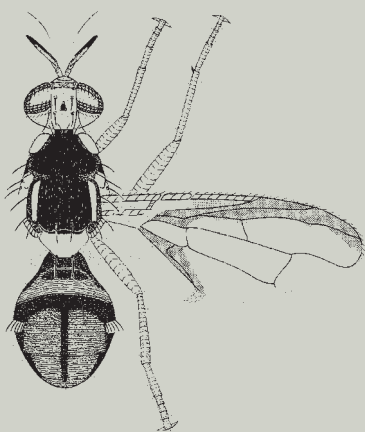# THE AUSTRALIAN HANDBOOK FOR THE IDENTIFICATION OF FRUIT FLIES

Version 1.0

Species: Bactrocera bryoniae
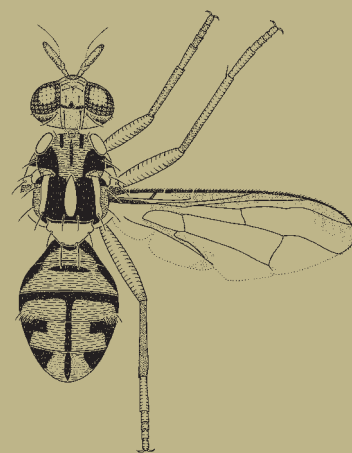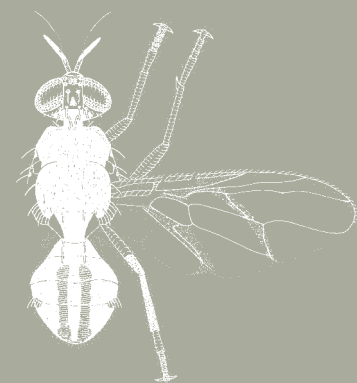
Species: Bactrocera frauenfeldi
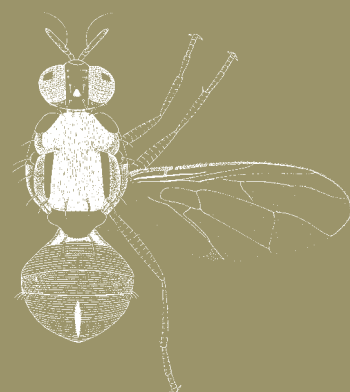
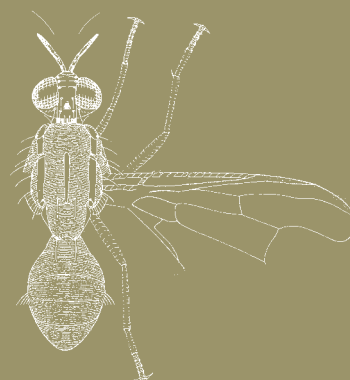*Species: Bactrocera kandiensis*

Species: Bactrocera tau

Species: Bactrocera trilineola

Species: Bactrocera umbrosa

Species: Bactrocera xanthodes

Species: Bactrocera newmani

Australian Government
Department of Agriculture, Fisheries and Forestry

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An electronic copy of this plan is available from the website listed above.

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This document has been made possible through consultation with and input from the following fruit fly entomologists, scientists, academics and diagnosticians:

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2   Foreword

The accurate identification of fruit flies is a key component of Australia's biosecurity system that underpins the domestic movement of fruit and vegetables, maintains international market access for Australian producers and protects Australia's borders from exotic pest incursion.

In Australia's tropics, routine surveillance of coastal and island communities results in a requirement to process and identify thousands of adult flies per hour. In some parts of southern Australia fruit fly sampling numbers are smaller, however diagnosticians still have to be skilled and equipped to identify a single fly of economic importance amongst a large range of native fruit flies that have no impact on commercial fruits and vegetables.

For the first time a document has been produced that integrates all the diagnostic techniques currently used in Australia for the identification of fruit flies. A new set of descriptions and photographs have been prepared to assist the identification of flies by adult morphology. In addition, current protocols used for the identification of fruit flies using molecular biology techniques are presented.

This document has been written by Australia's fruit fly diagnosticians for diagnosticians and it is my hope that the dialogue, sharing of information and experience, and constructive discourse that has resulted in this new publication will continue to grow. Together the combined expertise and knowledge of Australia's fruit fly researchers, academics, surveillance officers, diagnosticians and laboratory scientists make up a formidable national resource, which when networked and coupled with extensive fruit fly reference collections, provides a world-class national capability.

This valuable document provides a useful benchmark against which future updates and revisions can be developed and training programs can be delivered.

I would like to thank all the entomologists and scientists who have brought this document together and have the greatest pleasure in endorsing its adoption and use by practitioners and jurisdictions in Australia.

Professor Dick Drew

International Centre for Management of Pest Fruit Flies

Griffith University

3   Preface

The Australian Handbook for the Identification of Fruit Flies (v1.0)

- Was written by diagnosticians for diagnosticians;

- Collates current and existing practices and knowledge into a single document;

- Pools experience from Australia's network of fruit fly experts;

- Establishes a resource that can support and develop the confidence and expertise of all users;

- Provides a mechanism to possibly identify future information and research needs; and,

- Considers the potential of both morphological and molecular techniques.

The Handbook has been an important part of building a network of fruit fly diagnosticians across Australia and it is hoped that both the network and this document continue to grow and develop in the future. We also welcome feedback from fruit fly experts around the world.

The Handbook is a compilation of diagnostic techniques for some 47 fruit fly species, most of which are exotic to Australia. The Handbook is intended to facilitate rapid diagnosis of fruit fly species and be a comprehensive guide for Australian diagnosticians and field officers.

A copy of the Handbook can be downloaded by following the link below.

http://www.phau.com.au/go/phau/strategies-and-policy

This is the first version of *The Australian Handbook for the Identification of Fruit Flies.* It is provided freely as a reference resource with an expectation that it is appropriately acknowledged when it is used. As a living document it is designed to be continuously updated as more information becomes available through Australia's skilled network of fruit fly diagnosticians. For further information please contact the Office of the Chief Plant Protection Officer (OCPPO), Department of Agriculture, Fisheries and Forestry. Email: **ocppo@daff.gov.au**.

Funding for this important initiative was provided by the Australian Government. The Office of the Chief Plant Protection Officer would like to recognise the huge contribution made by researchers, academics, surveillance officers, diagnosticians and laboratory scientists who have collectively brought this valuable document into being. Thanks are also extended to Plant Health Australia for facilitating and coordinating the preparation of the Handbook.

Lois Ransom

Chief Plant Protection Officer

December 2011

Australian Government

**Department of Agriculture,
Fisheries and Forestry**

Contents

4   Introduction

Fruit flies are one of the world's most destructive horticultural pests and pose risks to most commercial fruit and vegetable crops. This has major implications for the sustainable production and market access of Australia's $4.8 billion horticultural industry. Worldwide there are some 4,000 species of fruit flies in the family Tephritidae of which around 350 species are of economic importance.

More than 280 species of fruit fly are endemic to Australia although only seven of these have been found to have significant economic impact. It is therefore important to be able to distinguish between those endemic species that pose a threat to production and domestic market access from those that do not.

Furthermore, Australia is free from many species that impact production elsewhere. Neighbouring countries in Southeast Asia and the South Pacific are home to numerous species of fruit fly that pose an immediate incursion risk to Australian quarantine. Rapid diagnosis of these flies should they arrive in Australia is therefore critical to containing and eradicating the populations before they establish.

Although a range of diagnostic methods are available that can be undertaken by a number of laboratories in Australia, there has not been an established agreement on (a) the number and type of tests that should be conducted to establish a positive identification, (b) the exact protocols that should be followed for specific diagnostic tests and, (c) agreement on the number and type of protocols that should be retained and maintained to facilitate a diagnosis at short notice.

This project was therefore undertaken to establish an agreed national standard that is able to facilitate rapid diagnosis and streamline a national response when suspected incursions occur, and include taxonomic identifications using morphological and molecular approaches.

PHA would like to acknowledge the support, encouragement and professional advice contributed by all participants to this process.

4.1   Background

Australia has a strong, internationally recognised capacity to diagnose fruit fly species and maintains a wide network of fruit fly traps as part of a national surveillance system. From the Northern Territory and the Torres Strait Islands to Tasmania, and from Perth to Melbourne, significant expertise is maintained in state and federal government departments, universities and in the private sector to support the identification of fruit fly species.

Supported by an extensive world class fruit fly collection (albeit split across various interstate locations), Australia is fortunate to have a group of entomologists and other scientists with extensive experience and knowledge of fruit fly diagnostics.

Not surprisingly, given the range of endemic and exotic fruit flies that can be encountered in different climatic zones, many jurisdictions have developed specialist expertise to identify species pertinent to regional production and quarantine requirements.

Against this background this project was undertaken to establish a diagnostic procedure that has a national focus and can assist all stakeholders to maintain the strongest capability to identify fruit flies.

This project also provides an opportunity to:

- collate current (existing) practices and knowledge base into a single document

- pool experience from all of Australia's experts in a collegiate manner

- facilitate and improve the constructive exchange of ideas and material across jurisdictions and entities

- establish a resource that can support and develop the confidence and expertise of all users

- provide a mechanism to possibly identify future information and research needs, and

- consider the potential of both morphological and molecular techniques as they are developed and become available

4.2 Coverage of this diagnostic handbook

To develop this document, a review was firstly conducted to establish those fruit fly species being targeted by jurisdictions in their current surveillance programs. These species were also reviewed against diagnostic tools (e.g. electronic and internet keys) already available and in use to support routine diagnosis. This review enabled the development of a proposed species list to be covered by this national protocol (Table 1)

Table 1. **Fruit flies covered in this diagnostic handbook**

Scientific name	Common name		Scientific name	Common name	
Anastrepha fraterculus	South American fruit fly	Exotic	*Bactrocera latifrons*	Solanum fruit fly	Exotic
Anastrepha ludens	Mexican fruit fly	Exotic	*Bactrocera melanotus*		Exotic
Anastrepha obliqua	West Indian fruit fly	Exotic	*Bactrocera musae*	Banana fruit fly	Present in Australia
Anastrepha serpentina	Sapote fruit fly	Exotic	*Bactrocera neohumeralis*	Lesser Queensland fruit fly	Present in Australia
Anastrepha striata	Guava fruit fly	Exotic	*Bactrocera occipitalis*		Exotic
Anastrepha suspensa	Caribbean fruit fly	Exotic	*Bactrocera papayae*	Papaya fruit fly	Exotic
Bactrocera albistrigata		Exotic	*Bactrocera passiflorae*	Fijian fruit fly	Exotic
Bactrocera aquilonis	Northern Territory fruit fly	Exotic	*Bactrocera philippinensis*	Philippines fruit fly	Exotic
Bactrocera atrisetosa		Exotic	*Bactrocera psidii*	South sea guava fruit fly	Exotic
Bactrocera bryoniae		Present in Australia	*Bactrocera tau*		Exotic
Bactrocera carambolae	Carambola fruit fly	Exotic	*Bactrocera trilineola*		Exotic
Bactrocera caryeae		Exotic	*Bactrocera trivialis*		Exotic
Bactrocera correcta	Guava fruit fly	Exotic	*Bactrocera tryoni*	Queensland fruit fly	Present in Australia
Bactrocera cucumis	Cucumber fruit fly	Present in Australia	*Bactrocera umbrosa*	Breadfruit fruit fly	Exotic
Bactrocera cucurbitae	Melon fly	Exotic	*Bactrocera xanthodes*	Pacific fruit fly	Exotic
Bactrocera curvipennis		Exotic	*Bactrocera zonata*	Peach fruit fly	Exotic
Bactrocera decipiens	Pumpkin fruit fly	Exotic	*Ceratitis capitata*	Mediterranean fruit fly	Present in Australia
Bactrocera dorsalis	Oriental fruit fly	Exotic	*Ceratitis rosa*	Natal fruit fly	Exotic
Bactrocera facialis		Exotic	*Dirioxa pornia*	Island fly	Present in Australia
Bactrocera frauenfeldi	Mango fruit fly	Present in Australia	*Rhagoletis completa*	Walnut husk fly	Exotic
Bactrocera jarvisi	Jarvis's fruit fly	Present in Australia	*Rhagoletis fausta*	Black cherry fruit fly	Exotic
Bactrocera kandiensis		Exotic	*Rhagoletis indifferens*	Western cherry fruit fly	Exotic
Bactrocera kirki		Exotic	*Rhagoletis pomonella*	Apple maggot	Exotic
Bactrocera kraussi		Present in Australia			

5 Detection

5.1 Plant products affected

Fruit flies can infest a wide range of commercial and native fruits and vegetables. Lists of hosts are provided in the data sheets contained in Section 7.

Fruit is increasingly likely to be attacked as it becomes more mature and as the fruit fly population increases during summer and autumn. A wide range of fruits are potentially vulnerable to fruit fly attack. In urban home gardens, and in orchards close to urban areas, fruit fly populations are often much higher than in outlying orchards.

Plant parts liable to carry the pest in trade or transport include fruiting bodies, in which eggs or larvae can be borne internally. The illegal movement or smuggling of non-commercially produced fruit is the major risk pathway for exotic fruit fly incursions (CABI 2007).

5.2 Signs and symptoms

The oviposition-site punctures in the fruit are commonly referred to as 'stings'. Stings are usually identified by making a shallow cut through the skin of the fruit and looking for the egg cavity containing eggs, larvae or the remains of hatched eggs. In fruits such as peaches, the stings are not very noticeable, while in pale, smooth-skinned fruits, the sting mark may be easily detected and can disfigure the fruit when marked by 'gum bleed'. Some fruits, such as avocado and passionfruit, develop hard, thickened areas where they are stung. In mature citrus, the sting mark may be a small brown depressed spot, or have an indistinct, bruised appearance, while on green citrus fruit the skin colours prematurely around the sting mark. In humid conditions, the fungi responsible for green mould in citrus and brown rot in stone fruit will readily infect stung fruit.

Fruit will fall from the tree as a result of larval infestation. The extent of the damage caused by larvae tunnelling through fruit varies with the type and maturity of the fruit, the number of larvae in it, and the prevailing weather conditions. Larvae burrow towards the centre in most fruits, with internal decay usually developing quickly in soft fruits. In hard fruits a network of channelling is usually seen, followed by internal decay. Larval development can be very slow in hard fruits such as Granny Smith apples.

5.3 Development stages

The following life history, from McKenzie *et al*. (2004), is based on the much studied Queensland fruit fly but is also relevant to most other fruit flies, although differences may occur with regard to host preference and the relationship between developmental rate and temperature.

Typically, fruit flies lay their eggs in semi-mature and ripe fruit. The female fruit fly has a retractable, sharp egg-laying appendage (the ovipositor) at the tip of the abdomen that is used to insert up to six eggs into a small chamber about 3 mm under the fruit skin.

Tephritid fruit fly eggs are white, banana shaped and nearly 1mm long. Infested fruit may show 'sting' marks on the skin and may be stung more than once by several females. In 2 or 3 days larvae (maggots) hatch from the eggs and burrow through the fruit. To the naked eye, the larvae resemble blowfly maggots. They are creamy white, legless, blunt-ended at the rear and tapered towards the front where black mouth hooks (cephalopharyngeal skeleton) are often visible. Female flies may have an association with bacteria resident in their gut in some regions of Australia, which they regurgitate onto the fruit before ovipositing (see Appendix 1). Most of the damage sustained by the fruit is actually caused by the bacteria and the maggots simply lap up the juice.

A pair of mouth hooks allows the larvae to readily tear the fruit flesh. The larvae develop through three larval stages to become about 9 mm long and pale yellow when fully grown. Several larvae can develop in each fruit, and when fully developed they leave the fruit, falling to the soil beneath the tree and burrowing down about 5 cm to form a hard, brown, barrel-like pupal case from its own skin where it completes its development. Many flies leave the fruit while it is already on the ground. Most insects cannot pupate successfully in the presence of excess moisture and fruit flies have a prepupal stage when they can 'flick' themselves over some distance, presumably to distance themselves from the host fruit.

The duration of pupal developmental is dependent on temperature with each stage taking from 9 days to several weeks to complete. Adult flies emerge from their pupal cases in the soil and burrow towards the surface where they inflate their wings and fly away. Adults are able to mate within a week of emerging, living for many weeks with females continuing to lay eggs throughout their lifecycle. Adult fruit flies feed on carbohydrates from sources such as fruit and honeydew, the sweet secretion from aphids and scale insects, as well as natural protein sources, including bird droppings and bacteria.

Fruit fly larvae can be attacked by parasitoids although they appear to have little impact on populations of most fruit flies, with 0-30% levels of parasitism typical (CABI 2007). However, mortality due to vertebrate consumption of infested fruit can be very high, as can pupal mortality in the soil, either due to predation or environmental factors.

5.4 Methods for detection

Monitoring is largely carried out by setting traps in areas of interest. However, there is evidence that some fruit flies have different host preferences in different parts of their range (CABI 2007). As such, host fruit surveys may be required in the event of an exotic incursion. Where known, specific lures are provided for each species in the data sheets contained in Chapter 4. The following information and images are taken from Lawson *et al.* (2003).

5.4.1 Trap types

LYNFIELD TRAP

The Lynfield lure trap (see Figure 1a) is a non-sticky disposable pot type trap for adult male flies. It consists of a modified clear plastic container, e.g. a 1 litre container with a 100 mm base, a 90 mm diameter top and depth 115 mm. There are four entry holes 25 mm in diameter evenly spaced 15 mm below the lip of the trap.

Cotton wicks containing the liquid lure are held together with a wire clip and hung from a wire loop under the lid of the trap.

Like the Lynfield and Paton traps, the hook holding the wick is formed by a wire inserted through the centre of the lid which extends about 25 cm above it so that it can be attached to the branch of a tree, allowing the trap to hang freely. A poison and information label is placed onto the trap body.

This trap is used in drier areas of Australia (eg. Townsville).

STEINER TRAP

The Steiner trap (see Figure 1b) is basically an open horizontal plastic cylinder within which a cotton wick impregnated with a mixture of attractant and insecticide is suspended.

This type of trap provides the flies with easy access into the trap whilst giving them protection from water and predator damage. They are popular in areas of high rainfall such as far north Queensland. The large openings at each end of the trap also allow the free movement of the attractant vapour from

the cotton wick. The cotton dental wicks provide absorption of the attractant and insecticide mix, yet still allow evaporation of the lure over relatively long periods, and are inexpensive.

PATON TRAP

The Paton trap (see Figure 1c) is used in areas of high rainfall or wind or where traps may be set longer periods (eg a month) between collections. They are generally used on Cape York Peninsula and the Torres Strait Islands in Queensland. They are very rain resistant, prevent flies falling out in windy situations and are able to hold about 10 000 flies (where the Steiner can only hold about 6000). They also have a wick impregnated with attractant and insecticide and labels on the outside (Poison, lure type, contact info) as per Lynfield and Steiner. They are often used with cardboard spacers to maintain the samples in good condition.

MCPHAIL TRAP

The McPhail trap (see Figure 1d) is essentially a glass or plastic flask-shaped container with an invaginated entrance at the base. It attracts both male and female flies to the trap, but in far fewer numbers than those which use male lures alone. It can be useful in attracting species that do not respond to male lures. Liquid attractants such as fruit juices and proteinaceous solutions are used to both attract and kill the flies (by drowning). These traps only catch a small number of flies due to the short range of attraction. They need to be cleared regularly to avoid deterioration of the specimens and to maintain their efficacy.

Figure 1. Types of fruit fly traps

| (a) Lynfield trap (*Image courtesy of NSW I&I*) | (b) Steiner trap |

| (c) Paton trap *(Image courtesy of AQIS)* | (d) McPhail trap |

5.4.2 Attractants

Attractants or lures are commonly used to trap fruit flies as they provide an easy way to collect large numbers of flies in a short period of time.

Food-based attractants, such as those used in McPhail traps, were widely used in the past. These are still in use today as they offer the advantage of attracting both sexes of many species, including those not attracted to male lures.

Males of many species respond to chemicals referred to as parapheromones. These lures attract flies from large distances. Cue lure (CUE) (Figure 2a) and methyl eugenol (ME) (Figure 2b) are two male attractants widely used in collecting *Bactrocera* spp. fruit flies. Most species appear to be attracted to one lure or the other, however other species are attracted to a combination of both lures (Dominiak *et al.*, 2011) (see Appendix 2). It should be noted that one of the breakdown products of CUE, raspberry ketone or Willison's lure, is itself an attractant (Metcalf *et al.*, 1983). Trimedlure/capilure is used to trap *Ceratitis* spp. All three lures are used in Lynfield and Steiner traps. Only ME and CUE are used in Paton traps in Australia (because these traps are used in the tropics and *Ceratitis* spp. cannot establish there).

