

A survey of *Ceratitis quinaria* (Bezzi) (Diptera: Tephritidae) in citrus production areas in South Africa

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The five-spotted fruit fly, *Ceratitis quinaria* (Bezzi) (Diptera: Tephritidae), is distributed in parts of eastern and western Africa, northern Africa and southern Africa (including South Africa). The species also occurs outside of Africa, in Yemen. The host range of *C. quinaria* is narrow, with *Mangifera indica* L. being the main host of commercial importance. *Ceratitis quinaria* was claimed to be associated with citrus in Sudan although this claim was never substantiated and there has never been any confirmed reared record of *C. quinaria* on citrus despite numerous surveys of citrus across Africa. In order to verify the type of association that *C. quinaria* has with citrus, field surveys were carried out in South Africa to determine the distribution and seasonal occurrence of *C. quinaria* in commercial citrus orchards and the possible natural infestation of citrus by this species. Surveys on distribution of *C. quinaria* across South Africa were carried out in 1999 and 2000 by trapping with Ceratitislure (containing protein hydrolysate and β -caryophyllene). The seasonal occurrence of *C. quinaria* in the northern areas of South Africa was determined over two years between 2015 and 2017 by trapping with Enriched Ginger Oil (EGO), a male lure containing α -copaene. In the same trapping period, citrus and other fruit were sampled to determine infestation by *C. quinaria*. Additionally between 2009 and 2018, citrus fruit was sampled from the trees and ground in other commercial and non-commercial areas in the north of South Africa. All fruit samples collected were incubated for at least five weeks to allow rearing of flies to the pupal and adult stages. Trapping surveys conducted between 1999 and 2000 showed the presence of *C. quinaria* only in the northern areas of South Africa. Catches of *C. quinaria* males in EGO-baited traps were low in commercial citrus orchards (peak of catches being lower than 0.05 flies/trap/day). Catches of *C. quinaria* were mainly recorded outside of the citrus ripening period. No *C. quinaria* was reared from any of the citrus fruit sampled, even in those areas where the presence of the species was demonstrated by catches in EGO-baited traps. The fruit surveys therefore demonstrated the absence of natural infestation of citrus with *C. quinaria* in South Africa and supported existing biological information that citrus is not a host for *C. quinaria*.

Key words: five-spotted fruit fly, citrus, trapping, fruit sampling, rearing.

INTRODUCTION

Ceratitis quinaria (Bezzi) (Diptera: Tephritidae), also known as the five-spotted fruit fly, belongs to the subgenus *Ceratalaspis* Hancock (De Meyer 1998; White & Elson-Harris 1994). As per the name, the species is differentiated from other *Ceratalaspis* species by the presence of five separate spots on the scutellum (Fig. 1) (De Meyer 1998). *Ceratitis*

quinaria is found in southern Africa (including South Africa), parts of East and West Africa, northern Africa (Ethiopia and Sudan) and Yemen (De Meyer & White 2004). The male attractants for this species are terpinyl acetate and Enriched Ginger Oil (EGO) (Manrakhan *et al.* 2017; Mwatawala *et al.* 2013; White & Elson-Harris 1994). Catches of the



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Fig. 1. *Ceratitis quinaria* (Bezzi) male (G. Goergen, IITA Cotonou). The image clearly illustrates the five spots on the scutellum (the region at the posterior end of the thorax) that are characteristic for the species.

species have also been recorded in protein-baited traps (Vayssières *et al.* 2007).

In Mali, *C. quinaria* was found to infest commercial mango (Vayssières *et al.* 2007). Other known host fruit species for *C. quinaria* are guava (*Psidium guajava* L.), peach (*Prunus persica* (L.) Batsch), apricot (*Prunus armeniaca* L.), cashew (*Anacardium occidentale* L.), Christ's thorn jujube (*Ziziphus spina-christi* (L.) Desf.), tallow wood (*Ximenia americana* L. var. *americana*) and shea tree (*Vitellaria paradoxa* C.F. Gaertn.) (De Meyer *et al.* 2002; De Meyer & White 2004).

