

OLIVE FRUIT (*OLEA EUROPAEA* L.) AS A HOST OF QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT) IN SOUTH EASTERN AUSTRALIA

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Summary

There is limited historical information for olive fruit as a host for Queensland fruit fly (*Bactrocera tryoni*). In 2015, five suspect samples from informal surveys of olive fruit from the Sunraysia district were examined for *B. tryoni* and found positive. Larval and adult identifications were confirmed using both morphological and molecular methods. Olive fruit were found to support and produce between 3.8 and 32.5 adults per kilogram of fruit, which is comparable to some citrus. There is a need to further develop a fruit fly standard for a host susceptibility index or host potential index. If producers are developing a systems approach to manage fruit fly, they need to be aware that olive fruit can act as an alternative host for *B. tryoni*.

Keywords fruit fly host, morphological identification, molecular, host susceptibility index

INTRODUCTION

Olive fruit, *Olea europaea* subsp. *europaea* L. (Oleaceae), have been grown in Australia since the early 1800's (Hamilton *et al.* 2011). The original commercial introduction was in about 1880, but the Australian olive fruit industry was slow to develop (Mailer *et al.* 2010, Spennemann and Allen 2000). In the early 1990's, olive fruit production rapidly increased with an estimated 12,000 tonnes of oil produced in 2008 (Mailer *et al.* 2010). Production of olive oil has increased to service the human health and cooking industries because olive oil has a high percentage of unsaturated fatty acids, phytosterols and other components that are important in human health and nutrition (Mailer *et al.* 2010). Olive fruit growing areas are mostly climatically equivalent to the traditional olive fruit growing areas in Europe, particularly the Mediterranean climate. Environments tend to be semi-arid to subhumid, with hot dry summers and winter dominant rainfall (Spennemann and Allen 2000). The states of Western Australia, South Australia, New South Wales (NSW) and Victoria are the main Australian olive fruit production areas (Mailer *et al.* 2007).

In many regions of the world, olive fruit are infested by fruit flies (Diptera: Tephritidae). In Tunisia, olive fruit fly (*Bactrocera oleae* Rossi) has been reported to infest olive fruit affecting the quantitative and qualitative composition of the oil (Mraicha *et al.* 2010). *Bactrocera oleae* is currently distributed through the Mediterranean basin and western Asia (Ramezani *et al.* 2015), South and Central Africa, California and Central America (Daane and Johnson 2010), but is not known to occur in Australia.

Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) is recognised as a pest of olive fruit (Hancock *et al.* 2000) although in Australia this species only occurs in Western Australia and sporadically in South Australia (Dominiak and Mapson 2017).

In eastern Australia, Queensland fruit fly, *Bactrocera tryoni* (Froggatt) is the most important fruit fly pest of horticulture (Dominiak and Daniels 2012). Much of the early fruit fly research focused on major commercial crops and particularly on higher value or more susceptible hosts. There are up to 243 known or suspected commercial and native *B. tryoni* host plants (Hancock *et al.* 2000). However, interactions of *B. tryoni* with less susceptible hosts, such as grapes, have only recently been considered (Dominiak, 2011). New hosts of *B. tryoni* are occasionally detected (Reynolds *et al.* 2015). Within the Oleaceae, olive fruit were not considered to be a host of *B. tryoni* (Anon. 1996), and consequently did not require postharvest treatment. Hancock *et al.* (2000) reviewed the historical reports of *B. tryoni* and reported that olive fruit (*O. europaea* and *Notelaea longifolia* Vent.) were hosts of *B. tryoni* based on May (1953). *Notelaea longifolia* is known as "large mock-olive" and is a shrub associated with rainforests in far north Queensland. However, May (1953) claimed that *O. europaea* was not usually infested, and that *N. longifolia* was a host. This view was based on Tryon (1927) who stated that *N. longifolia* was an indigenous host and *Olea europaea* L. was a cultivated host. May (1957, 1960) subsequently added other *B. tryoni* host details but there was no further clarification regarding olive fruit. In Victoria,

O'Loughlin (1964) also claimed olive fruit were a host of *B. tryoni* based on May (1953). The Australian Plant Pest Database (APPD 2015) has only a single example of *B. tryoni* being recorded in olive fruit: this was for "Oleaceae, *Olea*". The sample from Roma in Queensland was collected in 1925 and identified by R. Veitch. The understanding of olive fruit as a potential host for *B. tryoni* is therefore not clear, based on the scant historical data.