Figure 2. Chemical structure of cue lure and methyl eugenol

| (a) Cue lure | (b) Methyl eugenol |
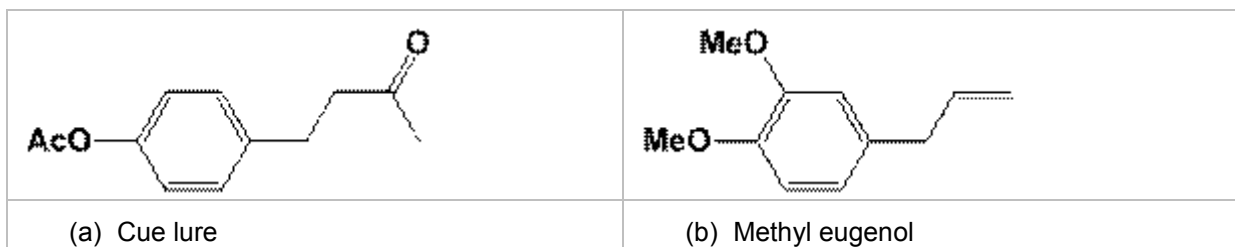
Attractants are generally highly volatile chemicals and need only to be used in small amounts to be effective. Generally, a wick is impregnated with a mixture of 4 mL attractant and 1 mL or less of 50% w/v concentrate of malathion or dichlorvos and is then suspended within the trap. It is very important that lure contamination does not occur along any step of the way from when the wicks are prepared through to when the traps are emptied. If this occurs then flies that are attracted to one lure may also end up in traps containing flies attracted to another. This can lead to confusion during identification.

5.5 Inspection of material, sample preparation and storage

Fruits (locally grown or samples of fruit imports) should be inspected for puncture marks and any associated necrosis. Suspect fruits should be cut open and checked for larvae. Infested fruit should be held in a container which has a gauze cover to allow aeration. Pupae need to develop in a dry medium such as sand or sawdust. Once flies start to emerge they need to be provided with access to water and sugar for survival and for colour development. After about 4 days they may be collected, killed and prepared for study (Lawson *et al.* 2003).

Fruit fly adults, larvae and eggs should never be handled live if there is any chance of the sample being involved with a quarantine breach. For the purposes of this protocol, all fruit fly samples, where the fruit fly adult, larva or egg has been removed from its substrate, should be placed in a sealed vial or container and either frozen (at -20°C) or stored in 100% ethanol. The sample vial should have labels stating the collection details including (at a minimum) the collector, collection date, host if known, place of collection and accession number(s).

Samples should be collected and despatched in a manner compliant with PLANTPLAN (with particular reference to sampling procedures and protocols for confirmation).

6   Identification

6.1   Overview

The National Handbook for the Identification of Fruit Flies in Australia (overview presented in Figure 3 and Figure 4) proposes that primary identification is undertaken using conventional taxonomy with the support of molecular genetic techniques for some species. The diagnostic methods available for each species are presented in Table 2 and covered in greater detail in sections 6.2. (Morphological), 6.3.1. (PCR amplification), 6.3.2 (DNA barcoding) and 6.4 (Allozyme Electrophoresis). These techniques are currently in use in Australia and form the basis of this national protocol. Section 7 contains data sheets with the specific morphological and molecular diagnostic information for each species.

Molecular techniques are best used to support or augment morphological identification. They can be used to identify early larval stages (which are hard to identify reliably on morphological features) and eggs. They can also be used for incomplete adults that may be missing specific anatomical features required for morphological keys, or specimens that have not fully developed their features (especially colour patterns). It should be recognised, however, that the success of a molecular diagnosis can be impacted by factors such as life stage, specimen quality or any delays in processing. As a result, the suitability of each method has been identified.

The molecular protocols require a laboratory to be set up for molecular diagnostics, but can be carried out by almost any laboratory so equipped. Access to published sequences is required for whichever protocol is being used[1].

Most molecular techniques presented in this standard involve the amplification of particular region(s) of the fly genome using a polymerase chain reaction (PCR). Often the target is the internal transcribed spacer region of the ribosomal RNA operon referred to as ITS1. Many species can be identified by the size of the ITS1 alone although similar species often produce fragments of the same size. In this case, restriction digestion of the ITS1 PCR product can be performed, using each of up to six different restriction enzymes. This approach is referred to as restriction fragment length polymorphisms (RFLP) analysis. This does not necessarily eliminate non-economic fruit flies but will identify if the restriction pattern produced conforms to that produced by a reference fly from an economically important species. If the species is still not identified, more comprehensive information can be obtained by undertaking nucleotide sequence analysis.

DNA barcoding, focusing on analysis of the mitochondrial gene for cytochrome oxidase subunit I (COI) is now available as an alternative to ITS-based techniques. This technology sometimes provides more accurate and consistent results than analysis of the ITS region, with less confusing overlap between taxa; however, inconsistencies and anomalies can still arise, particularly among closely related species complexes.

This national protocol is presented on the premise that ITS analysis and DNA barcoding are used alongside morphological methods. Most species can generally be resolved using traditional or molecular taxonomy without ambiguity. However, more difficult cases will only yield to a combination of both morphological and one or more molecular approaches.

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[1] Many of the DNA barcoding sequences were obtained by the CBOL tephritid fruit fly project (**www.dnabarcodes.org/pa/ge/boli_projects**), which examined all economically important tephritid fruit fly species known to be agricultural pests as well as many closely related species.

Figure 3. Overview of fruit fly diagnostic procedures (adult specimens)
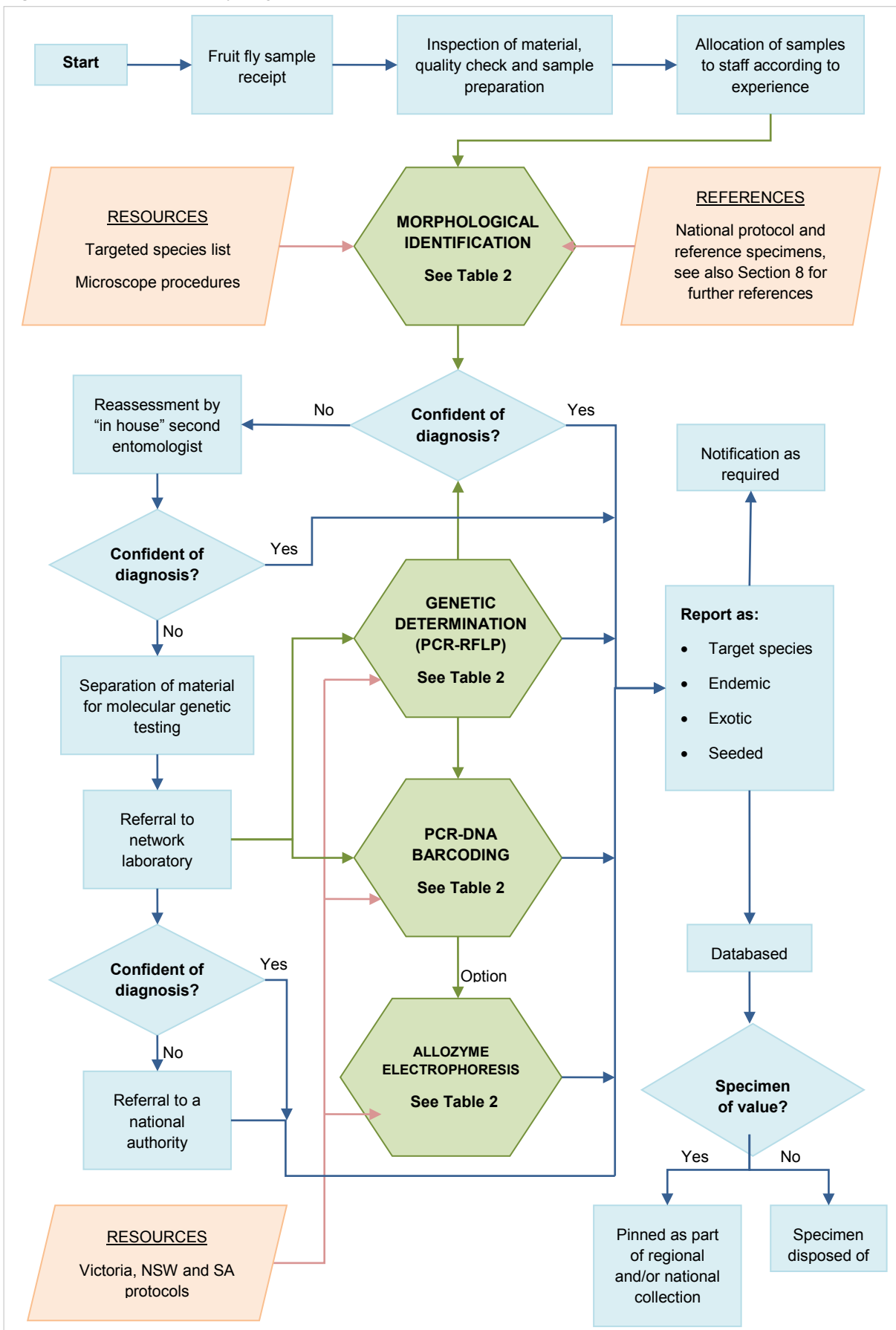
Figure 4. Overview of fruit fly diagnostic procedures (larval specimens)
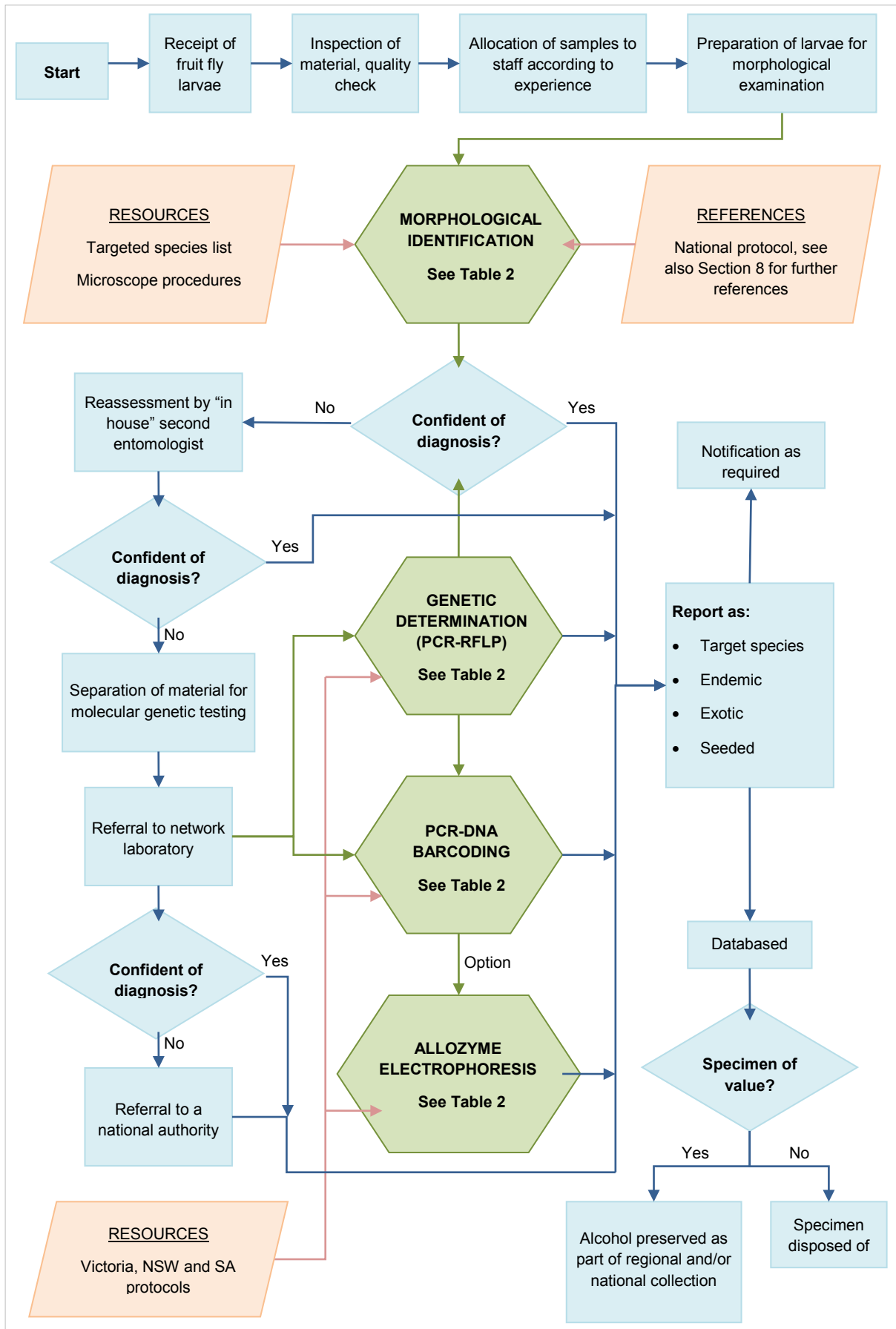
Table 2. Diagnostic methods used to identify fruit fly species

Scientific name	Morphological description (6.2)	PCR-RFLP (6.3.1)	PCR-DNA Barcoding[4] (6.3.2)	Allozyme Electrophoresis (6.4)
Anastrepha fraterculus	✓		✓ (14)	
Anastrepha ludens	✓	✓	✓ (10)	
Anastrepha obliqua	✓	✓	✓ (16)	
Anastrepha serpentina	✓	✓	✓ (13)	
Anastrepha striata	✓		✓ (14)	
Anastrepha suspensa	✓		✓ (7)	
Bactrocera albistrigata	✓	✓	✓ (1)	
Bactrocera aquilonis	✓	✓[1]	✓[1] (36)	
Bactrocera atrisetosa	✓		(0)[5]	
Bactrocera bryoniae	✓	✓	✓ (10)	
Bactrocera carambolae	✓	✓	✓ (10)	
Bactrocera caryeae	✓		✓ (1)	
Bactrocera correcta	✓		✓ (15)	
Bactrocera cucumis	✓	✓	✓ (8)	
Bactrocera cucurbitae	✓	✓	✓ (72)	
Bactrocera curvipennis	✓	✓	✓ (2)	
Bactrocera decipiens	✓		(0)[5]	
Bactrocera dorsalis	✓	✓[2]	✓[2] (29)	
Bactrocera facialis	✓	✓	✓ (1)	
Bactrocera frauenfeldi	✓	✓	✓ (16)	
Bactrocera jarvisi	✓	✓	✓ (6)	✓
Bactrocera kandiensis	✓		✓ (10)	
Bactrocera kirki	✓	✓	✓ (5)	
Bactrocera latifrons	✓	✓	✓ (20)	
Bactrocera melanotus	✓		✓ (3)	
Bactrocera musae	✓	✓[3]	✓ (5)	
Bactrocera neohumeralis	✓	✓[1]	✓[1] (4)	
Bactrocera occipitalis	✓		✓ (5)	
Bactrocera papayae	✓	✓	✓ (11)	✓
Bactrocera passiflorae	✓	✓	✓ (1)	
Bactrocera philippinensis	✓	✓[2]	✓[2] (9)	
Bactrocera psidii	✓	✓	✓ (2)	
Bactrocera tau	✓		✓ (5)	
Bactrocera trilineola	✓		✓ (2)	

Scientific name	Morphological description (6.2)	PCR-RFLP (6.3.1)	PCR-DNA Barcoding[4] (6.3.2)	Allozyme Electrophoresis (6.4)
Bactrocera trivialis	✓		✓ (3)	
Bactrocera tryoni	✓	✓[1]	✓[1] (12)	✓
Bactrocera umbrosa	✓	✓	✓ (9)	
Bactrocera xanthodes	✓	✓	✓ (7)	
Bactrocera zonata	✓	✓	✓ (22)	
Ceratitis capitata	✓	✓	✓ (120)	✓
Ceratitis rosa	✓	✓	✓ (24)	
Dirioxa pornia	✓	✓	✓ (3)	✓
Rhagoletis completa	✓		(0)[5]	
Rhagoletis fausta	✓		✓ (1)	
Rhagoletis indifferens	✓		(0)[5]	

[1] Species cannot be distinguished from each other at the ITS or COI region

[2] Species cannot be distinguished from each other at the ITS or COI region

[3] Requires full ITS sequencing to split *B. musae* from the *B. philippinensis*, *B. dorsalis* group

[4] Numbers in brackets refer to the number of individuals of that species with (COI) DNA barcodes of >500 bp on the Barcode of Life website (**www.boldsystems.org/views/taxbrowser.php?taxid=439**; as of 23 August 2011).

[5] DNA barcodes (COI) are available for other species in these genera. There are 86 species of *Bactrocera*, 65 species of *Dacus*, and 19 species of *Rhagoletis* that do have barcodes available (as of 23 August 2011).

6.2   Morphological identification

Approximately 90% of the dacine pest species can be identified accurately, and quickly, by microscopic examination of the adult. For these species there is no need for supporting evidence. The remaining 10% (mainly some *dorsalis* complex species) can be identified with this same method but require expert examination and may require additional supporting evidence such as the molecular diagnosis or host association records.

Only morphological diagnostic procedures and information for adult fruit flies are contained in this document. Aside from molecular techniques, larval diagnosis has been excluded from this protocol.

6.2.1   Procedure

The following apparatus and procedures should be used to prepare the specimen for identification (adapted from QDPIF 2002):

Apparatus:

- Stereoscopic microscope or Stereomicroscope with magnification range of 7X to 35X.

- Light source

- 90mm diameter petri dishes

- Forceps (Inox #4)

Preparation procedure:

1) Ensure the workstation is clean and clear of all flies before commencing.

2) Adjust chair height and microscope, and turn on the light source (refer to specific operating procedures for the microscope in use).

3) If applicable, record the lure and trap type or host material in which the specimen was found.

4) Carefully place the fruit fly into a plastic petri dish. If examining more than one fly at once ensure there is a single layer of flies only, with room to move flies from one side of the dish to the other.

5) While looking through the microscope check each fly individually. Manipulate them with the forceps so that diagnostic features are visible.

6.2.2   Identification

Key features (Figure 5, Figure 6, Figure 7 and Figure 8) used for the morphological diagnosis of adult fruit flies include:

- Wing morphology and infuscation

- Presence or absence of various setae, and relative setal size. (Note: Chaetotaxy, the practice of setal taxonomy, is not as important in this group as some others.)

- Overall colour and colour patterning

- Presence, shape and colour of thoracic vittae. A vitta is a band or stripe of colour.

Use the morphological diagnostic key and descriptions contained in Section 7 to identify the species of fruit fly under microscopic examination.

If identification cannot be made using this diagnostic procedure and/or the specimen is suspected to be of quarantine concern, it should be referred to either a State or National authority (see section 8.1 Key contacts and facilities). If the specimen is identified as an exotic fruit fly, it should be referred to a National Authority within 24 hours and the appropriate National Authority notified as required in PLANTPLAN.