In dealing with fruit fly observations in the Sudan during a survey conducted in 1966, Venkatraman & Khidir (1967) reported a direct host association between citrus fruit and *C. quinaria*, but this record comprises an anecdotal two-sentence statement contained in a two page report and is considered doubtful. The authors provided only a vague description of the fruit fly. The specimen was reported to be distinguished from *Ceratitis capitata* (Wiedemann) by its colour and size. No reference was made to the highly obvious and characteristic five scutellar spots of the species. The report indicates that adult flies were collected, seemingly with trimedlure trapping, but *C. quinaria* does not respond to trimedlure (White & Elson-Harris 1994). Venkatraman & Khidir (1967) also mentioned that they

reared flies from infested fruit in the laboratory. However, no voucher specimens for this record could be traced in any of the natural history museums or research institutions in Africa or the northern hemisphere (Europe/North America) to verify the identification.

There have been a number of surveys carried out across Africa to determine fruit flies infesting citrus (Mwatawala *et al.* 2006; Umeh *et al.* 2008; Mwatawala *et al.* 2009; N'dépo *et al.* 2010; Vayssières *et al.* 2010; Foba *et al.* 2012; Bawa *et al.* 2017; Theron *et al.* 2017). There was no record of *C. quinaria* in citrus in any of these surveys. In the comprehensive annotated check list of host plants for *Ceratitis* species in Africa, *C. quinaria* was listed as an associated species with *Citrus* sp. (De Meyer *et al.* 2002). De Meyer *et al.* (2002) defined an association as either an ambiguous record or a specimen found resting or collected in traps on the plant species. This type of association was clearly demarcated from confirmed rearing records (De Meyer *et al.* 2002). Despite the fact that there has never been any confirmed rearing of *C. quinaria* from citrus, national plant protection organisations of some countries and the global database of the European Plant Protection Organisation (<https://gd.eppo.int>) list citrus as a host for *C. quinaria*.

In order to verify the type of association that

C. quinaria has with citrus, surveys were carried out to determine (1) the distribution and seasonal occurrence of *C. quinaria* in citrus production areas in South Africa and (2) its presence in citrus fruit.

MATERIAL AND METHODS

Trapping surveys between 1999 and 2000

Between 1999 and 2000, trapping surveys were carried out to determine the distribution of *C. quinaria* in different citrus production areas across South Africa (Table 1). In each area, sites within mainly citrus plantings were selected. Two Sensus bucket traps were placed, each baited with Ceratitislure, which consisted of protein hydrolysate and β -caryophyllene (traps and Ceratitislure were both supplied at the time by Quest Developments, South Africa). The Sensus trap is a bucket 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 \times 0.8 cm) evenly spaced just underneath the lid (1.6 cm). Ceratitislure is an attractant developed for monitoring of *Ceratitis* species and was found to be effective for *Ceratitis cosyra* (Walker) (Grout et al. 2011), another species in the *Ceratalaspis* group present in South

Africa and closely related to *C. quinaria* (De Meyer 1998; Virgilio et al. 2017). Each trap contained a dichlorvos (195 g/kg of active ingredient) strip to kill any attracted flies. In the first year, traps were serviced once a month for three months between June and August (during citrus harvesting times). In the second year, traps were serviced monthly for a longer period (up to six months before peak of citrus harvest) with some sites starting as early as January. During trap servicing, traps were emptied and the flies were transported to a laboratory at Citrus Research International (CRI), Nelspruit, for identification to species and sex. The dichlorvos strips and Ceratitislure were replaced every eight weeks.