The Sunraysia district in north west Victoria is normally free from *B. tryoni*. In response to a high number of detections of *B. tryoni* in monitoring traps in Sunraysia after 2010-2011 (Dominiak and Mapson 2017), samples of suspect fruit including olive fruit were sent to diagnostic laboratories to establish if larval stages were present. In March 2015, olive fruit submitted by a member of the general public were found to contain *B. tryoni* larvae. Subsequent sampling in March, April and May 2015 was undertaken to obtain additional data. The main aim of this paper is to report on findings pertaining to olive fruit as a host of *B. tryoni* and to gain some indication of the number of fruit flies that could complete their life cycle from olive fruit.

MATERIALS AND METHODS

Olive fruit samples were collected from the Sunraysia region on the Murray River. In particular, the centre of a *B. tryoni* outbreak in Mildura (Victoria) and the adjacent town Buronga (NSW) were sampled five times between March and May 2015 (Table 1). All samples of suspect fruit were sent to the Diagnostic Laboratory, Agriculture Victoria, AgriBio, for morphological and molecular testing. Three small initial samples "1 - 3" (in March) were collected during fruit fly monitoring by Agriculture Victoria and two larger follow up samples "4 - 5" (in April and May) were collected by NSW Department of Primary Industries for identification and rearing of adults.

Rearing process and morphological identification

Fly specimens in the large follow up samples "4" and "5" were reared from olive fruit arranged in a single layer on paper towels above a layer of vermiculite, at 22°C, 60% humidity, and with a 16L:8D photoperiod. Immediately upon receipt olive fruit from sample 4 were held at +4°C for 4 days while an adequate rearing room and container were prepared for the sample. The adults were identified using dichotomous

keys and features described in Drew (1989), White and Elson-Harris (1992) and Plant Health Australia (2011). Larvae were identified morphologically using White and Elson-Harris (1992).

Molecular testing

DNA-based identification/confirmation was also conducted due to uncharacteristic colour variation in some adults, the number of pupae that failed to produce adults, and the number of larvae that were received in poor condition. DNA was extracted from specimens from each sample and sequenced for a section of the Cytochrome Oxidase I (COI) gene or Cytochrome b (Cytb) gene, or both (Table 2), following the methods outlined in Blacket *et al.* (2012). It was found necessary to sequence both the COI and Cytb genes for some samples, as the results obtained initially using only the COI gene produced an initial "uncertain" identification result (Table 2). Molecular species identification involved comparing the sequences obtained with other species on the public databases GenBank & BOLD Systems as outlined in Blacket *et al.* (2012).

Host susceptibility index

The host susceptibility index is the number of adult flies produced per gram of fruit subjected to an initial infestation rate of one egg per gram of fruit (Lloyd *et al.*, 2013). These conditions are usually controlled in a laboratory. Our samples were naturally infested in the field at an unknown rate, however, the number of adults emerging per gram of olive fruit was calculated. For sample 4 and 5, the number of adults was divided by the weight of olive fruit in the sample. Weights were not recorded in samples 1, 2 and 3, so the number of adults per olive was calculated (Table 1).

RESULTS

Morphological Identification

Larvae from the initial samples (1, 2 and 3) were morphologically identified as *B. tryoni* (Table 1). However, there was very little additional collection data available for these early samples. Follow up larval specimens from samples 4 and 5 were reared through to adults and identified as outlined below (Table 1). Larval specimens and reared adults from these samples (1 to 5) are lodged in the Victorian Insect Collection, Agriculture Victoria.

Table 1. Date of sampling, weight of sample or number of olive fruit, and number of each *B. tryoni* life stage recorded.