Figure 5. Adult morphology; head (top) and wing (bottom) (White and Elson-Harris 1992).

ar – arista	gn grv – genal groove	pafc – arafacial area
comp eye – compound eye	g ns – genal seta	ped – pedicel
fc – face	i vt s – inner vertical seta	poc s – postocellar seta
flgm 1 – 1st flagellomere	lun – lunule	pocl s – postcular setae
fr – frons	oc – ocellus	ptil fis – ptilinal fissure
fr s – frontal setae	oc s – ocellar seta	scp – scape
gn – gena (plural: genae)	o vt s – outer vertical seta	vrt – vertex
	orb s – orbital setae	

Figure 6. Adult morphology, Thorax; Dorsal features (White and Elson-Harris 1992).
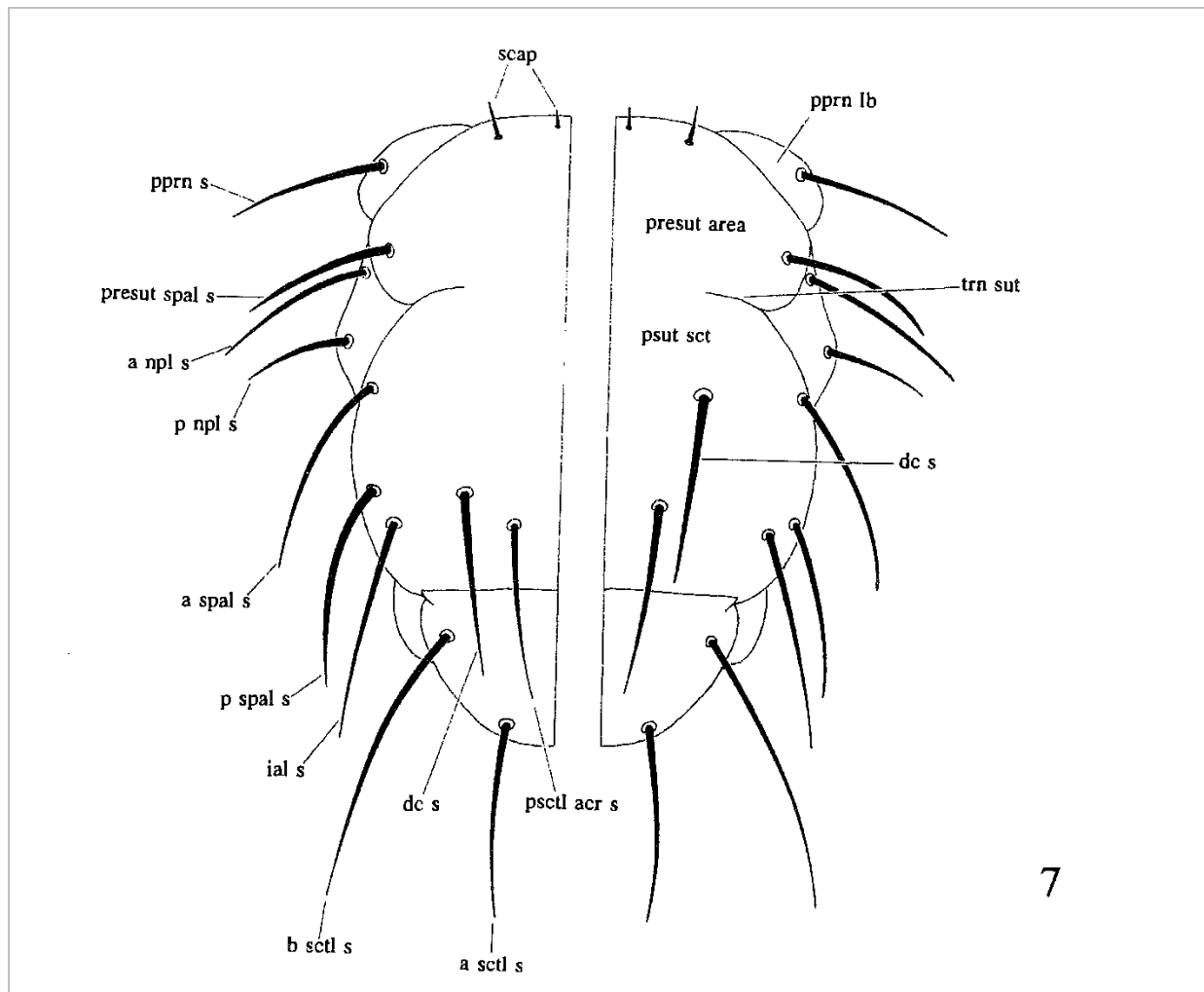
a npl s – anterior notopleural seta	hlt – halter or haltere	prepst – propisternum
a sctl s – apical scutellar seta	ial s – intra-alar seta	presut area – presutural area
a spal s – anterior supra-alar seta	kepst – katepisternum	presut spal s – preutural supra-alar seta
a spr – anterior spiracle	kepst s – katepisternal seta	
anatg – anatergite	ktg – katatergite	psctl acr s – prescutellar acrostichal seta
anepm – anepimeron	npl – notopleuron	psut sct – postcutural scutum
anepst – anepisternum	p npl s – posterior notopleural seta	sbsctl – subscutellum
anepst s – upper anepisternal seta	p spal s – posterior supra-alar seta	scape – scapula setae
b sctl s – basal scutellar seta		sctl – scutellum
cx – coax	p spr – posterior spiracle	trn sut – transverse scuture
dc s – dorsocentral seta	pprn lb – postpronotal lobe	
	pprn s – postporontal seta	

Figure 7. Adult morphology, thorax; lateral features (White and Elson-Harris 1992).

See Figure 5 for abbreviations.
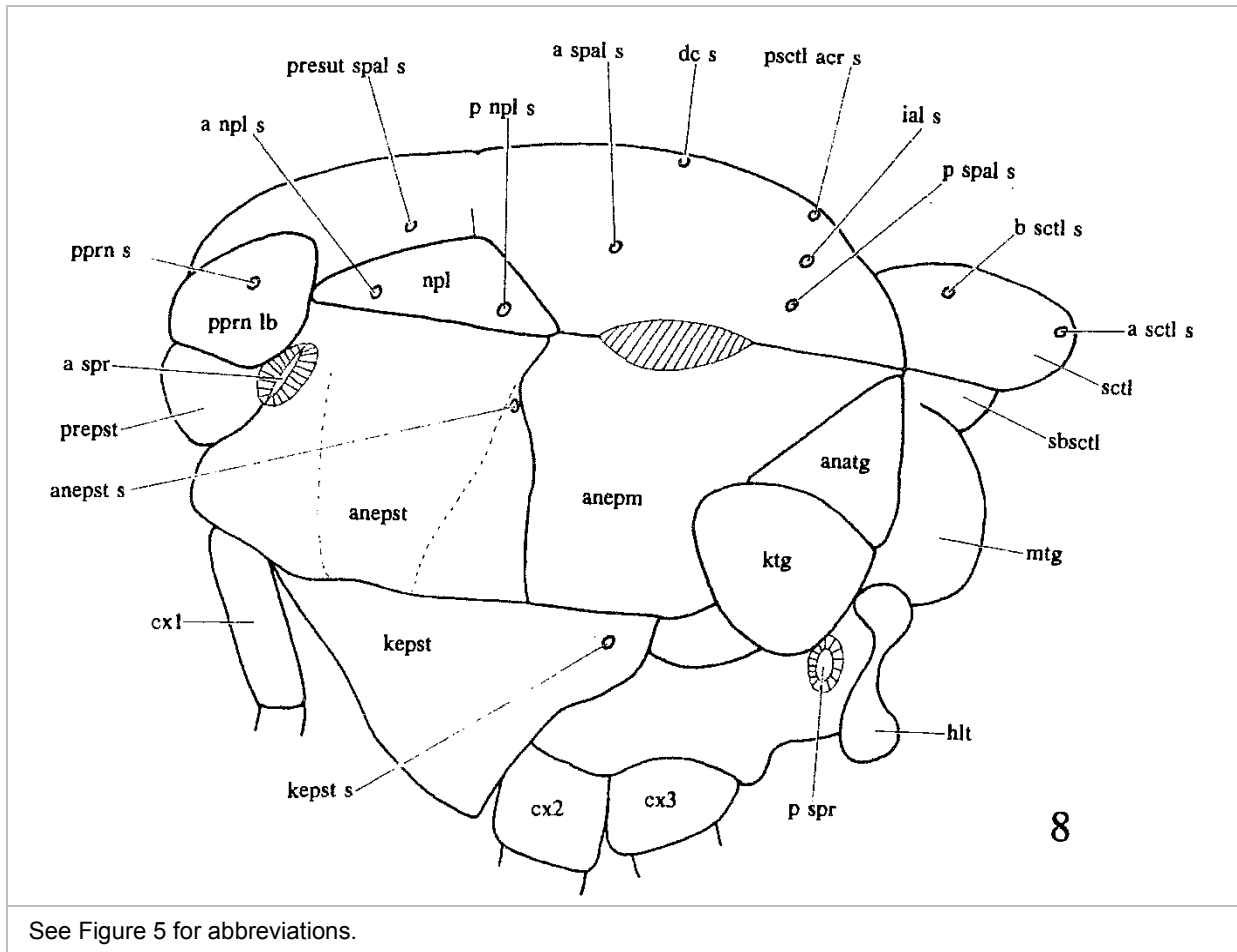
Figure 8. Adult morphology, abdomen; male with features of typical dacini (left), Female, with extended ovipositor (right) (White and Elson-Harris 1992).

acul – aculeus	ovsc – oviscape	tg – tergites where 1+2 are fused to form syntergosternite 1+2, followed by tergites 3-5 in the male and 3-6 in the female
ev ovp sh – eversible ovipositer sheath	st – sternites numbered 1-5 in the male and 1-6 in the female	
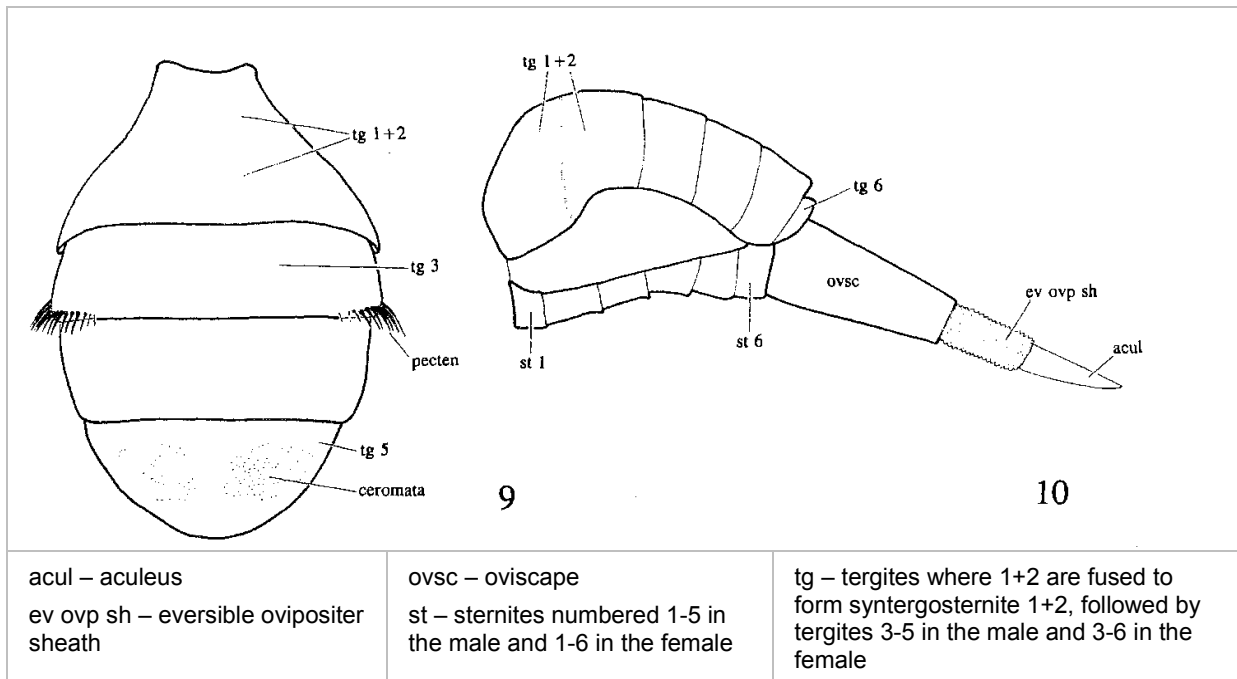
6.3   PCR – based identification

6.3.1   Restriction Fragment Length Polymorphism

Two Restriction Fragment Length Polymorphism (RFLP) tests are described below. In both tests, the internal transcribed spacer region (ITS1), part of the nuclear rRNA gene cluster, is amplified through Polymerase Chain Reaction (PCR) methods and then digested with various enzymes. Test 1 was developed by McKenzie *et al.* (2004). In this test a DNA fragment (600 to 1200 bp in length) is amplified and can be used for identification of at least 30 fruit fly species. Methods used in Test 2 are similar to Test 1 but the former amplifies a slightly larger (1.5-1.8 kb) DNA fragment, encompassing the 18S and the ITS1 genes (see figure below). Test 2 was originally developed by Armstrong and Cameron (1998) and included at least 31 economically significant fruit fly species. This test has been adopted and slightly modified by Linda Semeraro and Mali Malipatil, Victorian Department of Primary Industries (Semeraro and Malipatil 2005) to specifically identify only a few main fruit fly groups of interest (see Target below).

Figure 9. Part of the ribosomal RNA operon with the location of primer positions for Tests 1 and 2
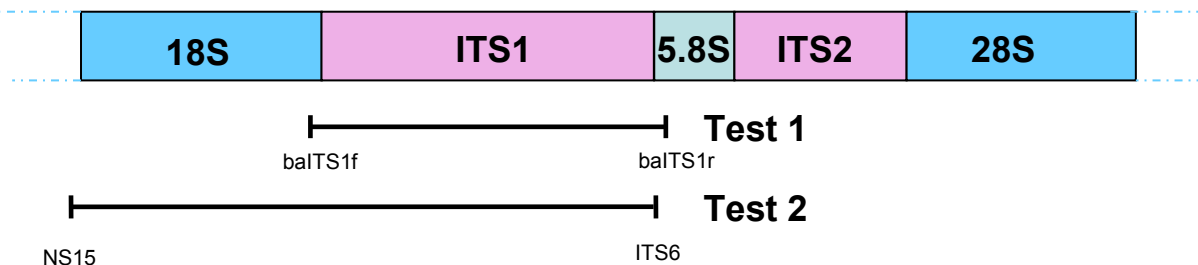
AIM

These tests aim to use a prescribed molecular protocol to identify DNA from targeted fruit fly species.

TARGETS

Despite there being many hundreds of species of fruit flies in the Australasian region Test 1 targets 30 species (Table 2) that have been assessed as being of the highest economic importance to Australia. The assessment of targets includes factors such as host range, frequency of interaction (trade, migration etc.) and prior incursions. This priority listing was assembled in consultation with fruit fly workers and quarantine authorities. Test 2 is used for the diagnosis of the *Bactrocera tryoni* group (including *B. tryoni*, *B. neohumeralis* and *B. aquilonis*) and *Ceratitis capitata* only.

SUITABILITY

Good/suitable for fresh adults, fresh larvae or fresh eggs but viability of this method requires specimens of adequate freshness so prior sample handling, storage and preparation very influential on diagnostic outcome.

Use of these tests cannot necessarily eliminate from the identification fruit flies of other less economically important species not included as targets. Host records (Section 7) for the target taxa may assist in the elimination of possible non target species. Fruit fly adults or larvae producing non-conforming restriction patterns can be assumed not to belong to the economically important species included in this key.

The amount of DNA extracted varies between adults and larvae but we have used these methods to analyse mature larvae (2nd & 3rd instars). The protocol should also work for small fruit fly larvae (1st and 2nd instar) and eggs if the extraction process is scaled down. This protocol is as effective for larvae as for adult flies.

RFLP TEST 1

6.3.1.1.1   *Procedure overview*

DNA is extracted from fruit flies (adults or larvae) using a commercially available kit. A region of the fly genome (an internal transcribed spacer region of the ribosomal RNA operon, referred to as ITS1) is amplified using the PCR. Some species can be identified based on the length of this fragment. Otherwise the ITS1 fragment is digested using each of up to six different restriction enzymes using a process known as analysis of Restriction Fragment Length Polymorphisms (or RFLP)[2].

6.3.1.1.2   *Sample handling*

Samples should be collected and despatched in a manner compliant with PLANTPLAN (with particular reference to sampling procedures and protocols for confirmation).

Fruit fly adults, larvae and eggs should never be handled live if there is any chance of the sample being involved with a quarantine breach. For the purposes of this protocol all fruit fly samples, where the fruit fly adult, larva or egg has been removed from its substrate should be placed in a sealed vial or container and either frozen (at -20°C) or stored in 100% ethanol. The sample vial should have labels stating the collection details including (at minimum) the collector, collection date, host if known, place of collection and accession number(s).

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[2] It is not clear that this method will reliably discriminate between *B. tryoni* and *B. aquilonis,* particularly if relying on agarose gel separation/detection as described in the protocol. The method relies on "specimens of utmost freshness so prior sample handling, storage and preparation are very influential on diagnostic outcome". The protocol states that if there is a size match for an unknown "it could be either a pest or non-pest species". There is an overlap in the size of the PCR product for *B. tryoni* and *B. aquilonis* (810-830 bp vs. 790-830 bp). Even with additional enzyme cleavage, which can sometimes discriminate PCR products of similar size, there is only one enzyme in the protocol that produces a difference between these two species (*Sauβ*aI), and that results in a 5 bp difference, which would not be discriminated by standard gel electrophoresis. In the event of follow-up DNA sequencing, there is still no guarantee that the identity of fruit fly would be confirmed as "…differences in DNA sequence ….in many species frequently presents problems with this approach". It is not clear in the protocol whether these two species were DNA sequenced, or whether they proved problematic. This could be explored further. As stated in the protocol, the molecular results are "designed to support morphological identification", and it's also suggested that they be taken in the context of differences in geographical distribution and hosts. *NB: Reference fragment lengths for each species are contained in the relevant data sheets in Section 7.*

Figure 10: Workflow of molecular procedures for fruit fly identification
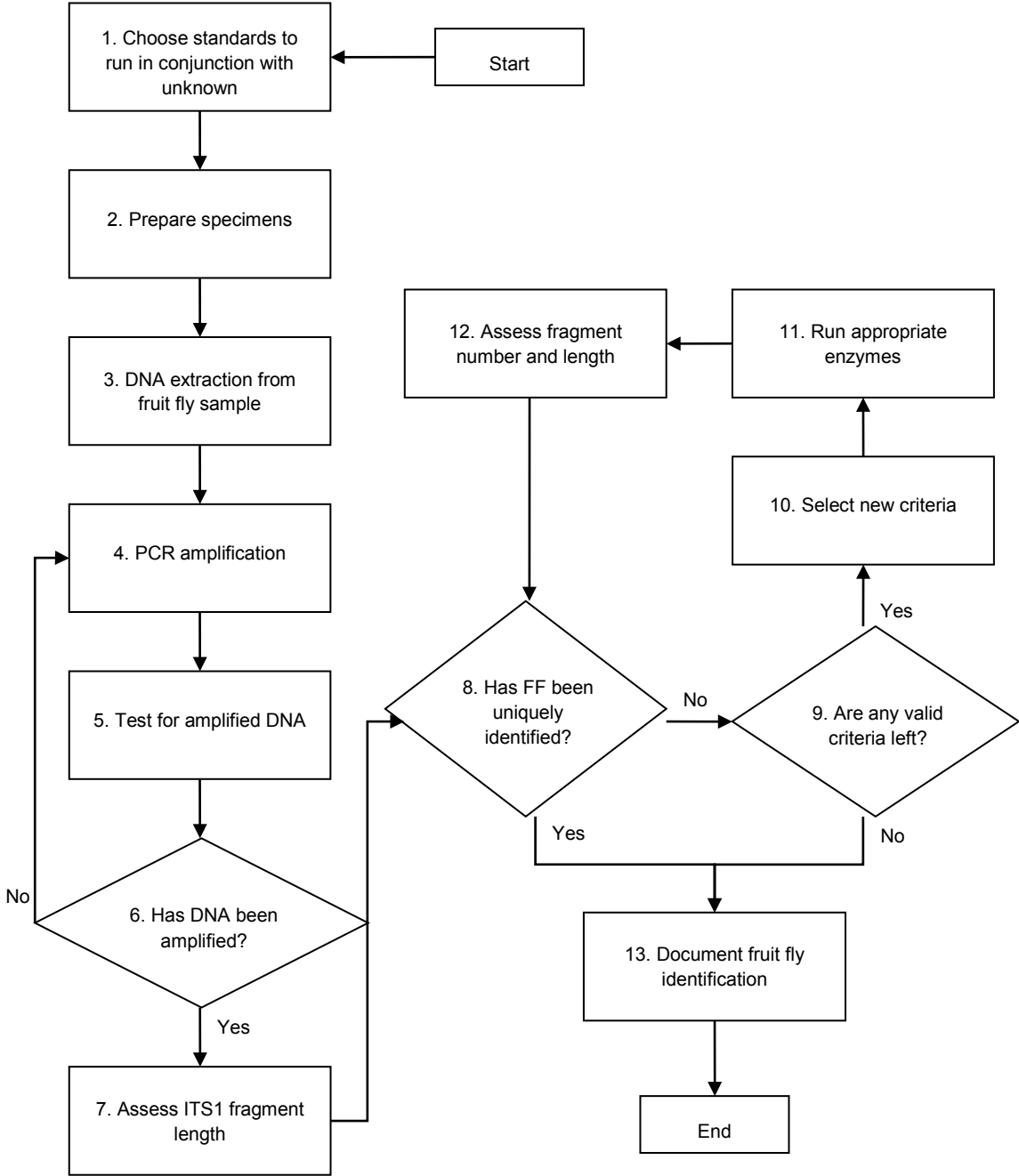
6.3.1.1.3   *Extraction of DNA from fruit fly material*

Equipment

- Pipettors and tips
- Sterile disposable microcentrifuge tubes
- Microcentrifuge
- Gel tank and power pack
- Latex or Nitrile gloves
- Microwave
- UV transilluminator with camera

Reagents

- DNeasy Tissue QIAGEN Kit (but other similar kits could be tried)
- 1 x PBS
- Ethanol (Reagent grade)
- Agarose (Amresco)
- 1 x TBE
- DNA molecular weight marker (aka 100 bp ladder)
- Ethidium bromide (Sigma), staining solution at 800 ng μL^{-1} final concentration

Method

Extraction is essentially as per manufacturer's recommendations.