Trapping and fruit sampling surveys between 2015 and 2016

The seasonal occurrence of *C. quinaria* and infestation of citrus and other fruit by this species was determined by surveys carried out between 2015 and 2016 in two of the northern provinces of South Africa; Limpopo and Mpumalanga. Surveys were carried out in four selected commercial citrus farms (Van Veijeren Landgoed: 23°38'45.6"S 30°42'30.1"E; Letaba: 23°51'14.3"S 30°14'54.7"E; Crocodile Valley: 25°27'38.8"S 31°03'13.3"E;

Table 1. Catches of *Ceratitis quinaria* in Ceratitislure-baited Sensus traps in different citrus production areas in South Africa between 1999 and 2000. Trapping of *C. quinaria* was carried out for a period of 3 months between June and August in 1999. In 2000, trapping was conducted between January and July, depending on areas.

Province	Area	GPS coordinates	<i>C. quinaria</i> flies/trap/day (trapping year)	
			1999	2000
Limpopo	Tshipise	22°40'47.5"S 30°05'03.4"E	NA*	0.00
	Letsitele	23°52'01.2"S 30°19'04.9"E	NA	0.00
	Hoedspruit	24°22'58.0"S 30°47'05.1"E	NA	0.00
Mpumalanga	Groblersdal	25°01'13.8"S 29°24'02.4"E	0.01	0.00
	Nelspruit	25°28'17.3"S 31°04'12.7"E	0.00	0.00
	Hectorspruit	25°25'11.3"S 31°40'08.8"E	0.00	0.00
KwaZulu-Natal	Nkwalini	28°45'11.2"S 31°31'46.6"E	0.00	NA
Eastern Cape	Fort Beaufort	32°46'33.9"S 26°38'08.7"E	0.00	NA
	Sundays River Valley	33°29'43.7"S 25°37'17.0"E	0.00	0.00
	Gamtoos River Valley	33°50'34.5"S 24°52'22.3"E	0.00	0.00
Western Cape	Knysna	34°00'06.8"S 23°00'16.7"E	0.00	0.00
	Paarl	33°43'26.1"S 18°57'14.8"E	0.00	NA
	Clanwilliam	32°09'26.6"S 18°53'17.5"E	NA	0.00
	Citrusdal	32°37'46.4"S 19°01'02.5"E	NA	0.00

*NA: not available.

Vergenoeg: 25°20'19.7"S 31°55'21.3"E). In the commercial farms, different citrus types were cultivated with the dominant citrus species being sweet orange (*Citrus sinensis* (L.) Osbeck). In one of the commercial citrus farms – Van Veijeren Landgoed, mango (*Mangifera indica* L.) was also cultivated in close proximity (within 1 km of trapping sites). In each commercial farm, three Sensus traps (River Bioscience (Pty) Ltd, Port Elizabeth, South Africa) baited with EGO Pherolure (Insect Science (Pty) Ltd, Tzaneen, South Africa) were allocated at random in each of three randomly selected rows. EGO Pherolure contained Enriched Ginger Oil (EGO) lure, which is a known male attractant for *C. quinaria*. EGO Pherolure was dispensed from a bulb-like septum with each septum containing 2 ml of lure. Each trap contained a dichlorvos strip. Traps were serviced on a monthly basis. During trap servicing, traps were emptied and the flies were transported to a laboratory at CRI Nelspruit, for identification. Dichlorvos strips were changed once a month while EGO Pherolure was changed every 24 weeks as per product label. In the laboratory, specimens were identified to species and sex. *Ceratitis quinaria* adults were confirmed by the presence of morphological characters provided in the key by De Meyer (1998).

During the trapping period, citrus and other fruit found on the ground in or near the trapping area were collected (Table 2) and taken to CRI Nelspruit for determination of fruit infestation. Fruit were counted, weighed and placed in containers covered with gauze. Fruit were incubated over a thin layer of sterilised sand. All fruit samples were kept for at least eight weeks in a room at ambient temperature. Containers were checked regularly for adult emergence. Emerged tephritid flies were aspirated and placed in an aerated container with water, sugar and yeast extract before being killed, identified, sexed and counted.