Sample number, location and date of collection	Description	Weight of sample and/ or number of olive fruit	Number and life stage/s	Number of specimens per gram or number per olive fruit
Sample 1 Mildura 24 March 2015	Olive fruit handed in by a member of the public *	Not recorded	29 larvae (3 rd instar)	NA
Sample 2 Buronga 26 March 2015	Olive fruit	8 x olive fruit	56 larvae (3 rd instar)	7 larvae per olive fruit
Sample 3 Buronga 26 March 2015	Olive fruit	1 x olive fruit	7 larvae (1x 2 nd instar, 6x 3 rd instar)	7 larvae per olive fruit
Sample 4 Buronga 16 April 2015	Olive fruit collected from the ground	144 x olive fruit; 1.23 kg	13 larvae 12 adult females 28 adult males	0.0325 adults per gram or 0.37 <i>B. tryoni</i> per olive fruit
Sample 5 Buronga 4 May 2015	Olive fruit	278 x olive fruit; 2.08 kg	>160 larvae, 4 adult females 4 adult males	0.0038 adults per gram** or 0.6 <i>B. tryoni</i> per olive fruit

* no further details were recorded

** high larval mortality in transit affected the number of adults emerging from this sample

Sample 4

Olive fruit, collected from the ground around a single tree on the border of a commercial plantation of olive fruit and near domestic citrus trees, were submitted on 16 April 2015. Adults emerged between 1 and 19 May 2015. Prior to rearing, 13 larval specimens, apparently 3rd instar *B. tryoni*, were removed from the sample and preserved in either 70% (for morphological examination) or 100% ethanol (for molecular analysis). For emerged flies, colouration differences were noted in these specimens compared with the description of *B. tryoni* in Drew (1989) (absence of a distinct medial longitudinal black marking on the pronotum). Molecular testing was therefore conducted to help confirm the species identification. One larva and five adult flies were tested using molecular methods. Additionally, following clean-up of the rearing container, further specimens were found dead and identification to species could not be confirmed based on morphology due to the poor condition of specimens.

Sample 5

Olive fruit were submitted on 5 May 2015. Upon receiving the olive fruit samples, many of the larvae

appeared to be dead (bloated and no longer moving). These larvae were removed and preserved in 70% ethanol. The preserved larvae (161 specimens) were identified as *B. tryoni* (3rd instar). Flies mostly emerged between 20 May – 18 June. Additionally, following clean-up of the rearing container, further specimens were found dead and morphological identification to species could not be confirmed due to their poor condition.

Molecular Identification

Tephritidae specimens from all five of the olive fruit samples tested using molecular methods were found to match the *B. tryoni* complex (i.e. they were positive for *B. tryoni*, $n = 6$ larvae & 7 adults, Table 2). Four larvae were confirmed as *B. tryoni* using the COI gene but results for the remaining specimens were uncertain. Two larvae and all seven adults were further tested using the Cytb gene and subsequent results obtained from these samples provided a positive match with the *B. tryoni* complex (Table 2). The colouration differences noted previously appear to be due to the teneral status of the adult flies, which had only recently emerged.

Table 2. DNA barcoding results for *B. tryoni* complex (following Blacket *et al.* 2012) samples from olive fruit.

Sample	Lifestage	COI	Cytb	Molecular Identification
1	Larva	+	not tested	<i>B. tryoni</i>
	Larva	+	not tested	<i>B. tryoni</i>
2	Larva	uncertain	+	<i>B. tryoni</i>
	Larva	uncertain	+	<i>B. tryoni</i>
3	Larva	+	not tested	<i>B. tryoni</i>
4	Larva	+	not tested	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>
5	Adult	uncertain	+	<i>B. tryoni</i>
	Adult	uncertain	+	<i>B. tryoni</i>

Legend:

“+” indicates that a DNA sequence was successfully obtained for this locus/specimen;

“uncertain” indicates DNA sequence was obtained but resulted in an inconclusive match with *B. tryoni*;

“not tested” indicates there was no DNA sequence obtained for this locus/specimen.

DISCUSSION

This is the first paper in nearly a century to provide data on olive fruit as a host for *B. tryoni*. Importantly, we have confirmed in this small study that olive fruit can support *B. tryoni* and are an alternative host. Identification of *B. tryoni* was confirmed through both morphological and molecular methods. DNA sequence identification (DNA barcoding) confirmed larval and teneral adult identifications. The initial uncertain results obtained using COI were likely due to the presence of a nuclear copy of the COI gene being amplified (i.e., a numt) as in Blacket *et al.* (2012), Krosch *et al.* (2018). The most complete emergence data was obtained from Sample 4, with emergence continuing for 19 days. Given that rearing was undertaken in controlled conditions, the trigger to the variability of adult emergence times must have occurred in the field. We suspect this could be caused by developmental differences between individuals or by differences in the timing of egg laying in the field.