1) Use aseptic technique to place <50 mg insect into a pre-labelled sterile 1.5 mL microcentrifuge tube (one adult fly = 4 mg).
2) Add 180 µL of 1 x PBS and grind with a sterile disposable pestle.
3) Add 20 µL of Proteinase K solution.
4) Add 200 µL of Buffer AL.
5) Incubate at 70°C for at least 10 min.
6) Add 200 µL of ETOH and mix well, then pipette all the mixture into a prepared DNeasy Spin Column.
7) Centrifuge for 1 min at 13000 rpm.
8) Discard flow through and collection tube. Put the spin column into a new collection tube.
9) Add 500 µL of the prepared AW1 buffer. Centrifuge for 1 min at 13000 rpm.
10) Discard flow through and collection tube. Put the spin column into a new collection tube.
11) Add 500 µL of the prepared AW2 buffer. Centrifuge for 1 min at 13000 rpm and carefully discard the flow through centrifuge for a further 3 min.
12) Carefully put the column into a new sterile labelled Eppendorf tube without contacting the flow through or touching the base on anything that is not sterile.
13) Add 50-100 µL (depending on the amount of starting material) of Buffer AE directly onto the centre of the column membrane. Incubate at room temperature for 1 min.
14) Centrifuge for 3 min at 10000 rpm.
15) Discard the column and store the eluted DNA at -20°C until required.

16) Check DNA quality on a 1% agarose gel made up in 1X TBE. Load 1-5 µL DNA solution + 2 µL Gel Loading Buffer in each well, and run at 80 V x 60 min or 120 V x 30 min. Post-stain in a 1 mg L^{-1} ethidium bromide solution.

6.3.1.1.4 *Amplification of ITS1 region from fruit fly material using the polymerase chain reaction*

Equipment

- Pipettors and tips
- Sterile disposable microcentrifuge tubes
- Microcentrifuge
- Gel tank and power pack
- Latex or Nitrile gloves
- Microwave
- UV transilluminator with camera
- Thermocycler
- Personal protective equipment including lab coat, eye protection, gloves

Reagents

- Primer sequences are:
  baITS1f 5' GGA AGG ATC ATT ATT GTG TTC C 3' (McKenzie *et al.* 1999)
  baITS1r 5' ATG AGC CGA GTG ATC CAC C 3' (McKenzie *et al.* 1999)
- 1X TBE buffer
- 1% (w/v) agarose gel: 1 g DNA grade agarose per 100 mL 1X TBE
- 6X Loading dye
- DNA molecular weight marker (aka 100 bp ladder)
- Ethidium bromide staining solution (final concentration 800 ng µL^{-1})

Method

In pre-PCR cabinet:

1) Label sterile 0.2 mL PCR tubes.

	Final concentration	Each
Manufacturer's reaction buffer (10X)	1X	5 µL
MgCl$_2$ (50 mM)	1.5 mM	1.5 µL
dNTP's (2 mM)	200 µM	5 µL
Forward primer (10 µM)	1 µM	5 µL
Reverse primer (10 µM)	1 µM	5 µL
H$_2$O		20.25 µL
Taq polymerase enzyme (5U µL^{-1})		0.25 µL
Total volume		**42 µL**

2) Store "Master Mix" on ice in sterile 1.5 mL centrifuge tube.

3) Add 8 µL of sterile dH$_2$O to the first negative control tube.

In BSC:

1) Add the *Taq* polymerase to the Master Mix in the BSC.

2) Aliquot 42 µL Master Mix to the each PCR tube.

3) Add 8 µL of DNA extract to each sample tube as appropriate.

4) Add 8 µL of positive control DNA appropriate tube(s).

5) Add 8 µL of sterile dH$_2$O to the second negative control tube.

6) Cycle the tubes using the following program:

Cycle 1	Step 1	94°C	2 min
Cycles 2 to 35	Denaturing	94°C	1 min
	Annealing	60°C	1 min
	Extension	72°C	1 min
Final extension 5 min		72°C	

7) Place reaction products on ice or freeze until ready to analyse.

8) Mix 3 µL of each PCR sample with 2 µL loading dye.

9) Load samples and 100 bp DNA ladder onto separate wells of 1% (w/v) agarose gel in 1X TBE.

10) Electrophorese in 1X TBE buffer at 100 V for around 40 min.

11) Stain the gel in ethidium bromide, according to local Standard Operation Procedure.

12) Visualise bands and capture image using the Gel Documentation System.

Analysis of ITS fragment length

The expected size of the amplified product is between 600 and 1200 bp, depending on the species. Some species can be differentiated from others on the target list simply by the size of their ITS1 fragment, particularly if combined with other data on host or geographic origin (Section 7).

Sizes of ITS1 fragments for the species in the target list are shown in Table 3. Sizes are given as a range to reflect that sizing is approximate when using low-resolution gel electrophoresis systems such as these.

Table 3. Approximate size in bp of the ITS1 region for each species

Species	Fragment range	Species	Fragment range
A. ludens	640-680	*B. latifrons*	760-780
A. obliqua	650-690	*B. moluccensis*	800-820
A. serpentina	740-760	*B. musae*	770-790
B. albistrigata	840-860	*B. neohumeralis*	810-840
B. aquilonis	790-830	*B. papayae*	800-840
B. bryoniae	790-830	*B. passiflorae*	810-840
B. carambolae	830-860	*B. philippinensis*	800-840
B. cucumis	760-770	*B. psidii*	780-800
B. cucurbitae	590-610	*B. tryoni*	810-830
B. curvipennis	830-860	*B. umbrosa*	750-780
B. dorsalis	800-820	*B. xanthodes*	670-700
B. endiandrae	770-800	*B. zonata*	820-850
B. facialis	750-780	*C. capitata*	890-900
B. frauenfeldi	830-860	*C. rosa*	1000-1040
B. jarvisi	800-840	*R. pomonella*	740-780
B. kirki	840-860	*D. pornia*	500-520

6.3.1.1.5 *Restriction digestion of PCR product*

If the species of fly is not identified by the size of the ITS1 fragment, a restriction digest on the ITS1 PCR product is performed to differentiate between species. These data are self-contained, and the table could be used as the only tool to identify an unknown fly. Flies producing fragments of less than 700 bp or greater than 900 bp are segregated and then restriction enzymes are used in series to differentiate the species.

Enzymes were also selected based on the requirement for differences in fragment sizes to be easily detected by visual examination of an agarose gel.

The scheme developed, particularly the use of a combination of enzymes in series, allows definitive identification of the majority of the species. This powerful combination eliminates the reliance on discrete restriction sites and limits the likelihood of false negatives that may arise through a rare recombination event.

Restriction endonucleases used are *Vsp*I, *Hha*I, *Ssp*I, *Hinf*I, *Bsr*I, *Sna*BI and *Sau*3aI. During the development of this standard enzymes purchased from New England Biolabs were used but other brands would work equally well.

Since the time this protocol was developed, nucleotide sequencing has also become much more routine and affordable and this type of analysis may be more applicable to laboratories with this capacity.

Equipment

- Pipettors and tips

- Sterile disposable microcentrifuge tubes

- Microcentrifuge

- Dry heating block, waterbath or similar

- Gel tank and power pack

- Latex or nitrile gloves

- Microwave

- UV transilluminator with camera and image capture and analysis software

- Personal protective equipment including lab coat, eye protection, gloves

Reagents

- Sterile distilled water

- Bovine serum albumin (BSA, 10 µg µL^{-1}) (comes supplied with NEB enzymes)

- Restriction enzymes *Vsp*I, *Hha*I, *Ssp*I, *Hinf*I, *Bsr*I, *Sna*BI, and *Sau*3aI

- Restriction buffer supplied with enzyme

- Ethidium bromide solution, 800 ng µL^{-1} final concentration

Method

1) Label microcentrifuge tubes.
2) To each centrifuge tube add:

Water	2.3 µL
10X buffer	2 µL
BSA (10 ug µL^{-1}) 0.2 µL	
PCR product	5 µL
Restriction enzyme	0.5 µL

3) Mix reagents and place tubes in a waterbath preheated to 37°C for 2 h.
4) Store tubes on ice or at -20°C until ready to load on agarose gel.
5) Add 3 µL of 6X loading buffer to each tube.
6) Load the entire volume of each sample (23 µL) into a lane of a 2% (w/v) high resolution blend agarose gel.
7) Load 100 bp DNA molecular weight marker into one or two wells of the gel.
8) Analyse products by electrophoresis at 100 V for 50 min.
9) Stain the gel with ethidium bromide.
10) Visualise fragments using a UV transilluminator.
11) Capture gel image using gel documentation system.

Analysis of RFLP products

In analysis of RFLP profiles for diagnostic purposes, bands under 100 bp and over 1500 bp in size are disregarded. The molecular weights of experimental bands are calculated with reference to the DNA molecular weight standards loaded on the same gel.

Table 4 summarises the data for the ITS1 fragment length and the six restriction enzymes used within this diagnostic procedure.

6.3.1.1.6 *Nucleotide sequencing analysis of entire ITS1 fragment*

The PCR product can also be sequenced to confirm the identity of fruit fly if required, however a region near one end that is AT-rich in many species frequently presents problems with this approach.

Nucleotide sequencing can be done in-house or outsourced; details of the reaction chemistry and fragment resolution are not presented here.

Equipment

- Pipettors and tips
- Sterile disposable microcentrifuge tubes
- Microcentrifuge
- Gel tank and power pack
- UV transilluminator with camera
- Personal protective equipment including lab coat, eye protection, gloves
- PC with internet access
- Software programs for analysis

Reagents

- Primers:

    baITS1f 5' GGA AGG ATC ATT ATT GTG TTC C 3' (McKenzie *et al.* 1999)

    baITS1r 5' ATG AGC CGA GTG ATC CAC C 3' (McKenzie *et al.* 1999)

- 1X TBE buffer
- 1% (w/v) Agarose gel
- Loading dye
- Molecular mass DNA ladder (Invitrogen)
- Ethidium bromide staining solution (final concentration 800 ng mL^{-1})
- JetQuick™ PCR Purification Kit (Astral Scientific)
- Nucleotide sequencing kit

Table 4. Analysis of RFLP products from ITS1 fragments from fruit flies

Species	ITS1*			Hinfl		Vspl		Hhal		Sspl		Bsrl		SnaBl		Sau3al	
	<700	700-900	>900	DNC	Cuts	DNC	Cuts	DNC	Cuts	DNC	Cuts	DNC	Cuts	DNC	Cuts	DNC	Cuts
A. ludens	X				550		550	X		X		X		X		X	
A. obliqua	X				450, 270		550	X			550, 150	X		X			450, 200
A. serpentina		X		X			420, 250	X		X		X		X			530, 200
B. albistrigata		X		X		X			670, 180		620, 180	X		X			450, 400
B. aquilonis		X			770	X			640, 190		570, 180		600, 200	X			415
B. bryoniae		X			760	X			620, 200		560, 180		600, 230	X			400
B. carambolae		X		X			480, 350		680, 200	X			650, 250		530, 350		450, 400
B. cucumis		X		X		X			550, 180	X		X		X		X	
B. cucurbitae	X			X		X			400, 180	X		X		X		X	
B. curvipennis		X		X		X			620, 170		550, 200		570, 250	X			420
B. dorsalis		X			770	X			650, 190	X			650, 260		540, 320	X	
B. facialis		X		X		X			600, 180	X			600, 200	X			390
B. frauenfeldi		X		X		X				X	620, 180			X			450, 400
B. jarvisi		X			770	X			640, 180		700		600, 250	X			420
B. kirki		X		X		X			680, 190		620, 180	X		X			450, 400
B. latifrons		X		X		X			600, 190	X			600, 200	X		X	
B. musae		X		X		X			635, 220	X			600, 250		520, 320	X	
B. neohumeralis		X			770	X			640, 190		570, 180		600, 200	X			420
B. papaya		X			770	X			650, 190		750		650, 260		535, 320	X	
B. passiflorae		X			770	X			650, 190		750		650, 270	X		X	
B. philippinensis		X			770	X			650, 190		750		630, 250		535, 320	X	
B. psidii		X		X		X			640, 190		570, 250	X		X		X	
B. tryoni		X			770	X			640, 190		570, 180		600, 200	X			420
B. umbrosa		X			730	X			600, 190		680	X		X			380
B. xanthodes		X			680	X			670, 200		380, 250	X		X		X	
B. zonata		X		X		X			680, 190		750		600, 200		535, 330	X	
C. capitata			X	X			650, 200	X			520, 160	X		X		X	
C. rosa		X			800, 200		600, 300	X			570, 480	X		X		X	
Di. pornia	X			X		X		X			300, 220	X		X		X	

* The length of the ITS1 fragment and the response of each to seven restriction enzymes (*Hinfl, Vspl, Hhal, Sspl, Bsrl, SnaBl, Sau3al*) are indicated for each of the target species. ITS1 fragment length is scored as one of three classes (approximate length in bp). Enzyme responses are measured in two classes - either does not cut (DNC) or cuts (Cuts – this column shows the length of each fragment in bp).

Method

1) Amplify the ITS1 region as per section 6.3.1.1.4 (Amplification of ITS1 region from fruit fly material using the polymerase chain reaction).

2) Clean the amplified DNA away from other reaction components using JetQuick™ Spin kit as per manufacturer's instructions (or other similar process).

3) Load a fraction of the cleaned DNA onto an agarose gel against DNA mass standards to quantitate the concentration of DNA in the cleaned PCR product (ng µL^{-1}).

4) Prepare cleaned PCR products for sequencing as per the manufacturer's instructions.

5) Perform nucleotide sequencing reaction and resolve products.

6) Consolidate forward and reverse reactions for each fragment to determine fragment sequence.

7) Compare fragment sequences against all sequences posted on the GenBank database (**www.ncbi.nlm.nih.gov/genbank**) using the program BlastN (Altschul *et al.* 1997), to determine if the sequence is Tephritidae and which species.

6.3.1.1.7   *Composition of reagents*

5X TBE buffer

- 450 mM Tris base
- 450 mM Boric acid
- 10 mM EDTA (pH 8.0)
- Store at room temperature

Dilute to 1X TBE with millipore water prior to use.

1% Agarose gel

1) 1 g of DNA grade agarose per 100 mL of 1X TBE.
2) Melt in a microwave.
3) Pour into a prepared gel tray when agarose has cooled sufficiently.
4) Allow the gel to set at room temperature for at least 30 min before use.

6 x Loading dye

- 1X TBE buffer
- 0.25% (w/v) Bromophenol Blue
- 0.25% (w/v) Xylene cyanol FF
- 30 % (v/v) Glycerol

Store at room temperature.

10X PBS

- 1.37 M NaCl
- 27 mM KCl
- 43 mM $Na_2HPO_4.7H_2O$
- 14 mM KH_2PO_4

1) Autoclave.

2) Store at room temperature.

3) Dilute to 1X PBS with sterile water for use.

4) Store 1X PBS buffer at room temperature in sterile bottle.

RFLP TEST 2

6.3.1.1.8   *Procedure overview*

As in Test 1, fruit fly DNA is extracted using a commercially available kit. The nuclear internal transcribed spacer (ITS1) and partial *18S* genes are amplified using PCR techniques. The PCR product is then digested using four recommended restriction enzymes and the fragments of different sizes are visualised on a gel. Digested fragment patterns are then compared to those of *B. tryoni* and *C. capitata*.

NB: Reference fragment lengths for each species are contained in the relevant data sheets in Section 7 and gel image provided in Section 6.3.1.1.12 below.

6.3.1.1.9   *Sample handling*

Refer to details in Test 1. Live larvae can be placed directly into boiling water for fixing. Larvae are then placed into 100% ethanol and if not used immediately for extraction are stored in -20°C or -80°C. Adults may be stored dry or in 100% ethanol but preferably stored at -20°C freezer.

Figure 11: Workflow of molecular diagnostic procedure for fruit fly identification

Receive samples of presumed fruit fly larvae for identification

Morphological examination conducted to ascertain whether specimens are fruit flies –
1. If YES, determine whether QFF.
2. If identification to species is not possible (based on morphology), then proceed further

Molecular diagnostic
Test (PCR-RFLP)

DNA Extraction

(QIAGEN DNeasy ®
Blood and Tissue Kit)

PCR amplification
(ITS1 + 18S)
(include QFF +ve ctrl)

PCR product
positive = band at
~1500 bp

Restriction Fragment
Length Polymorphism (RFLP)
(digest PCR product using four enzymes- *Alu*I, *Dde*I, *Rsa*I and *Ssp*I)

Gel Analysis – compare size of fragment/s with restriction profile for QFF group and MedFly

Specimen profile = QFF/MedFly or not. Species diagnosis completed

6.3.1.1.10 *Extraction of DNA from fruit fly material*

Materials and Equipment

- blotting paper (or kimwipes)
- blades
- Eppendorf tubes (1.5 mL) and racks
- ethanol (100%)
- heating block
- ice
- forceps
- plastic pestles (0.5 mL)
- pipettes (0.02-2 µL, 2-20 µL, 20-200 µL, 200-1000 µL)
- pipette tips – aerosol resistant

Preparation of specimens for extraction

Larvae

i. Blot specimens on towel paper and leave to dry for at least 1 min until ethanol evaporates.

ii. Cut mid-section of larva (use middle 1/3 of specimen) and place in a 1.5 mL Eppendorf tube (use equal quantity of material for each sample if possible).

iii. Place head (anterior 1/3, with spiracles and mouth-hooks) and posterior part of abdomen (posterior 1/3, with spiracles and anal lobes) into a separate 1.5 mL Eppendorf tube in 100% ethanol to be stored at -20°C for future reference.

iv. Keep specimens on ice until ready for grinding.

Adults

i. Blot specimens if stored in ethanol.

ii. Remove head or legs for extraction.

iii. Pin/ dry mount remaining specimen or place back into ethanol (with cross-referenced labels) for future reference.

iv. Centrifuge Eppendorf tube (with dissected insect section).

Note:

i. Allow approximately 1-3 h for processing 1-10 specimens.

ii. Before starting heat water bath or heating block to 55°C.

QIAGEN Kit extraction

Refer also to instructions in QIAGEN DNeasy ® Blood and Tissue kit handbook for guide to animal tissue extractions but note any differences in instructions below indicated by an asterisk.

v. Add 5 µL of Buffer ATL and 5 µL of Proteinase K to sample. Grind specimen using 0.5 mL plastic pestle until there are no large fragments visible.

vi. Add 195 µL of Buffer ATL and 15 µL of Proteinase K and vortex for 5 s. Incubate for 1-1 ½ h at 55°C.

vii.   Vortex for 15 s. Then add 200 µL of Buffer AL. Vortex and incubate for 10 min.

viii.   Add 200 µL of ethanol and vortex again for 5 s.

ix.   Pipet mixture into QIAGEN column and centrifuge at ~6000 *g* for 1 min. Discard flow-through.

x.   Place column into a new collection tube. Add 500 µL of Buffer AW1. Centrifuge at ~6000 *g* for 1 min and discard flow-through.

xi.   Place column into a new collection tube. Add 500 µL of Buffer AW2. Centrifuge at ~20, 000 *g* and centrifuge for 3 min. Discard flow-through.

xii.   Place column into a new 1.5 mL tube and add AE buffer. *If part specimen (such as 1/3 of the larva, or the adult fly head, or legs of adults), use only half of the elution buffer recommended in the DNeasy® Blood and Tissue Handbook (for Spin Column protocol) (i.e. only use 100 µL or less for each elution step.

xiii.   Incubate at room temperature for 1 min and then centrifuge for 1 min at 6000 *g*.