Trapping and fruit sampling surveys between 2016 and 2017

Trapping and fruit sampling surveys to determine seasonal occurrence of *C. quinaria* and fruit infestation by this species expanded and continued from June 2016 until May 2017. This time, surveys were carried out in nine commercial citrus farms (Ryton Estates: 25°36'10.8"S 30°27'59.0"E; Joubert & Seuns Citrus: 25°24'37.5"S 30°37'31.1"E;

Siyalima Boerdery: 25°41'08.9"S 31°11'26.6"E; Vergenoeg (same coordinates as mentioned above); Whisky: 25°23'09.7"S 31°51'18.3"E; Hectorspruit: 25°27'32.3"S 31°40'16.1"E; Schoeman Boerdery: 25°02'02.6"S 29°24'17.9"E; Van Veijeren Boerdery (same coordinates as mentioned above) and Unifrutti: 24°22'13.0"S 30°42'51.5"E) located in the Mpumalanga and Limpopo Provinces of South Africa. In all farms, trapping with EGO Pherolure baited Sensus traps (three traps per farm) and fruit sampling (Table 2) were carried out as described above.

Fruit surveys 2011–2018

As part of a host survey on economically important fruit fly species in South Africa undertaken by the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC), Nelspruit, South Africa, mature fruit of different citrus types were sampled from the ground and trees from two non-commercial sites in Mpumalanga Province (ARC-TSC: 25°27'19.2"S 30°58'12.6"E; Bushbuckridge: 24°50'03.5"S 31°04'15.6"E) as well as non-commercial (Levubu: 23°03'45.9"S 30°03'39.8"E) and commercial orchards in Limpopo Province (Hoedspruit: 24°22'45.5"S 30°52'55.7"E; Letsitele: 23°38'12.1"S 30°40'45.3"E) from 2009 to 2018. Collected fruit were transported to laboratories at Tzaneen and the ARC-TSC Nelspruit, weighed and counted. Fruit were placed on chicken wire mesh in 5- to 10-litre cylindrical plastic containers containing sterilised sand. Mesh fitted lids were placed on containers to ensure enough ventilation. Fruit were held at room temperature for approximately 8 weeks.

Another small survey of citrus fruit from the area surrounding Pretoria (between latitudes 25°41'S and 25°48'S and longitudes 28°03' and 28°17'E), Gauteng Province, South Africa, was performed during the citrus fruiting season in September 2018 by the Department of Zoology and Entomology at the University of Pretoria. Citrus fruit were sampled from trees in home gardens (non-commercial) in the area. Collected fruit were washed in an antifungal solution (1 ml/l water; Sporekill, ICA International Chemicals (Pty) Ltd, Stellenbosch, South Africa), weighed and counted. Fruit were placed in black plastic seedling trays with additional holes drilled into the base. These were suspended over dry sand in white plastic crates. The white plastic crates were covered and sealed with white voile curtain fabric

for ventilation. Fruit were held in a climate room maintained at $25 \pm 1^\circ\text{C}$ for approximately 5 weeks.

In both fruit surveys, each container with incubated fruit was checked for presence of pupae and adult fruit flies at the end of the incubation period. Flies found were identified and counted.

Data analysis

In trapping surveys conducted in 1999 and 2000, the relative abundance of *C. quinaria* was presented for each area. Data for all sites and all trapping months in an area were pooled due to very low catches of the target species. For trapping surveys conducted between 2015 and 2017, annual profiles of abundance of *C. quinaria* were presented with data analysed only from orchards where *C. quinaria* was trapped. Orchards were

considered as replicates. All trapping data were presented as flies/trap/day. This was calculated as the total number of flies collected over the total number of traps and over the total number of trapping days.

Fruit infestation data were presented as the total number of flies per species that emerged from each fruit type. The degree of fruit fly infestation of a fruit type was presented as the number of flies/kg of sampled fruit.