Regarding the potential of olive fruit to host *B. tryoni*, one large (7.5 – 8.5 grams) olive fruit can host up to at least seven 3rd instar larvae. We calculated that one kilogram of olive fruit can support the emergence of between 3.8 and 32.5 adult *B. tryoni* (Table 1).

Past fruit fly research has focused on pest distribution (Dominiak and Daniels 2012), host range (Hancock *et al.* 2000) and control options (Dominiak and Ekman 2013). However, little work has been done on the probability of infestation, or on the potential of hosts

to produce variable numbers of fruit flies. This current work has at least provided an initial evaluation of the potential of olive fruit to produce *B. tryoni*. Similar work needs to be conducted on commercial and native hosts to better understand the capacity to produce pest fruit flies.

Surveillance for alternative hosts for *B. tryoni* remains important for NSW, Victoria and any production regions producing fruit fly free commodities, particularly with the globalisation of trade. The regional fruit fly eradication scheme began in 1996 and led to the establishment of the Fruit Fly Exclusion Zone (FFEZ). However, following the wettest two-year period on record (Webb, 2012), the legislation supporting the movement controls in the FFEZ was withdrawn in NSW in July 2013 and on May 2015 in Victoria (Dominiak and Mapson 2017). Domestic trade has since moved away from regional pest freedom programs to localised fruit fly management programs such as areas of low pest prevalence (Dominiak *et al.* 2015).

Many growers are not aware that olive fruit could act as a host for *B. tryoni* and hence olive trees do not receive any fruit fly control treatments. Our findings will inform growers that hosts such as olive trees do need to be considered in fruit fly management, either for spraying or removal if the trees are unwanted. Information about additional hosts of *B. tryoni* may assist in maintaining domestic and international market access into the future. Additionally, feral olive

trees arising from seed dispersed by fruit-eating birds and foxes are becoming a problem (Spennemann and Allen 2000). These feral trees may harbour diseases and pests of commercial plantings (Mailer *et al.* 2010). Feral olive trees are also a serious threat for indigenous woodland floras as olive trees shade out native species (Hamilton *et al.* 2011).

Our results of olive fruit as a host of *B. tryoni* need to be placed in context. Firstly, the ability of *B. tryoni* to complete its life cycle under natural conditions are consistent with the definition within ISPM37 for the determination of host fruit to fruit flies (ISPM 2016). While our work has found olive fruit were a host of *B. tryoni*, we also aimed to assess how effective olive fruit were as a host. It should also be noted that olive fruit are a later season host and are unlikely to contribute to fruit fly risk for early season crops such as cherries. Lloyd *et al.* (2013) noted that there was no international standard to determine a host susceptibility index (HSI) for Queensland fruit fly. They proposed and defined a HSI as the number of adult flies produced per gram of fruit that was subjected to an infestation rate of one egg per gram of fruit. While our results and those of Lloyd *et al.* (2013) were obtained under different conditions, there are valid grounds for comparisons to be made. Based on our data, olive fruit would have a HSI range 0.0038 to 0.0325. This may be a conservative estimate, since sample 5 returned a very low HSI due to many of the 161 larvae recovered from the sample, apparently dying before assessment could be completed. Our preliminary HSI results reported here compare to 0.005 for lemons (*Citrus limon* L.), a

recognised poor host, and to 0.026 for Ellendale mandarin (*C. reticulata* Blanco), which was the most susceptible citrus species assessed by Lloyd *et al.* (2013). For non-citrus, fruit with the highest HSI were guava (*Psidium guajava* L.) with 0.318, cherry guava (*P. littorale* Raddi) with 0.226 and mulberry (*Morus nigra* L.) with 0.209. Based on the HSI of Lloyd *et al.* (2013) and our results, olive fruit have a similar susceptibility to citrus generally and a much lower susceptibility than some other fruit. While we have used the HSI of Lloyd *et al.* (2013), Bellamy *et al.* (2013) used the Host Potential Index (HPI) based on the number of insects per kilogram. If the HPI was used, olive fruit would have a HPI of between 3.8 and 32.5.

Olive fruit could be regarded as an alternative but not a major host. It is recognised that susceptibility of different fruit commodities changes seasonally during the year. More research needs to be conducted on HSI or HPI and the role that these parameters might play in a systems approach for the management of fruit fly (Dominiak, 2019). Any future index standards should include laboratory and field standards.

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