6.3.1.1.11   *Amplification of ITS1 and 18S gene region from fruit fly material using the polymerase chain reaction*

Note: A single PCR product should be between 1.5 – 1.8 *kb* in size

Materials and equipment

- centrifuge
- Eppendorf tubes (0.5 mL and 0.2 mL)
- ice
- PCR machine
- pipettes (various sizes incl. 2.0-20 µL, 20-200 µL, 200-1000 µL)
- pipette tips – aerosol resistant
- plastic storage racks
- vortex

Chemicals and reagents

- dNTPs (2.5 µM)
- nuclease free H_2O
- primers
  - (10 µM) - NS15 5' CAATTGGGTGTAGCTACTAC 3'
  - (10 µM) - ITS6 5' AGCCGAGTGATCCACCGCT 3'
- NEB *Taq* polymerase or (5U µl^{-1})
- NEB Thermpol buffer (X10)

Master mix recipe	Final Concentration	Per reaction (µL)
dd H_2O		30.6
10X Thermpolbuffer	1 X	5
2.5 µM dNTP's	200 µM	4
10 µM (ITS6)	0.5 µM	2.5
10 µM (NS16)	0.5 µM	2.5
2 Units NEB Taq Polymerase	2 Units	0.4
Template DNA	5	
Total		**50**

Amplification

1. Vortex extractions for 5-8 s.

2. Aliquot 5 µL of DNA template to a 0.2 mL tube. Note: Include at least one positive control (QFF or Medfly) and one negative control in the test.

3. Prepare Master Mix using recipe above.

4. Aliquot 45 µL of master mix to each 0.2 mL tubes (containing 5 µL of template DNA).

5. Mix product and reagents well (or vortex) and centrifuge for 3-5 s.

6. Place samples in PCR machine and program the following temperature profile (based on Armstrong and Cameron 1998):

Step 1

94°C / 2 min　　　} x1 cycle

Step 2

94°C / 15 s

60°C / 30 s　　　} x40 cycles

68°C / 2 min

Step 3

72°C / 5 min　　　} x1 cycle

23°C / ∞

Check product yield after PCR by visualising PCR products on a 1.5% agarose gel and add 1 µL of loading dye to 5 µL of PCR product. Use a 100 bp ladder for measuring product size. Run gel at 100 V (see instructions in Section 6.3.1.1.15 for preparing and setting up a gel). If product visible at 1.5-1.8 kb then proceed to Section 6.3.1.1.12 – restriction digest.

6.3.1.1.12 *Restriction digestion of PCR product*

Materials and equipment

- Eppendorf tubes (0.2 mL)
- ice
- pipettes (various sizes including 2.0-20 µL, 20-200 µL, 200-1000 µL)
- pipette tips – Aerosol resistant
- incubator
- centrifuge
- vortex

Chemicals and Reagents

*Alu*I, *Dde*I and *Rsa*I (10 U/µl) and *Ssp*I (5U/µl)

nuclease free H_2O

100X BSA

10X Buffer

Restriction enzymes	Incubation Temperature	Time
*Alu*I	37°C	2-3 h
*Dde*I	37°C	2-3 h
*Rsa*I	37°C	2-3 h
*Ssp*I	37°C	2-3 h

Master mix recipe for restriction enzymes (based on recommendations by Melissa Carew, CESAR, Melbourne University)

	µL per reaction
dd H_2O	7.2
10X Buffer	2
100X BSA	0.2
Enzyme	0.6
PCR product	10
Total	**20**

Restriction Digest method

i. Digest each sample with each of the four enzymes.

ii. For each samples aliquot 10 µL of PCR product into a 0.2 µL tube (repeat for four tubes in total).

iii. Prepare master mix (following recipe above) for each enzyme.

iv. Aliquot 10 µL of each Master mix solution to 10 µL PCR product.

v. Mix reagents and PCR product and centrifuge briefly for 3-5 s.

vi. Place samples in incubator at temperature recommended for each enzyme.

vii. Prepare a 2-3% agarose gel (see Section 6.3.1.1.15 D) to visualise fragment pattern and use a 100 bp ladder for determining fragment sizes

viii. See Section 6.3.1.1.13 for expected fragment pattern and size of *Bactrocera tryoni* group and *Ceratitis capitata*.

Note: Also, compare results with positive controls and check fragment pattern as in Armstrong and Cameron (1998).

6.3.1.1.13 *Nucleotide sequencing analysis of entire ITS1 fragment*

Species	Pattern	Fragment sizes (bp; as in Armstrong and Cameron 1998)
***Alu*I enzyme**		
B. tryoni group	**C3**	780-770, 240-230*, 170, 130 120 110
C. capitata	**D3**	1300, 130, 120, 110
***Dde*I enzyme**		
B. tryoni group	**A5**	1000-980*, 270, 220, 170-160
C. capitata	**D**	1150, 270, 220,130
***Rsa*I enzyme**		
B. tryoni group	**C1**	530-500*, 460-440*, 410, 290
C. capitata	**K**	450, 380, 290, 260, 240, 210
***Ssp*I enzyme**		
B. tryoni group	**G1**	1000, 550, 100
C. capitata	**G2**	1020, 520, 100

* = sometimes double band present

Restriction enzyme pattern types are represented by letters as used in Armstrong and Cameron (1998).

	*Alu*I	*Dde*I	*Rsa*I	*Ssp*I
Bactrocera tryoni group (includes *B. aquilonis*, *B. neohumeralis* and *B. tryoni*)	C3	A5	C1	G1
Ceratitis capitata	D3	D	K	G2

Species	Distinct enzyme profiles
Bactrocera tryoni group	**Unique pattern type** for *Ssp*I (*C. capitata* is probably the closest to QFF for this enzyme, but look at results for *Alu*I, *Dde*I and *Rsa*I which clearly separates these two groups).
Ceratitis capitata	**Unique pattern** for *Dde*I, *Rsa*I and *Ssp*I also useful.

Comments:

1. Variations - for *Ssp*I, the smallest fragment (100bp) is not clearly visible on all gels and often the *Ssp*1 enzyme does not fully digest the entire PCR product, thus a full size band (around 1500 bp) may also appear.

2. It is a good idea to compare the results with fragment enzyme patterns as presented in Armstrong and Cameron (1998). Some patterns may appear very similar amongst species for some enzymes but by using at least four enzymes, a unique combination of patterns helps distinguish QFF group and Medfly from each other and other species tested.

6.3.1.1.14 *Restriction digest patterns on gel*
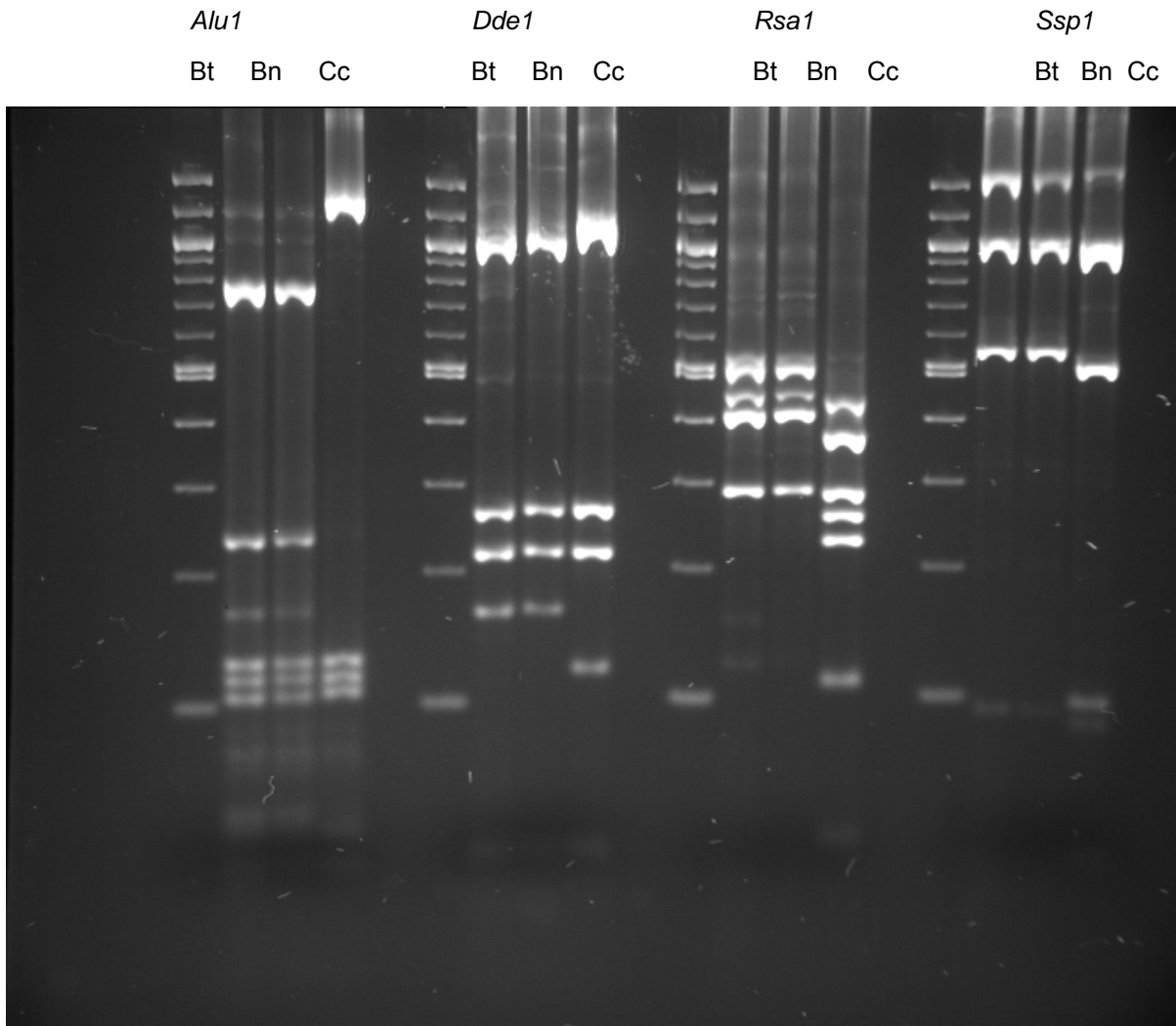
Bt = *Bactrocera tryoni* **(Queensland fruit fly)**

Bn = *Bactrocera neohumeralis* **(Lesser Queensland fruit fly)**

Cc = *Ceratitis capitata* **(Meditteranean fruit fly)**

(see previous page for haplotype patterns and fragment length sizes)

6.3.1.1.15   *Composition of reagents and preparation*

A. Preparing Primers

Preparing Primers for a 10 µM concentration

i. Add nuclease free water to primer stock. To calculate quantity of water to add, find the nmol reading for each primer and move decimal place forward (to right) of the nmol reading eg. 51.7 nmol = 517 µL of nuclease free water to be added to primer stock.

ii. Prepare a 1 in 10 dilution of primer for final stock. eg. if preparing 500 µL, then add 450 µL of nuclease free water and 50 µL of primer from original stock.

B. Preparing dNTP's

The final dNTP stock is prepared using individual nucleotide stocks each with initial concentration of 100 mM. To prepare dNTP's for a 400 µl final stock with final concentration of 2.5 mM use the following steps:

- Prepare Eppendorf tubes (1.5 ml) for final stock

- Add 360 µl nuclease free water to each Eppendorf tube

- Add 10 µl of each dNTP to each final stock eg. 10 µl of A, 10 µl of C, 10 µl of G, 10 µl of T, to each tube. Mix well

- This should make a 1 in 10 dilution = 2.5 mM.

C. Preparing molecular weight marker (100 bp ladder)

For 250 µg mL^{-1} concentration of marker:-

- 400 µL marker

- 200 µL loading dye

- 600 µL ddH$_2$O

Proportion is 2:1:3 respectively

D. Preparing a gel - basic equipment and materials

- agarose powder

- electrolytic buffer (TAE or TBE)

- SYBR® Safe DNA gel stain (or ethidium bromide if alternative is not available)

- loading dye (X6)

- large glass flask and plastic jar (250 mL)

- molecular weight marker (100 bp ladder)

- microwave

- plate and combs

- pipette tips - non-aerosol

- pipettes

Preparation of gel

Gel size may be 50 mL, 100 mL, or 200 mL.

1. Use a 1.5% agarose gel for PCR product and a 2-3 % gel for digest products.

2. Add TBE to agarose powder.

3. Microwave until boiling point and solution is clear.

4. Pour agarose into a plastic beaker and add SYBR® Safe to solution once the agarose is heated. Add 1 µL of SYBR® Safe to 10 ml of agarose solution. If using ethidium bromide, add directly to glass beaker and use the same quantity per mL, but make sure to use a 1 in 10 ethidium bromide solution.

5. Pour gel onto plate and allow to cool for 30 min or until gel is opaque.

6. Run electrophoresis machine. Time will vary depending on size and density of gel eg. 250 mL gel can run at 90 V for 3-4 h. A 50 mL gel may run for 30 min to 1 h at 100 V.

<u>Viewing Results</u>

i. For gels with SYBR Safe, place onto the Safe Imager Transilluminator (with amber filter unit) and cover with camera box for viewing image on screen. If using ethidium bromide place gel onto a UV light trans-illuminator and cover with camera box.

ii. Save gel image electronically or print.

iii. A bright band at around 1500 bp indicates a positive PCR result (i.e. successful amplification for fruit fly DNA).

iv. For digests, use a 100 bp ladder to determine size of fragments and compare with fragment size chart above in Section 6.3.1.1.13. If specimens do not match QFF group or Mediterranean FF, compare with other fragment profiles in Armstrong and Cameron (1998).

6.3.2 DNA barcoding of tephritid fruit flies

This test was developed by Mark Blacket, Linda Semeraro and Mali Malipatil, Victorian Department of Primary Industries (Blacket *et al.,* 2012).

INTRODUCTION

Tephritid fruit fly adult specimens are primarily identified through an examination of diagnostic morphological characters (Table 2). Other life stages are more problematic, with only third instar larvae (and sometimes pupae) usually identified through visual examination. Identification of earlier life stages (early instars, eggs), and morphologically ambiguous adult specimens, generally requires the use of molecular techniques.

DNA barcoding is a molecular method that is routinely being applied at DPI Vic to identify such morphologically problematic specimens. This technique involves obtaining a DNA sequence of a specific gene (usually the mitochondrial COI gene) from a specimen to compare with a database of reference specimens. There are currently many reference DNA barcoding sequences available; most of these were obtained through the Tephritid Fruit FIy Project[3], which examined all known tephritid fruit fly species known to be agricultural pests as well as many closely related species. However, there are currently no peer-reviewed published DNA barcoding laboratory protocols covering all of the targeted tephritid species listed in Table 2 (although there have been some studies that have tested this approach on a limited number of species e.g. Armstrong and Ball 2005). The method outlined below utilises the reference information that is publicly available through the Bar Code of Life website[4] to assist in identifying specimens using DNA barcoding.

DNA barcoding should ideally obtain DNA using relatively non-destructive techniques, to ensure that a voucher specimen is available for future morphological examinations (Floyd *et al*. 2010). Several suitable DNA extraction methods are currently available to retain voucher specimens after DNA

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[3] **www.dnabarcodes.org/pa/ge/boli_projects**

[4] **www.boldsystems.org/views/login.php**

extraction. For fruit fly adults a leg or the head of a specimen can be used while retaining many other valuable morphological features of the specimen (such as wings, thorax and abdomen). For larvae, anterior and posterior sections can be sectioned off and retained, preserving the morphologically valuable mouthparts and spiracles. Alternative even less destructive DNA extraction methods, involving Proteinase K digestion, will no doubt prove valuable in the future (e.g. Gilbert *et al.* 2007).

AIM

This test aims to identify fruit fly species through DNA sequencing and comparison with reference sequences of the DNA barcoding region, i.e. the COI gene.

TARGETS

This method utilises a publicly available database of reference DNA sequences from almost all of the relevant species of fruit flies from the Australasian region (Table 2). The small number of species (x 4, Table 2) that have not been sequenced to date belong to genera where many other species have been examined, allowing a DNA barcoding approach to at least place these species to the appropriate genus[5].

PROCEDURE

DNA is extracted from fruit flies (adults or larvae) using a commercially available kit. It is possible to obtain suitable DNA from larval specimens that have been blanched in hot water during morphological examination prior to freezing or storing in ethanol (preferably 100%). A region of the fly genome, the mitochondrial Cytochrome Oxidase I (COI) gene, is amplified using PCR. This region is then sequenced and compared with other publicly available reference sequences to assist in species identification. However, some species, such as *Bactrocera tryoni*, are members of very closely related species complexes, and are thus reported as being identified to a species group (e.g. QFF group, rather than *B. tryoni*).

This document provides supporting information for a two-step process involving:

1. DNA Extraction Protocol for DNA barcoding – Fruit fly larvae and adults.

2. Polymerase Chain Reaction (PCR) of the mitochondrial barcoding gene (COI) from Fruit fly DNA.

Additional steps: Agarose checking gel & PCR purification (see relevant protocols)

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[5] Species *B. aquilonis* and *B. tryoni* cannot be distinguished from each other at the ITS or COI region

6.3.2.1.1 *DNA extraction protocol for DNA barcoding – fruit fly larvae and adults*

Equipment and/or material needed

- Blotting paper (or tissues)

- Scalpel blades (if sub-sampling each sample)

- Micro-centrifuge

- Eppendorf tubes (1.5 mL)

- QIAGEN extraction kit (DNeasy® Blood and Tissue kit)

- Heating block to 56°C (or waterbath)

- Forceps

- Vortex

- Bead-Mill

- 3 mm solid glass beads

- QIAGEN DNeasy® Blood and Tissue Kit Handbook, July 2006 (for reference if required)

Methods

1) Allow several hours for processing (Proteinase K digest time dependent).
2) Heat heating block to 56°C (or use a water bath at 56°C).
3) Add two glass beads (3 mm solid beads, acid-washed in 10 % HCl prior to use) to a clean Eppendorf tube. Add 20 µL of Proteinase K to tube.
4) Remove samples (e.g. larva, adult head or leg) from 100% ethanol and dry on blotting "tissue" until ethanol evaporates (approximately 1 min). If samples were "dry" frozen omit this step.
5) Add sample to tube, cleaning forceps (ethanol wipe or flame) in between samples to prevent cross-contamination. Grind specimen in "Bead-Mill" (1 min @ 30 MHz).
6) Quick-spin in centrifuge (up to 10,000 rpm).
7) Rotate previously "outer" samples to "inner" position of the Bead-Mill (i.e. swap the inner Eppendorf tube insert around). Repeat Bead-Mill shaking (1 min @ 30 MHz).
8) Repeat Bead-Mill and centrifuge steps until samples contain no large visible fragments.
9) Add 180 µL of Buffer ATL and vortex.
10) Incubate for 1-1 ½ h at 56°C (possibly overnight for complete digestion).
11) Vortex, quick-spin to remove liquid from inner lid of Eppendorf tubes.
12) Add 200 µL of Buffer AL and 200 µL of ethanol (Buffer AL and ethanol can be premixed in a large tube for multiple samples, and then dispensed [400 µL] to each sample). Vortex.
13) Pipette mixture to QIAGEN kit column and centrifuge at ~6000 x g for 1 min. Discard lower collection tube.
14) Place column into a new collection tube. Add 500 µL of Buffer AW1. Centrifuge at ~6,000 x g for 1 min. Discard lower collection tube.
15) Place column into a new collection tube. Add 500 µL of Buffer AW2. Centrifuge at ~20,000 x g (17,000 x g is acceptable) and centrifuge for 3 min. Discard lower collection tube (making sure no AW2 Buffer splashes onto the base of column).
16) Label the top and side of clean 1.5 mL Eppendorf tube with the sample "VAITC" number.
17) Place column into the new Eppendorf tube. Add 100 µL of AE buffer (this buffer must come into direct contact with the column filter). Incubate at room temperature for 1 min, then centrifuge for 1 min at 6000 x g.

18) Repeat elution a second time.

19) Discard column and retain Eppendorf tube containing 200 µL of DNA in AE Buffer.