RESULTS AND DISCUSSION

In all trapping surveys, very few *C. quinaria* were captured in different citrus growing areas in South Africa (Table 1, Fig. 2 and Fig. 3). In 1999, *C. quinaria* was only trapped in Groblersdal located in the

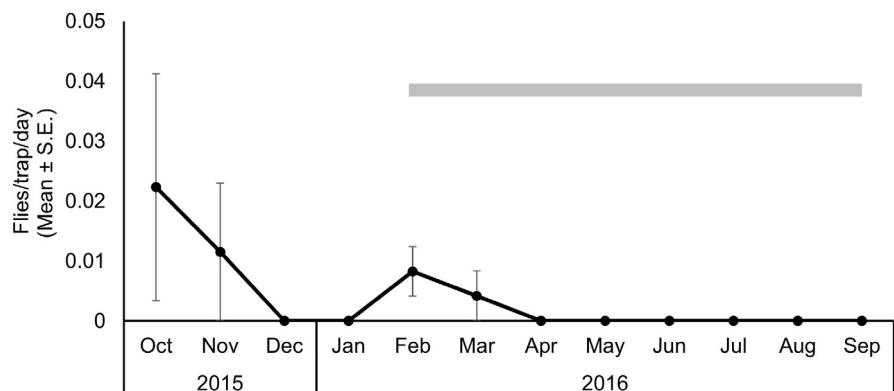


Fig. 2. Seasonal variation in catches of males of *Ceratitis quinaria* in EGO lure-baited Sensus traps in three commercial citrus orchards of Limpopo and Mpumalanga between October 2015 and September 2016 where *C. quinaria* was trapped. No captures of *C. quinaria* were recorded in one of the commercial orchards sampled (Vergenoeg). Citrus harvest period is indicated by a grey bar.

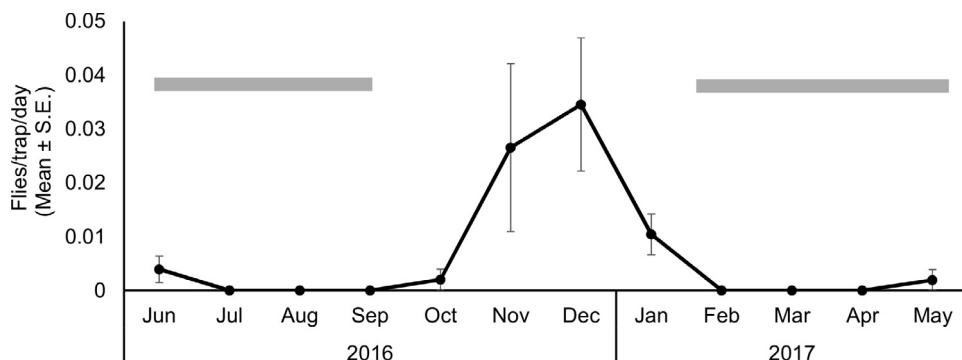


Fig. 3. Seasonal variation in catches of males of *Ceratitis quinaria* in EGO lure-baited Sensus traps in six commercial citrus orchards of Limpopo and Mpumalanga between June 2016 and May 2017 where *C. quinaria* was trapped. During this survey, no captures of *C. quinaria* were recorded in three commercial orchards sampled: Ryton Estates, Joubert & Seuns Citrus and Siyalima Boerdery. Citrus harvest period is indicated by grey bars.

north of South Africa (Table 1). In 2000, no *C. quinaria* was trapped in any of the sampled sites (Table 1).

In the survey conducted between 2015 and 2016 in the northern areas of South Africa, *C. quinaria* was only trapped in three out of four farms. In the survey conducted between 2016 and 2017, *C. quinaria* was trapped in six out of nine farms. For both survey periods, in farms where *C. quinaria* was trapped, there was no increase in abundance of *C. quinaria* during the citrus ripening and harvesting season, which is between February and September (Fig. 2 and Fig. 3). This is in contrast to profiles of catches of fruit-infesting fruit flies such as *C. capitata* on citrus or *Bactrocera dorsalis* (Hendel) on mango (Katsoyannos *et al.* 1998; Vayssières *et al.* 2009; Manrakhan *et al.* 2017). For those species, male and female catches were found to increase during fruit-ripening periods.

