons to *C. capitata* to be associated with over-ripeness and high *C. capitata* population pressure (USDA 2008). The Animal and Plant Health Inspection Service (APHIS) of USDA subsequently applied this recognition of non-host status of lemons to new lemon fruit import regulations, as is evident in a pest risk analysis conducted on the import of citrus from Uruguay (USDA-APHIS 2012). In 2013, USDA promulgated a final rule which included authorising the importation of lemon fruit from Uruguay (Federal Register 2013). The final rule in the USA Federal Register (Volume 78, No. 132), specifies that lemons from Uruguay, may be imported into continental U.S.A. if harvested when green and do not require disinfestation treatment for *C. capitata* and the South American fruit fly, *Anastrepha fraterculus* (Wiedemann) (Federal Register 2013). USDA APHIS also conducted a pest risk analysis on the import of lemons from Argentina (USDA-APHIS 2015). In 2016, USDA promulgated a final rule in the US Federal register (Volume 81, No. 247) to authorise import of lemons from Argentina (Federal Register 2016). In the final rule, it was stipulated that lemons harvested green and within a specified time period, can be imported into U.S.A. without the need for application of a disinfestation treatment (81 Federal Register 2016).

The findings in this study provide (1) experimental evidence, compliant with international

standards for phytosanitary measures, that commercially produced export standard Eureka lemon fruit, qualifies as a non-host for *B. dorsalis*, *C. capitata*, *C. quilicii* and *C. rosa* and, (2) technical justification for exempting South African commercially produced export grade Eureka lemons from post-harvest fruit fly disinfestation treatments.

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