20) Store DNA in -20°C freezer.

6.3.2.1.2 *Polymerase chain reaction of the mitochondrial barcoding gene (COI) from fruit fly DNA*

Equipment and/or material needed

Primers:

- Forward: FruitFlyCOI-F (FFCOI-F) 5'-GGAGCATTAATYGGRGAYG-3' (Blacket *et al.,* 2012[6])

- Reverse: HCO 5'- TAAACTTCAGGGTGACCAAAAATCA-3' (Folmer *et al.* 1994)

PCR Master Mix:

- BSA [1X] (diluted with dH$_2$O from 100X BSA stock)

- NEB 10X Buffer (Cat# M0267S)

- dNTP's [2.5 mM]

- MgCl$_2$ [25 mM]

- Primers [10 µM] (working primer concentration is 10 µM, store stocks at 100 µm, -20°C)

- NEB *Taq* DNA Polymerase (Cat# M0267S)

- DNA template (see Section 6.3.2.1.1*. DNA extraction protocol for DNA barcoding*)

- QIAGEN QIAquick Spin Handbook, March 2008 (for reference if required)

Methods

1) Extract fruit fly DNA for use as template (see Section 6.3.2.1.1*. DNA extraction protocol for DNA barcoding*).

2) Set up Master mix (keeping all reagents on ice during setup).

3) Master Mix (25 µL reaction volume):

	X 1 reaction µL
1X BSA	17
10X Buffer	2.5
dNTP's	2
MgCl$_2$	0.5
FFCOI-F	1.25
HCO	1.25
NEB Taq	0.2
DNA Template	2

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[6] This primer is a fly-specific primer that was initially successfully tested on *Bactrocera*, *Ceratitis* (Tephritidae) and *Calliphora* (Calliphoridae) species.

4) PCR Conditions (use "T800", Eppendorf (epgradient S) Thermocycler):

1 x cycle	94 °C, 2 min
40 x cycles	94 °C, 30 s
	52 °C, 30 s
	72 °C, 30 s
1 x cycles	72 °C, 2 min
1 x hold	15 °C, indefinitely

5) After PCR is complete, load 5 µL of the PCR product (plus 2 µL loading dye) onto a 2% agarose checking gel (use 5 µL of SYBR Safe [Cat# S33102] per 50 mL liquid gel mix, before casting gel). Mix PCR product and dye together in plastic "gel loading" plate, using a new pipette tip for each sample. Run agarose gel at 100 V, for 30 min. Visualise and photograph gel on light box.

6) Clean successful PCR products using QIAquick PCR Purification Kit (QIAGEN, Cat# 28104), elute final volume in 30 µL of EB Buffer. Estimate PCR product concentration from agarose gel photo (weak PCR ~20 ng µL^{-1}, strong PCR >100 ng µL^{-1}).

7) Send to external facility (e.g. Micromon, Monash University/Macrogen, Korea) for DNA sequencing.

8) After high quality DNA sequences have been obtained (preferably with a QV or Phred score of greater than 20) they can are compared with a public database (i.e. BarCode of Life website[7]) to identify species as outlined in Section 6.3.2.1.3.

6.3.2.1.3 *Data analysis – DNA barcoding identification*

Method

1) Go to the Barcode of Life website (**www.boldsystems.org/views/login.php**).
2) Click on the "Identify Specimen" tab (**www.boldsystems.org/views/idrequest.php**).
3) Paste the DNA sequence (use only the high quality section of the DNA sequence) into the "Enter sequences in fasta format:" box (please note: there is no requirement for the sequence to actually be in FASTA format).
4) Click the "Submit" button.
5) The top 20 matches are displayed, together with the "Specimen Similarity" score (as a %).
6) The matches with the highest percentage similarity (listed from highest to lowest) are the reference sequences that best match the unknown specimen being identified.
7) It is a good idea to view the best matches as a phylogenetic tree using the "Tree based Identification" button.
8) Click "View Tree" to view a PDF of the phylogenetic tree.
9) The specimen being identified is referred to as the "Unknown Specimen" (written in red) on the tree (indicated with arrows in Figure 12 and Figure 13), and is shown closest to the reference specimens that it best matches (Figure 12).
10) The specimen can now be assigned to the species that it is most similar to. However, please note that sometimes specimens can only be assigned to species groups (i.e. a closely related species complex, Figure 13) that are unable to be distinguished using DNA barcoding.

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[7] http://www.boldsystems.org/views/idrequest.php

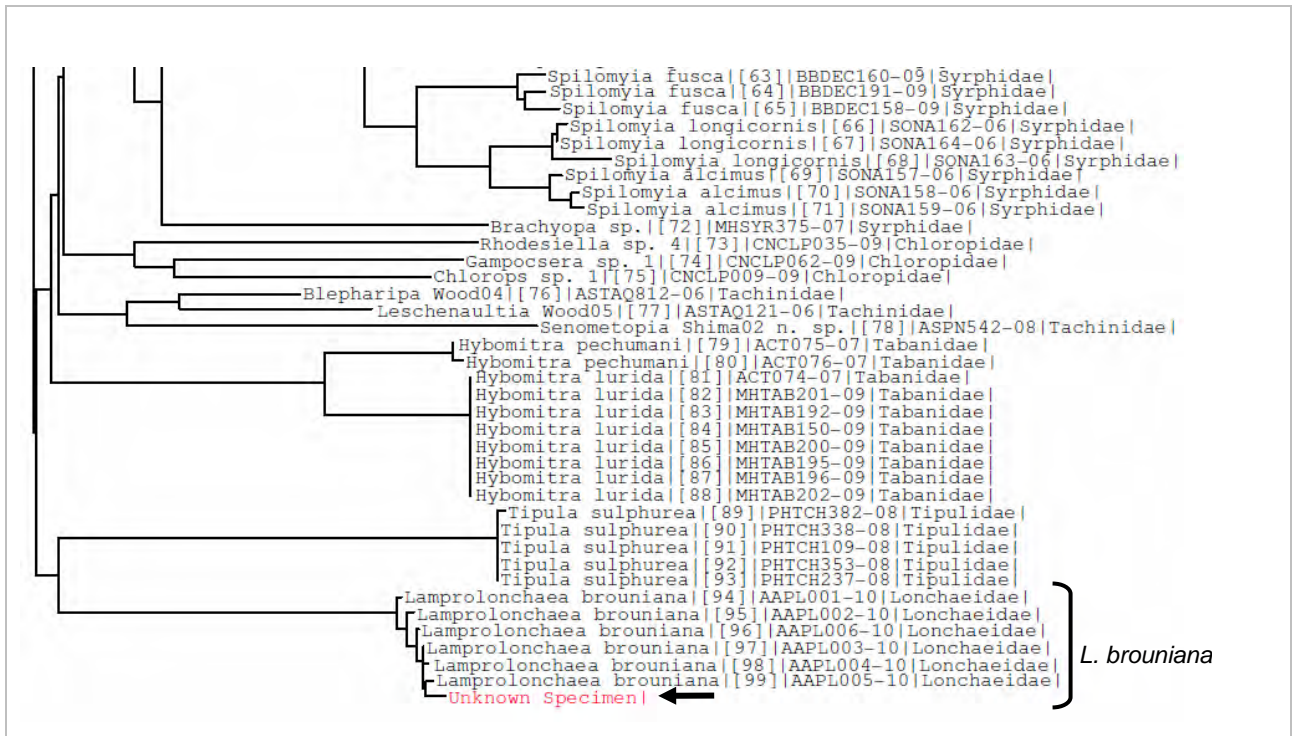**Figure 12.** Specimen confidently assigned to species (*Lamprolonchaea brouniana*)

Figure 13. Specimen only confidently assigned to species group (Queensland Fruit Fly group), due to three closely related species (*B. tryoni*, *B. aquilonis*, *B. neohumeralis*) being "mixed together" (i.e. non-monophyletic) on the phylogenetic tree
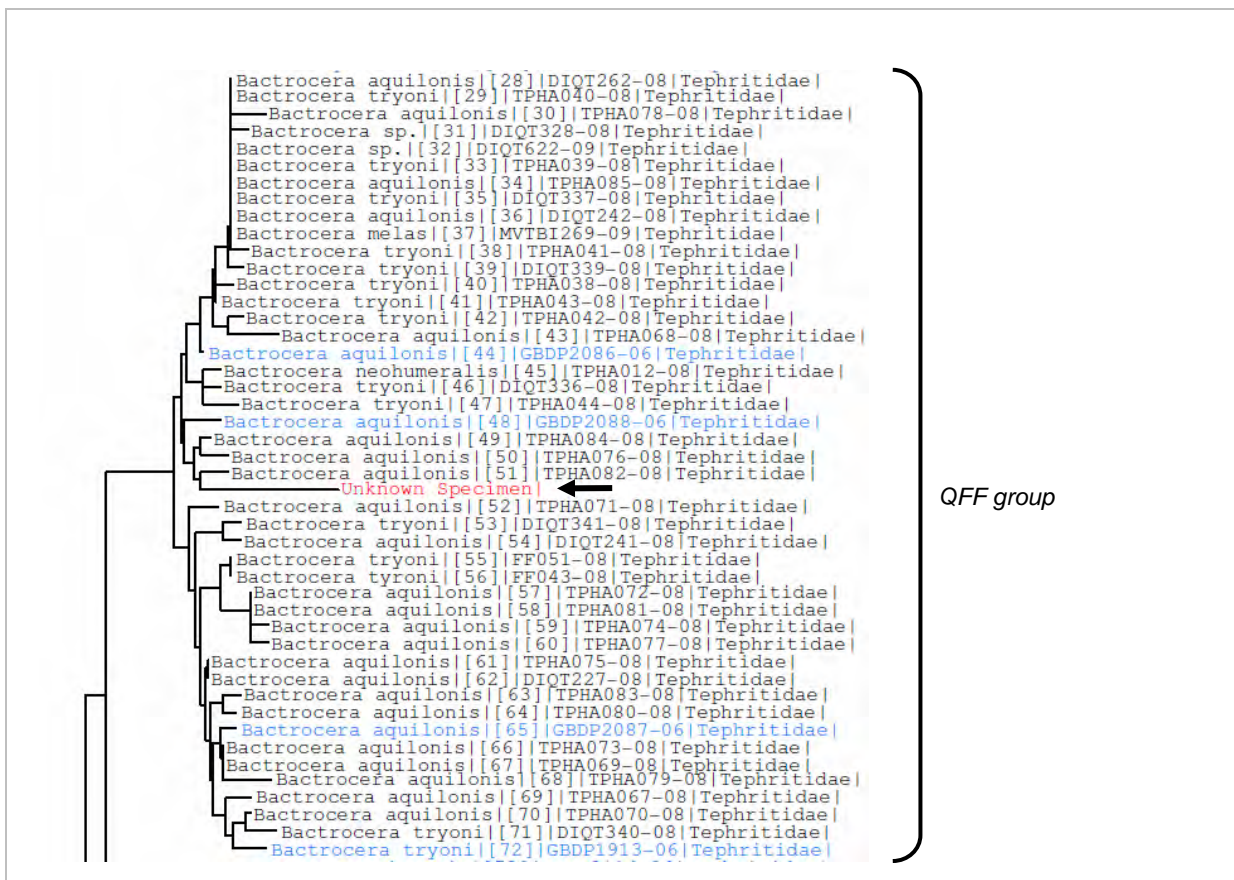
6.4 Allozyme electrophoresis

6.4.1 Aim

Allozyme electrophoresis provides a method for the rapid molecular identification of various species of fruit fly.

6.4.2 Targets

Routinely targets *Bactrocera tryoni*, *Ceratitis capitata*, *Dinoxia pornia*, *Bactrocera papayae* and *Bactrocera jarvisi* (Table 2). Additional species can be incorporated where suitable reference material is provided as freshly-frozen specimens.

6.4.3 Suitability

Suitable for the comparison of soluble protein from live, recently-dead, or freshly-frozen larvae or adults. The service is currently routinely provided by the South Australia Museum's Evolutionary Biology Unit laboratory. The procedures take 2-3 hours to complete for a single screen of up to 20 specimens for 10 different genes.

Given the comparative nature of the technique and its continued reliance on reference samples, it is important to note that additional species can only be identified as "new" (i.e. not one of the five reference species) unless suitable, known-identity samples can also be provided for a putative match. Moreover, the incorporation of additional species into the routine screening procedure may also require a re-evaluation of which enzyme markers are diagnostic for the species concerned, in order to satisfy the minimum requirement of three diagnostic genetic differences between every pair of species.

6.4.4 Procedure overview

Crude extracts of soluble protein from live, recently-dead, or freshly-frozen larvae or adults are compared electrophoretically against known-identity extracts representing these five species.

Test samples are readily identifiable by their comparative allozyme profile (i.e. relative band mobility) at a suite of six enzyme markers, encoded by a minimum of 10 independent genes, and together able to unambiguously diagnose the five reference species from one another at a minimum of three genes[8].

B. aquilonis is not listed in the five target species, so this method is **not** designed to differentiate between *B. tryoni* and *B. aquilonis*.

SPECIMEN PREPARATION

Test specimens

- Need to be supplied either (a) alive, (b) freshly dead and kept cool and moist, or (c) frozen when alive and not allowed to thaw until tested (dry ice required for transport; ice is not suitable)

- Can represent any life history stage

---

[8] *B. aquilonis* is not listed in the 5 target species, so this method is **not** designed to differentiate between *B. tryoni* and *B. aquilonis*.

Reference specimens

- Frozen specimens representing the species requiring discrimination must be available. When kept at -70 ^{o}C, these remain suitable for use as controls for at least 10 years

- A single homogenized larva provides enough homogenate to act as a reference specimen on up to eight separate occasions. Once prepared, these reference homogenates can be stored at -20 ^{o}C as separate ~5 µL aliquots inside glass capillary tubes. Thus reference specimens for any test run are usually available as pre-prepared homogenates straight from the freezer

Specimen Preparation (ideally in cold room at 4°C)

- Specimens are hand-homogenized in an equal volume of a simple homogenizing solution (0.02 M Tris-HCl pH 7.4 containing 2 g PVP-40, 0.5 mL 2-mercaptoethanol and 20 mg NADP per 100 mL)

- ~0.5 µL of homogenate loaded directly onto each gel

- The remaining homogenate is transferred as a series of ~5 µL aliquots into individual glass capillary tubes and stored at -20°C. These samples can either be subjected to further allozyme analysis if doubt remains as to species identity, or used as fresh reference material for the species thus identified (activity declines over a 12 month period at -20°C)

ELECTROPHORESIS (IDEALLY IN COLD ROOM AT 4°C)

Allozyme analyses are conducted on cellulose acetate gels (Cellogel^{TM}) according to the principles and procedures of Richardson *et al.* (1986). Table 5 indicates the suite of enzymes most commonly used for fruit-fly genetic identifications and details the electrophoretic conditions employed for each.

GEL INTERPRETATION

The interpretation of allozyme gels requires some expertise; the intensity of allozyme bands changes over time after a gel is stained, plus banding patterns can be affected by the "freshness" of the specimen and by what type of gut contents are present (some plants contain compounds which affect fruit fly enzymes once the sample is homogenised. Richardson *et al.* (1986) devote an entire section to the interpretation of allozyme gels, but there is no substitute for experience.

RECORDING OF RESULTS

All gels are routinely scanned several times over the time course of stain incubation and the resultant JPG files archived as a permanent record.

Table 5. Enzymes most commonly used for fruit-fly genetic identifications

Enzyme	Abbr	E.C. No.	No. of genes	Buffer[1]	Run time	Stain	Species delineated
Aconitase hydratase	ACON	4.2.1.3	2	B	1.5 h @ 250 V	Richardson *et al.* (1986)	*B. tryoni* vs *B. jarvisi* vs *B. neohumeralis*?
Aminoacylase	ACYC	3.5.1.14	1	C	1.5 h @ 250 V	Manchenko (1994)	*C. capitata* vs *B. tryoni* vs *B. jarvisi / B. papayae* vs *D. pornia*
Alcohol dehydrogenase	ADH	1.1.1.1	2	B	1.5 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni* vs *D. pornia*
Aspartate aminotransferase	GOT	2.6.1.1	2	B	1.5 h @ 250 V	method 3; Manchenko (1994)	*C. capitata* vs *B. tryoni / B. papayae* vs *B. jarvisi* vs *D. pornia*
Glycerol-3-phosphate dehyrogenase	GPD	1.1.1.8	1	C	1.5 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni / B. jarvisi / B. papayae* vs *D. pornia*
Glucose-6-phosphate isomerase	GPI	5.3.1.9	1	B	1.5 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni / B. papayae* vs *D. pornia*
3-Hydroxybutyrate dehydrogenase	HBDH	1.1.1.30	1	B	1.5 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni / B. papayae / B. jarvisi* vs *D. pornia* vs *B. neohumeralis*?
Isocitrate dehydrogenase	IDH	1.1.1.42	2	B	1.5 h @ 250 V	Richardson *et al.* (1986)	*B. tryoni* vs *B. papayae*
Malate dehydrogenase	MDH	1.1.1.37	2	C	1.5 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni / B. papayae / B. jarvisi* vs *D. pornia*
Dipeptidase	PEPA	3.4.13.	2	C	1.4 h @ 250 V	Richardson *et al.* (1986)	*C. capitata* vs *B. tryoni / B. jarvisi / B. papayae* vs *D. pornia*

[1]Code for buffers follows Richardson *et al.* (1986).

7 Diagnostic Information

The family Tephritidae can be separated from all other Diptera by the shape of the subcostal vein, which bends abruptly through a right-angle and fades to a fold before reaching the wing edge, combined with the presence of setulae (small setae) along the dorsal side of vein R_1.

7.1 Simplified key to major pest fruit fly genera (after White and Elson-Harris 1992)

1 Vein Sc abruptly bent forward at nearly 90°, weakened beyond the bend and ending at subcostal break; dorsal side of vein R_1, with setulae. Wing usually patterned by coloured bands. Wing cell cup with an acute extension…………………......................................***TEPHRITIDAE*** .**2**

- Vein Sc not abruptly bent forward, except in the *Psilidae*, which lack both dorsal setulae on vein R_1, and frontal setae. Species associated with fruit very rarely have any wing patterning. Wing cell cup usually without an acute extension (exceptions include some *Otitidae* and *Pyrgotidae*)……………..………………………………………..**Families other than Tephritidae**

2 Cell cup very narrow and extension of cell cup very long. 1st flagellomere (3rd segment of antenna) at least three times as long as broad. Wing pattern usually confined to a costal band and an anal streak. (Tropical and warm temperate Old World; adventive species in Hawaii and northern South America)…………….……………………***BACTROCERA* and *DACUS* (p. 54)**

- Cell cup broader and the extension shorter. 1st flagellomere shorter. Wing pattern usually includes some coloured crossbands…………………………………………………….………………………….**3**

3 The wing vein that terminates just behind the wing apex (vein M) is curved forwards before merging into the wing edge. Wing pattern usually similar to Figure 85. (South America, West Indies and southern USA)…………………..………………………….……………***ANASTREPHA* (p. 162)**

- The wing vein that terminates just behind the wing apex (vein M) meets the wing edge at approximately a right angle. Wing pattern usually similar to Figure 80…………………............**4**

4 Cell cup, including its extension, shaped as Figure 80. Basal cells of wing usually with spot- and fleck-shaped marks, giving a reticulate appearance. Scutellum convex and shiny. (*Ceratitis capitata* is found in most tropical and warm temperate areas; other spp. are African)…………………………………………………..………..………………***CERATITIS* (p. 154)**

- Cell cup, including its extension, shaped as Figure 91-Figure 95 Basal area of wing not reticulate. Scutellum fairly flat and not shiny. (Larvae develop in the fruits of Berberidaceae, Caprifoliaceae, Cornaceae, Cupressaceae, Elaeagnaceae, North temperate regions and South America)……………………………………………………………***RHAGOLETIS* (p. 185)**

7.2 Guide to PCR-RFLP molecular information

ITS1 Frag length - gel: size in base pairs (bp) (visual estimate) of amplified ITS fragment, on an agarose gel

HinfI: approximate size of fragment(s) in bp for restriction enzyme *Hinf*I

VspI: approximate size of fragment(s) in bp for restriction enzyme *Vsp*I

Ssp1:   approximate size of fragment(s) in bp for restriction enzyme *Ssp*I

Bsr1:   approximate size of fragment(s) in bp for restriction enzyme *Bsr*I

Sau3a1:   approximate size of fragment(s) in bp for restriction enzyme *Sau3a*I

SnaB1: approximate size of fragment(s) in bp for restriction enzyme *SnaB*I

Where a restriction enzyme does not cut the ITS1 sequence, or cuts only once and that is within about 90bp of the terminus, the enzyme is scored as 'DNC' (does not cut).