The fruit sampling survey conducted between 2015 and 2017 in commercial citrus orchards where *C. quinaria* was trapped showed no infestation of citrus by this species (Table 2). The species was also not reared from other fruit types sampled in the survey between 2015 and 2016 (Table 2). During fruit surveys conducted between 2009 and 2018 in Limpopo, Mpumalanga and Gauteng provinces, no *C. quinaria* was reared from a total of 76.4 kg of *C. sinensis*, 38.0 kg of *Citrus limon* (L.) Burm. f., 15.4 kg of *Citrus paradisi* MacFad, 0.6 kg of *Citrus reticulata* Blanco, 0.3 kg of *Citrus japonica* Thunb. and 1.5 kg of *Citrus jambhiri* Lush. sampled in commercial and non-commercial sites.

Ceratitidis quinaria was also never reared in surveys of citrus carried out in Tanzania, Ivory Coast and Benin where the species is also known to occur (Mwatawala *et al.* 2006; N'dépo *et al.* 2010; Vayssières *et al.* 2010). In a recent survey of fruit flies infesting citrus in the northern areas of South Africa, no *C. quinaria* was recorded in any of the citrus sampled (Theron *et al.* 2017). The only reliable host records for *C. quinaria* from South Africa are for *P. persica* (De Meyer *et al.* 2002) from Roodeplaat (near Pretoria) and Nylsvley Reserve (near Mookgopong/Naboomspruit), in the northern parts of South Africa, dating back to 1957 and 1959, respectively. In South Africa, *C. quinaria* has also been reported from *P. armeniaca*, *P. guajava* and *Ficus* sp. (unknown locality in South or southern Africa, given as 'widespread bushveld species' by Hancock (1989)). The latter records, however, require confirmation.

Based on its reared records from nine species and seven plant families excluding citrus (Family Rutaceae) (De Meyer *et al.* 2002; De Meyer & White 2004), *C. quinaria* fits in the category of polyphagous or generalist species which has the ability to exploit plants in many families (Aluja & Mangan 2008; Clarke 2017). *Ceratitidis quinaria*, however, does not exhibit extreme polyphagy (Normark & Johnson 2011) in comparison to its congener *C. capitata* which has a record of more than 140 species in more than 40 families in Africa (De Meyer *et al.* 2002). So *C. quinaria* can be categorised as an intermediate polyphage. Polyphagous fruit flies were shown to be able to exploit fruit outside of their normal host range in absence of their preferred hosts, albeit under laboratory conditions with detached, cut and pierced fruit (Fitt 1986). For highly polyphagous fruit flies, the acceptance of fruit outside of their host range was suggested to be linked to their ability to be less discriminatory in their recognition of host volatiles and their continuous oocyte development (Bernays 2001; Fitt 1986). Oocyte development in the absence of preferred hosts may, however, not be continuous for all polyphagous species. *Bactrocera jarvisi* (Tryon), an intermediate polyphage similar to *C. quinaria*, was found to inhibit oocyte development in the absence of its preferred host and this was in contrast to the highly polyphagous *Bactrocera tryoni* (Froggatt) which continued oocyte development and was able to readily accept fruit outside of its normal host range, particularly when deprived of its known hosts for a few days (Fitt 1986).

The hypothetical question that arises is whether the intermediate polyphagous *C. quinaria* could explore citrus as a novel host in case of shortage of its known hosts in the area. There are two factors that favour a negative answer to the above question. First, the observed low abundance of *C. quinaria* in citrus orchards in the northern areas of South Africa in combination with the mismatch of its phenology and ripening of citrus render the exploration of mature citrus fruit as a novel host by *C. quinaria* highly unlikely due to the lack of co-occurrence in time of the species and the fruit at the mature stage. Co-occurrence of the plant (host) and insect in time and space is one of the steps involved in a novel herbivore–plant interaction (Pearse *et al.* 2013). The second factor that favours a negative answer to the above hypothetical question is the mechanical and chemical resis-

Table 2. Fruit fly infestation of fruit collected from the ground from commercial citrus farms in Mpumalanga and Limpopo provinces, South Africa, between October 2015 and May 2017 where *C. quinaria* was trapped.

Province	Farm	Sampling period	Fruit species	Total weight of fruit sampled (kg)	Fruit fly infestation level (flies/kg of sampled fruit)	Infestation level by <i>C. quinaria</i> (C. quinaria/kg of sampled fruit)	Total number of <i>C. quinaria</i> trapped over one year (3 traps per site)
Mpumalanga	Crocodile Valley	2015–2016	<i>C. paradisi</i> <i>C. sinensis</i> <i>Sclerocarya birrea</i> (A. Rich.) Hochst.	1.10 20.95 4.96	0.00 0.00 0.81*	0.00 0.00 0.00	1
Hectorspruit		2016–2017	<i>C. sinensis</i>	1.02	0.00	0.00	6
Vergenoeg			<i>C. sinensis</i>	2.87	0.00	0.00	10
Whisky			<i>C. sinensis</i>	2.71	0.00	0.00	9
Limpopo	Letaba	2015–2016	<i>C. sinensis</i> <i>Citrus</i> sp. <i>M. indica</i>	27.01 14.81 1.08	0.00 0.00 0.00	0.00 0.00 0.00	3
	Unifrutti	2016–2017	<i>C. limon</i> <i>C. sinensis</i>	13.98 1.49	0.00 0.00	0.00 0.00	3
Schoeman Boerdery		2016–2017	<i>C. limon</i> <i>C. sinensis</i>	18.65 3.09	0.00 0.00	0.00 0.00	3
Van Veijeren Boerdery		2015–2016	<i>C. limon</i> <i>C. paradisi</i> <i>C. sinensis</i> <i>Ficus</i> sp. <i>Mangifera indica</i> L. <i>S. birrea</i>	10.73 11.30 4.26 4.75 22.41 15.56	0.00 0.00 0.00 0.00 6.17** 3.08***	0.00 0.00 0.00 0.00 0.00 0.00	11
		2016–2017	<i>C. limon</i> <i>C. paradisi</i> <i>C. sinensis</i>	16.30 4.74 3.13	0.00 0.00 0.00	0.00 0.00 0.00	16

*Emerged adults identified as *C. cosyra*.

**Emerged adults identified as *Bactrocera dorsalis* (Hendel) and *C. cosyra*.

***Emerged adults identified as *C. cosyra* and *Ceratitis rosa* Karsch.

tance of intact citrus fruit particularly in the peel region to attack by fruit flies (Greany *et al.* 1983). Citrus would therefore not be a susceptible fruit for *C. quinaria* to explore in case of shortage of fruit within its host range.

Reliable host records for pests are important to support appropriate phytosanitary decision-making (Aluja & Mangan 2008). As such, natural host records should only be based on original results of fruit surveys that test direct host associations. It is important to avoid referencing databases as evidence of host status if they are not based on reliable original data that positively establishes direct host association.

An international standard providing guidelines for determination of host status of fruit to fruit flies – International Standard for Phytosanitary Measures (ISPM) 37 – was recently adopted (FAO 2016). The steps involved in the determination of host status of fruit to fruit flies involve (1) the collection of existing biological and historical information to establish natural non-host or host status and (2) in case biological and historical information are inconclusive, to conduct field surveillance and field trials to confirm non-host, host or conditional host status. In the case of *C. quinaria*, there was already sufficient evidence in existing biological information (absence of confirmed rearing records from citrus despite numerous surveys of citrus across Africa) that there is no natural infestation of citrus by this species and that citrus qual-

fies, as such, as a non-host for *C. quinaria*. The fruit surveys carried out over a span of nine years in different regions in the northern parts of South Africa further confirmed the absence of natural infestation of citrus by *C. quinaria*.

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