7.3 *Bactrocera*

7.3.1 *Bactrocera* (*Bactrocera*) *albistrigata* (de Meijere)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus albistrigatus

Dacus (*Bactrocera*) *albistrigata*

DIAGNOSIS

7.3.1.1.1 *Morphological - Adult*

A medium sized species; face fulvous with a pair of circular to oval black spots; postpronotal lobes yellow (anteromedial corners black); notopleura yellow; scutum mostly black; lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe reaching to anterior *npl.* seta dorsally; scutellum yellow with a broad black basal band; wing with a narrow fuscous costal band which is extremely pale beyond extremity of cell sc to apex of wing, a narrow dark fuscous transverse band across wing enclosing r-m and dm-cu crossveins, a broad fuscous to dark fuscous anal streak; cells bc and c pale fuscous; microtrichia in outer corner of cell c only; abdominal terga III-V orange-brown with a narrow to medium width medial longitudinal dark fuscous to black band over all three terga and lateral dark markings which vary from narrow anterolateral dark fuscous to black corners on all three terga to broad lateral longitudinal dark fuscous to black bands over all three terga; posterior lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.1.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.1.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*1: DNC   SnaB1: DNC

*Hha*I: 670, 180   Ssp1: 180, 620

*Hinf*l: DNC   Vspl: DNC

*Sau*3a1: 400, 450

See also **PCR-DNA barcoding** (Section 6.3.2.).

HOST RANGE

Bactrocera albistrigata has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Apocynaceae, Combretaceae, Moraceae, Myrtaceae and Verbenaceae (for a full list of recorded hosts see Allwood *et al*. 1999).

Major commercial hosts (Allwood *et al*. 1999; pers. comm. Drew 2010):

Scientific name	Common name	Scientific name	Common name
Mangifera indica	mango	*Syzygium malaccense*	malay-apple
Syzygium aqueum	watery rose-apple	*Syzygium samarangense*	water apple

DISTRIBUTION

Andaman Islands, central to southern Thailand, Peninsular Malaysia, East Malaysia and Kalimantan (Borneo), Singapore, Indonesia east to Sulawesi, Christmas Island (pers. comm. Drew 2010).

REMARKS

Bactrocera albistrigata belongs to the *frauenfeldi* complex described by Drew (1989). The other species in the complex, *B. caledoniensis*, *B. frauenfeldi*, *B. parafrauenfeldi* and *B. trilineola* all possess the same basic body and wing colour patterns, however, *B. albistrigata* is the only species that occurs in South-East Asia and is distinguished by having a combination of moderately broad and elongate lateral postsutural vittae, face with a pair of black spots and abdominal terga III-V fulvous with dark colour patterns (not entirely black) (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- Medium level pest species

ATTRACTANT

Cue lure.

FIGURES

Figure 14. *Bactrocera albistrigata*

Images courtesy of Ken Walker, Museum Victoria, **www.padil.gov.au** (as of 22 August 2011))

Figure 15. *Bactrocera albistrigata*

Image courtesy of S. Phillips and the International Centre for the Management of Pest Fruit Flies, Griffith University
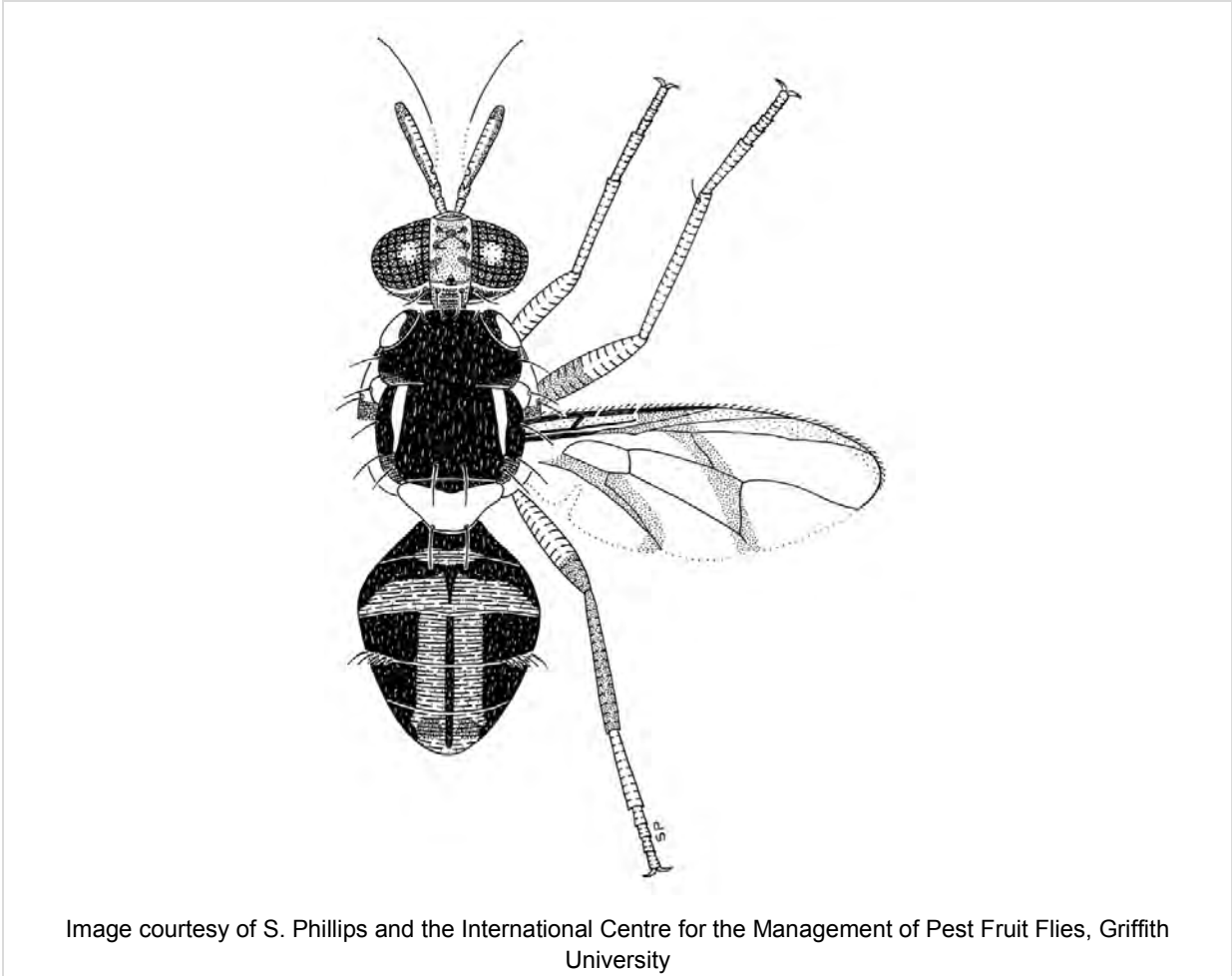
7.3.2 *Bactrocera* (*Bactrocera*) *aquilonis* (May)

TAXONOMIC INFORMATION

Common name: Northern Territory fruit fly

Previous scientific names:

Strumeta aquilonis

Dacus (*Bactrocera*) *aquilonis*

DIAGNOSIS

7.3.2.1.1   *Morphological - Adult*

Medium sized species; large black facial spots present; postpronotal lobes and notopleura yellow; scutum pale red-brown with fuscous markings, mesopleural stripe reaching almost to anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, cells bc and c fuscous, microtrichia covering cell c and most of cell bc; abdominal terga III-V pale orange-brown with pale fuscous along anterior margin of tergum III and widening over lateral margins of that tergum, a medial longitudinal pale fuscous band on terga III to V; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.2.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.2.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*1:	600, 200	*Sna*B1:	DNC
*Hha*I:	650, 200	*Ssp*1:	570, 180
*Hinf*I:	770	*Vsp*I:	DNC
*Sau*3a1: 420			

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera aquilonis has been recorded on hosts from a wide range of families. These include: Annonaceae, Arecaceae, Chrysobalanaceae, Combretaceae, Curcurbitaceae, Ebenaceae, Elaeocarpaceae, Euphorbiaceae, Lauraceae, Meliaceae, Myrtaceae, Rosaceae, Rubiaceae, Rutaceae, Santalaceae and Sapotaceae (for a full list of recorded hosts see Smith *et al.* 1988; Hancock *et al.* 2000).

Major commercial hosts (Hancock *et al.* 2000; pers. comm. Drew 2010):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew nut	*Flacourtia rukam*	rukam
Annona muricata	soursop	*Fortunella x crassifolia*	meiwa kumquat
Annona reticulata	bullock's heart	*Lycopersicon esculentum*	tomato
Annona squamosa	sugarapple	*Malpighia glabra*	acerola
Averrhoa carambola	carambola	*Malus domestica*	apple
Blighia sapida	akee apple	*Mangifera indica*	mango
Capsicum annuum	bell pepper	*Manilkara zapota*	sapodilla
Chrysophyllum cainito	caimito	*Prunus persica*	peach
Citrus limon	lemon	*Psidium guajava*	guava
Citrus maxima	pummelo	*Psidium littorale* var. *longipes*	strawberry guava
Citrus reticulata	mandarin	*Spondias dulcis*	otaheite apple
Citrus x paradisi	grapefruit	*Syzygium aqueum*	watery rose-apple
Eriobotrya japonica	loquat	*Syzygium jambos*	rose apple
Eugenia uniflora	Surinam cherry	*Syzygium malaccense*	malay-apple
Flacourtia jangomas	Indian plum	*Ziziphus mauritiana*	jujube

DISTRIBUTION

Northern areas of Western Australia and the Northern Territory (Hancock *et al.* 2000).

REMARKS

In the Northern Territory this species dramatically increased its host range during 1985. Since *B. aquilonis* and *B. tryoni* will produce viable offspring when crossed in the laboratory (Drew and Lambert 1986), hybridisation with *B. tryoni* was strongly suspected and might explain this increase (Smith and Chin 1987; Smith *et al.* 1988). By 1997, most but not all commercial production areas and larger towns supported populations of this fly, which attacks a wide range of cultivated hosts. Therefore, many of the Northern Territory host records for *B. aquilonis* since March 1985 are attributed to the suspected hybrid *B. aquilonis* x *B. tryoni* and are recorded under *B. tryoni* and now under *B. aquilonis* in the above table (Hancock *et al.* 2000).

Bactrocera aquilonis and *B. tryoni* are very similar in general body and wing colour patterns. *Bactrocera aquilonis* differs in being an overall paler colour with the scutum pale red-brown and the abdominal terga generally fulvous without distinct fuscous markings. However, these differences are not easily observed. These species can also be separated on the differences on the ovipositors: apex of aculeus rounded and spicules with 7-10 uniform dentations in *B. tryoni* compared with the more pointed aculeus and uneven dentations in *B. aquilonis* (Drew 1989).

A recent molecular genetic study of northwestern Australian fruit fly populations (Cameron *et al.* 2010) concluded that there is no genetic evidence supporting *B. aquilonis* as a distinct species from *B. tryoni.* They conclude that the recent increase in host range of fruit flies in northwestern Australia is due to local populations of *B. tryoni* (= *B. aquilonis*) utilising additional food resources from increased agricultural production in this region.

- Endemic
- Minor pest species

ATTRACTANT

Cue lure.

FIGURES

Figure 16. *Bactrocera aquilonis*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
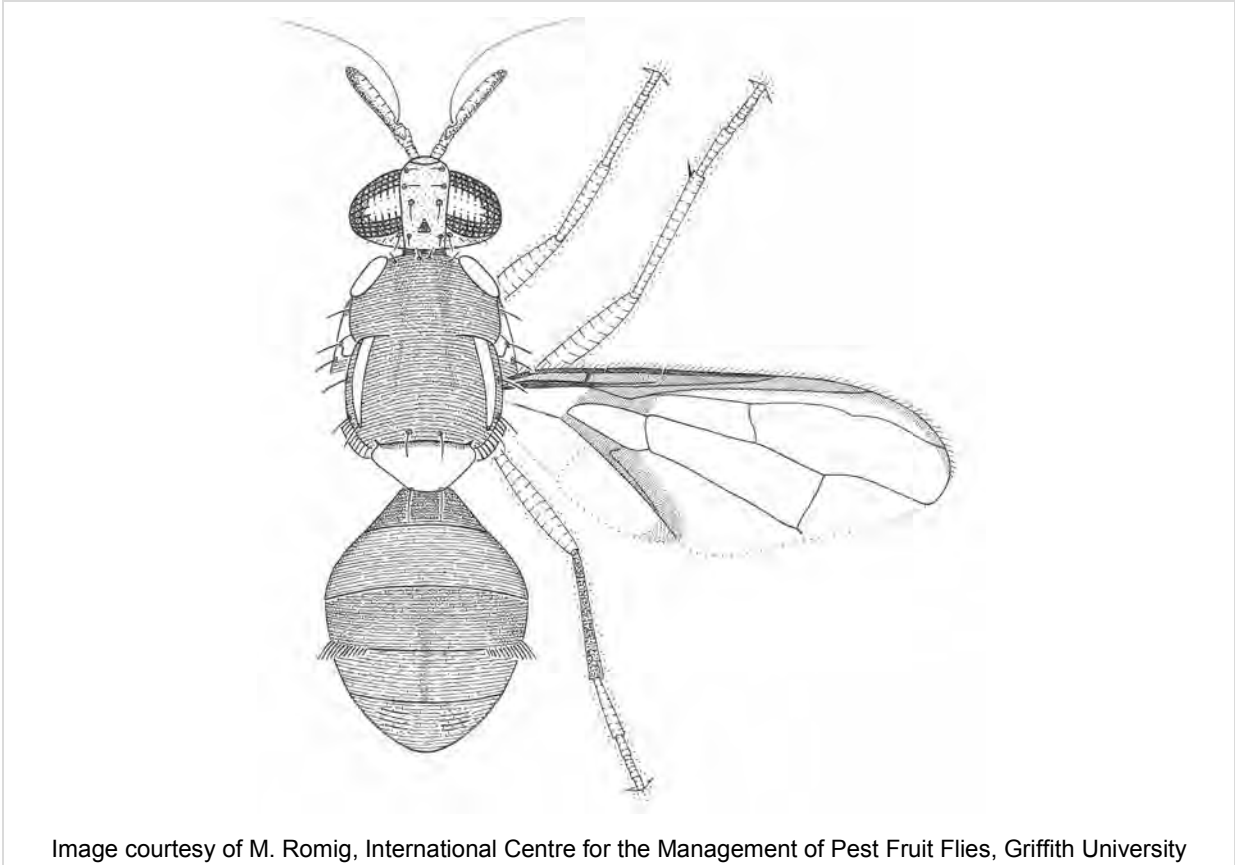
Figure 17. *Bactrocera aquilonis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.3 *Bactrocera* (*Paratridacus*) *atrisetosa* (Perkins)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Zeugodacus atrisetosus

Melanodacus atrisetosus

Bactrocera (*Paratridacus*) *atrisetosa*

DIAGNOSIS

7.3.3.1.1 *Morphological - Adult*

Medium sized species; small fuscous facial spots present; postpronotal lobes and notopleura yellow; scutum red-brown with irregularly shaped fuscous markings, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae beginning anterior to mesonotal suture, medial postsutural vitta present, scutellum yellow; wing with a narrow fuscous costal band and anal streak, cells bc and c pale fulvous with microtrichia in outer ½ of cell c only; abdominal terga III-V orange-brown occasionally with fuscous on lateral margins of tergum III and generally with narrow medial fuscous band on tergum V; posterior lobe of male surstylus long; female with aculeus tip blunt trilobed (Drew 1989; pers. comm. Drew 2010).

7.3.3.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.3.1.3 *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2.).

HOST RANGE

This species has been reared from eight host species in seven genera and three families, and is mainly associated with cucurbits: watermelons, honeydew and rock melons, cucumbers, pumpkins, zucchini, luffa and tomatoes (PaDIL 2007).

Major commercial hosts (per. comm. Drew 1989):

Scientific name	Common name	Scientific name	Common name
Citrullus lanatus	watermelon	*Cucurbita pepo*	ornamental gourd
Cucumis sativus	cucumber	*Lycopersicon esculentum*	tomato

DISTRIBUTION

Known only from Papua New Guinea where if occurs at higher altitudes (Drew 1989).

REMARKS

Bactrocera atrisetosa is distinguished in having the costal band narrow (just overlapping R_{2+3}), scutum red-brown with fuscous patterns, wings colourless, cells bc and c pale fulvous, abdominal terga III-V orange-brown except for a narrow medial longitudinal fuscous band on tergum V and lateral margins of tergum III fuscous (Drew 1989).

Bactrocera atrisetosa is very similar in appearance to the endemic *B. cucumis*. However it differs in having prescutellar and supra-alar setae present. In common with *B. cucumis* it also lacks pecten.

PEST STATUS

- Exotic.
- Medium level pest species

ATTRACTANT

No known record.

FIGURES

Figure 18. *Bactrocera atrisetosa*

Image courtesy of Mr. S. Wilson and the International Centre for the Management of Pest Fruit Flies, Griffith University

Figure 19. *Bactrocera atrisetosa*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.4   *Bactrocera* (*Bactrocera*) *bryoniae* (Tryon)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Chaetodacus bryoniae

Strumeta bryoniae

Dacus (*Strumeta*) *bryoniae*

DIAGNOSIS

7.3.4.1.1   Morphological - Adult

Large species; irregularly circular black facial spots present; postpronotal lobes and notopleura yellow; scutum dull black, mesopleural stripe slightly wider than notopleuron, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a broad fuscous costal band and anal streak, cells bc and c fulvous, microtrichia covering outer ½ of cell c only; abdominal terga III-V orange-brown with a medial and two lateral longitudinal dark bands joined along anterior margin of tergum III; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.4.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.4.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I: 600, 230   *Sna*BI: DNC

*Hha*I: 620, 200   *Ssp*I: 560, 180

*Hinf*I: 760   *Vsp*I: DNC

*Sau*3A1: 400

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera bryoniae has been recorded on hosts from five families. These include: Curcurbitaceae, Loganiaceae, Musaceae, Passifloraceae and Solanaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major commercial host (Drew 1989; Hancock *et al.* 2000):

Scientific name	Common name	Scientific name	Common name
Capsicum annuum	chilli		

Infests wild species of Cucurbitaceae and Passiflora (Hancock et al. 2000). Records from capsicum thought to be erroneous.

DISTRIBUTION

Widespread and common all over Papua New Guinea (every province except Bougainville and Manus), and Australia (Northern Western Australia, Northern Territory, east coast south to Sydney, New South Wales, and the Torres Strait Islands) (SPC 2006).

REMARKS

There are a number of species in Southeast Asia and the South Pacific with broad costal bands. However, *Bactrocera bryoniae* differs from these species in having costal band confluent with R_{4+5}, lateral postsutural vittae ending at upper *pa*. seta, abdominal terga III-V red-brown with a broad, dark fuscous band along anterior margin of tergum III and covering lateral margins, anterolateral corners of terga IV and V fuscous and a medial longitudinal dark fuscous band over all 3 terga (Drew 1989).

PEST STATUS

- Endemic
- Low level pest species in Queensland but not in Western Australia or the Northern Territory

ATTRACTANT

Cue lure, Willison's lure.

FIGURES

Figure 20. *Bactrocera bryoniae*

Image courtesy of S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and the Queensland Museum
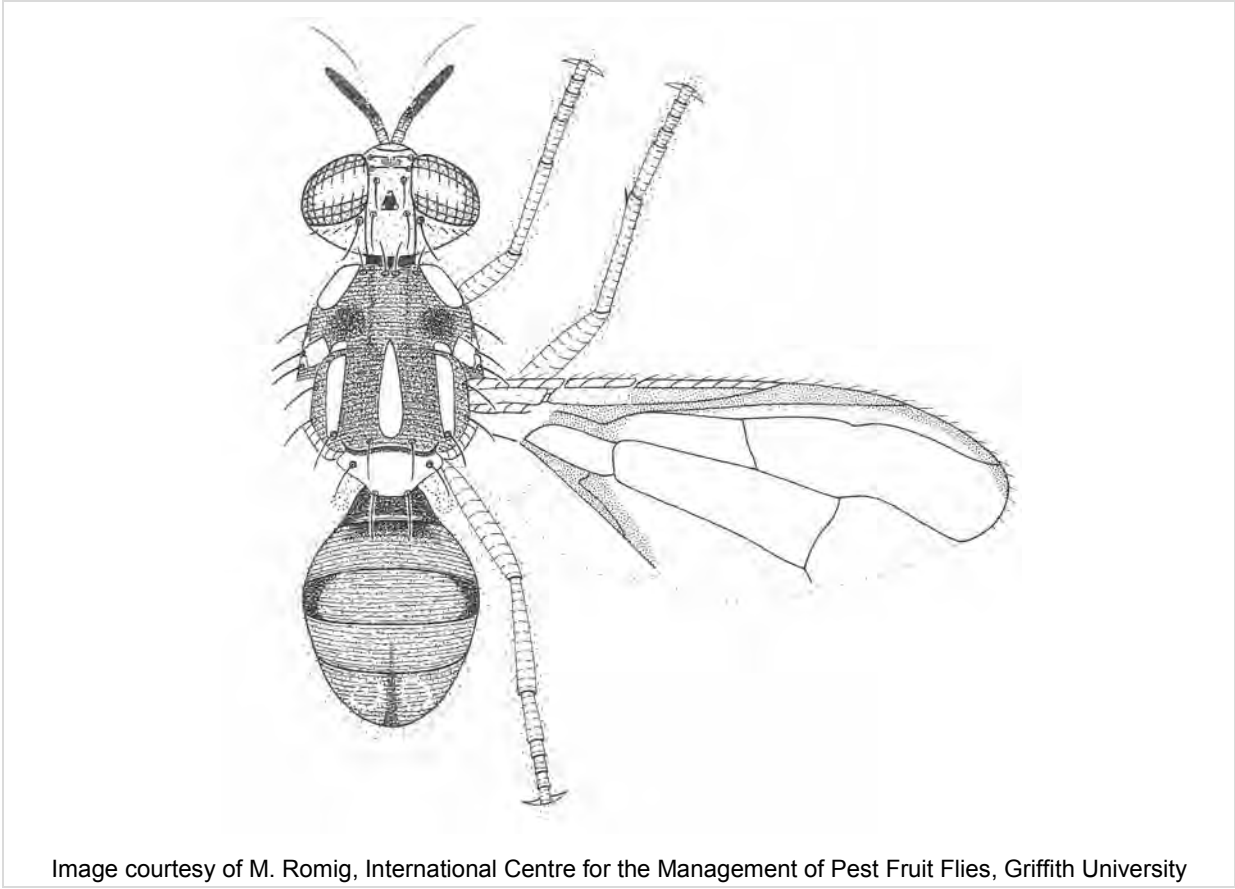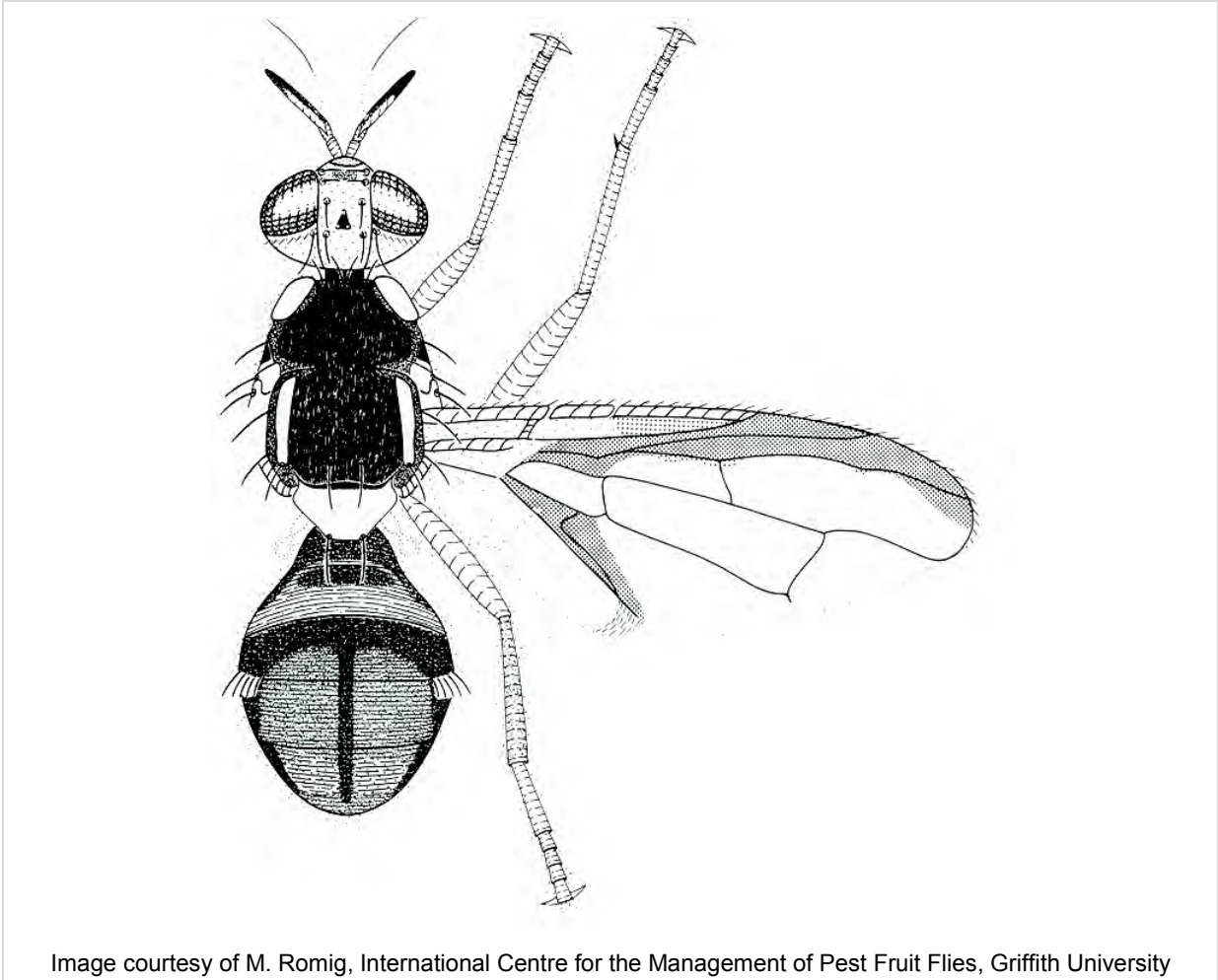
Figure 21. *Bactrocera bryoniae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.5   *Bactrocera* (*Bactrocera*) *carambolae* Drew and Hancock

TAXONOMIC INFORMATION

Common name: Carambola fly

Previous scientific names:

Bactrocera sp. *near dorsalis*

DIAGNOSIS

7.3.5.1.1   *Morphological - Adult*

Face fulvous with a pair of medium sized oval black spots; scutum dull black with brown behind lateral postsutural vittae, around mesonotal suture and inside postpronotal lobes; postpronotal lobes and notopleura yellow; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta dorsally; two broad parallel sided lateral postsutural vittae ending at or behind *ia.* seta; medial postsutural vitta absent; scutellum yellow; legs with femora fulvous with a large elongate oval dark fuscous to black preapical spot on outer surfaces of fore femora in some specimens, tibiae dark fuscous (except mid tibiae paler apically); wings with cells bc and c colourless, microtrichia in outer corner of cell c only, a narrow fuscous costal band slightly overlapping R_{2+3} and expanding slightly beyond apex of R_{2+3} across apex of R_{4+5}, a narrow fuscous anal streak; supernumerary lobe of medium development; abdominal terga III-V orange-brown with a 'T' pattern consisting of a narrow transverse black band across anterior margin of tergum III and widening to cover lateral margins, a medium width medial longitudinal black band over all three terga, anterolateral corners of terga IV dark fuscous to black and rectangular in shape and anterolateral corners of tergum V dark fuscous, a pair of oval orange-brown shining spots on tergum V; abdominal sterna dark coloured; posterial lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.5.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.5.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*I:	650, 250	*Sna*BI:	350, 530
*Hha*I:	680, 200	*Ssp*I:	DNC
*Hinf*I:	DNC	*Vsp*I:	355, 485
*Sau*3A1:	400, 450		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera carambolae has been recorded on hosts from a wide range of families. These include: Alangiaceae, Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Clusiaceae, Combretaceae, Euphorbiaceae, Lauraceae, Loganiaceae, Meliaceae, Moraceae, Myristicaceae, Myrtaceae, Oleaceae, Oxalidaceae, Polygalaceae, Punicaceae, Rhamnaceae, Rhizophoraceae, Rutaceae, Sapindaceae, Sapotaceae, Simaroubaceae, Solanaceae and Symplocaceae (for a full list of recorded species see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Averrhoa carambola	carambola	*Syzygium jambos*	rose apple
Manilkara zapota	sapodilla	*Syzygium malaccense*	malacca apple
Psidium guajava	guava	*Syzygium samarangense*	wax apple
Syzygium aqueum	watery rose-apple		

DISTRIBUTION

Southern Thailand, Peninsular Malaysia, East Malaysia, Kalimantan (Borneo), Singapore, Indonesian islands east to Sumbawa, Andaman Islands, Surinam, French Guiana, Brazil (pers. comm. Drew 2010).

REMARKS

Bactrocera carambolae is similar to *B. propinqua* and some specimens of *B. papayae* in possessing broad parallel sided or subparallel lateral postsutural vittae, costal band slightly overlapping R_{2+3}, abdominal terga III-V with narrow to medium width dark lateral margins, shining spots on abdominal tergum V pale (orange-brown to fuscous), femora entirely fulvous or with, at most, subapical dark spots on fore femora only, in addition to the general characteristics of the *dorsalis* complex.

It differs from *B. papayae* in having a broad medial longitudinal black band on abdominal terga III-V, a broader costal band apically, and shorter male aculeus and female ovipositor and from *B. propinqua* in having a narrower medial longitudinal black band on abdominal terga III-V (in *B. propinqua* this band is very broad) and apex of the aculeus needle shaped (in *B. propinqua* the apex of the aculeus is trilobed) (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- High priority pest identified in the Tropical Fruit Industry Biosecurity Plan (IBP; Plant Health Australia)
- This species is a major economic pest throughout the region where it occurs

ATTRACTANT

Methyl eugenol.

Figure 22. *Bactrocera carambolae*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
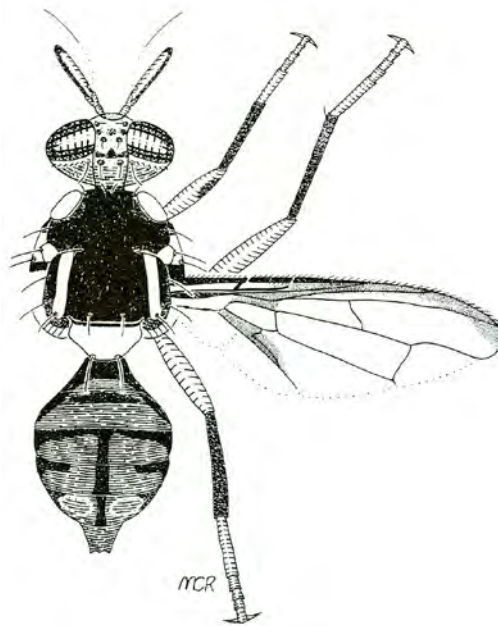
Figure 23. *Bactrocera carambolae*

Image courtesy of M. Romig, the International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.6   *Bactrocera* (*Bactrocera*) *caryeae* (Kapoor)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus (*Strumeta*) *caryeae*

Dacus (*Bactrocera*) *caryeae*

Bactrocera (*Bactrocera*) *caryeae*

DIAGNOSIS

7.3.6.1.1   Morphological - Adult

Face fulvous with a pair of large elongate oval black spots; scutum black with a small area of dark brown posterolateral to lateral postsutural vittae; postpronotal lobes yellow (except anterodorsal corners fuscous); notopleura yellow; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl*. seta dorsally; two narrow lateral postsutural vittae which are either parallel sided or narrowing slightly posteriorly to end at or just before *ia*. seta; medial postsutural vitta absent; scutellum yellow with a broad black basal band; legs with femora fulvous with large dark fuscous to black preapical spots on outer surfaces of fore femora and inner surfaces of mid and hind femora, fore tibiae fuscous, mid tibiae fulvous, hind tibiae dark fuscous; wings with cells bc and c colourless, sparse microtrichia in outer corner of cell c only, a very narrow fuscous costal band confluent with R_{2+3} and remaining very narrow around apex of wing, a narrow fuscous anal streak contained within cell cup; supernumerary lobe of medium development; abdominal terga III-V orange-brown with dark fuscous to black across anterior 1/3 to 1/2 of tergum III, two broad lateral longitudinal dark fuscous to black bands and a narrow medial longitudinal black band over all three terga, a pair of oval orange-brown shining spots on tergum V; abdominal sterna dark coloured; posterial lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.6.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.6.1.3   Molecular

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera caryeae has been recorded on hosts from six families. These include: Anacardiaceae, Lecythidaceae, Malpighiaceae, Myrtaceae, Rutaceae and Sapotaceae (for a full list of recorded species see Allwood *et al*. 1999).

Major commercial hosts (Allwood *et al*. 1999):

Scientific name	Common name	Scientific name	Common name
Citrus maxima	pummelo	*Mangifera indica*	mango
Citrus reticulata	mandarin	*Psidium guajava*	guava

DISTRIBUTION

Southern India and Sri Lanka (pers. comm. Drew 2010).

REMARKS

Bactrocera caryeae is similar to *B. kandiensis* and *B. arecae* in possessing narrow parallel sided lateral postsutural vittae, preapical dark markings on at least one pair of femora in addition to the general characteristics of the *dorsalis* complex. It differs from *B. arecae* in possessing preapical dark markings on all femora (in *B. arecae* the preapical dark markings are on fore femora only) and from *B. kandiensis* in possessing a broad medial longitudinal dark band and broad lateral longitudinal dark bands over abdominal terga III-V (pers. comm. Drew 2010).

PEST STATUS

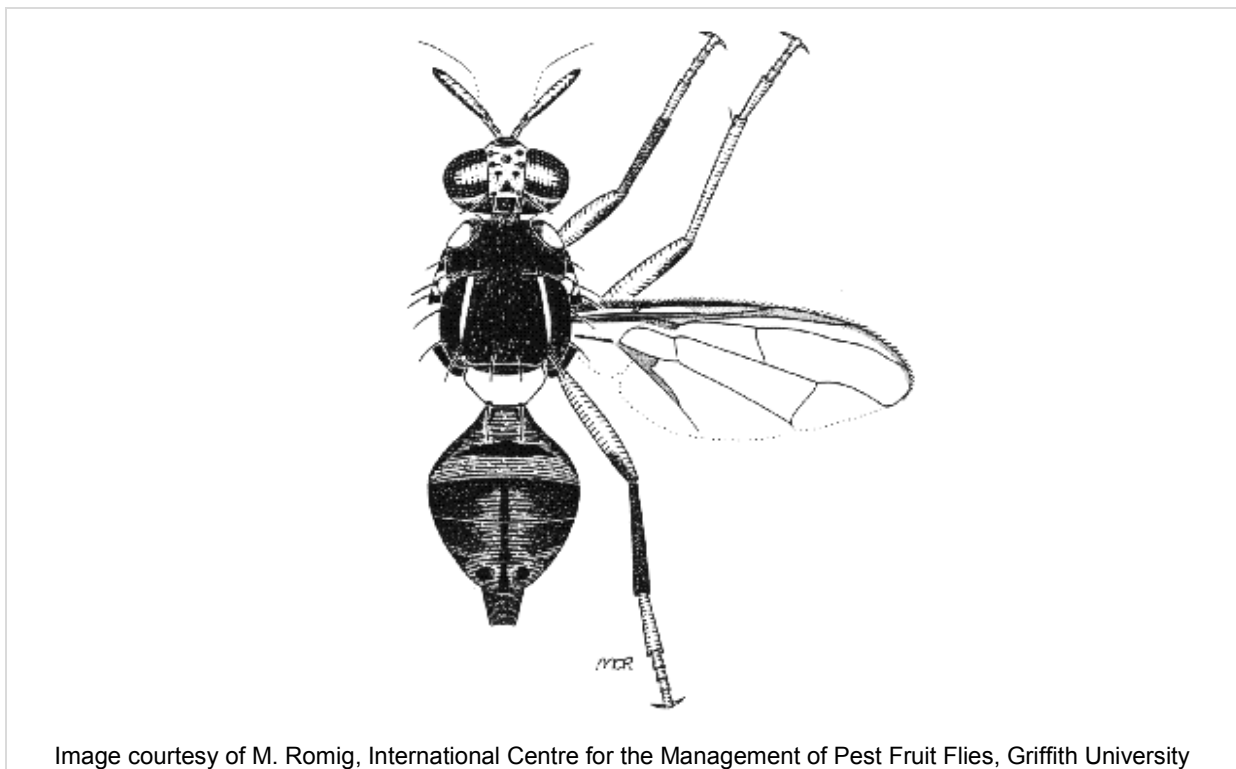- Exotic

- *Bactrocera caryeae* occurs in very large populations in many fruit growing areas of southern India and is probably responsible for much of the damage generally attributed to *Bactrocera dorsalis*

ATTRACTANT

Methyl eugenol.

FIGURES

Figure 24. *Bactrocera caryeae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.7 *Bactrocera* (*Bactrocera*) *correcta* (Bezzi)

TAXONOMIC INFORMATION

Common name: Guava fruit fly

Previous scientific names:

Chaetodacus correctus

Dacus (*Strumeta*) *correctus*

Bactrocera (*Bactrocera*) *correcta*

DIAGNOSIS

7.3.7.1.1 *Morphological - Adult*

Face fulvous with a pair of transverse elongate black spots almost meeting in centre; scutum black with dark red-brown along lateral and posterior margins; postpronotal lobes and notopleura yellow; mesopleural stripe reaching almost to anterior *npl.* seta dorsally; broad parallel sided lateral postsutural vittae ending behind *ia.* seta; medial postsutural vitta absent; scutellum yellow with narrow black basal band; legs with all segments entirely fulvous except hind tibiae pale fuscous; wings with cells bc and c colourless, both cells entirely devoid of microtrichia, a narrow pale fuscous costal band confluent with R_{2+3} and ending at apex of this vein, a small oval fuscous spot across apex of R_{4+5}, anal streak absent but with a pale fuscous tint within cell cup; supernumerary lobe of medium development; abdominal terga III-V red-brown with a 'T' pattern consisting of a narrow transverse black band across anterior margin of tergum III and a narrow medial longitudinal black band over all three terga, narrow black anterolateral corners on terga IV and V, a pair of oval red-brown shining spots on tergum V; posterior lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.7.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.7.1.3 *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera correcta has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Cactaceae, Capparaceae, Caricaceae, Combretaceae, Curcurbitaceae, Dipterocarpaceae, Elaeocarpaceae, Euphorbiaceae, Flacourtiaceae, Lecythidaceae, Malpighiaceae, Meliaceae, Moraceae, Musaceae, Myristicaceae, Myrtaceae, Olacaceae, Oxalidaceae, Rhamnaceae, Rosaceae, Rutaceae, Sapindaceae, Sapotaceae and Simaroubaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew nut	*Psidium guajava*	guava
Mangifera indica	mango	*Syzygium samarangense*	water apple
Manilkara zapota	sapodilla	*Terminalia catappa*	Singapore almond
Mimusops elengi	Spanish cherry	*Ziziphus jujuba*	common jujube
Muntingia calabura	Jamaican cherry		

DISTRIBUTION

Sri Lanka, India, Nepal, Pakistan, Myanmar, northern Thailand, southern China, Bhutan, Vietnam (pers. comm. Drew 2010).

REMARKS

Bactrocera correcta is similar to *B. dorsalis* in the general colour patterns of the body, wings and legs but differs from *B. dorsalis* in possessing transverse facial spots and an incomplete costal band. It is also similar to *B. penecorrecta* in the general colour patterns of the body and wings but differs from this species in having abdominal terga III-V mostly pale coloured (not mostly black as in *B. penecorrecta*) and the scutellum with a narrow black basal band (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- Major pest species, particularly in Vietnam

ATTRACTANT

Methyl eugenol.

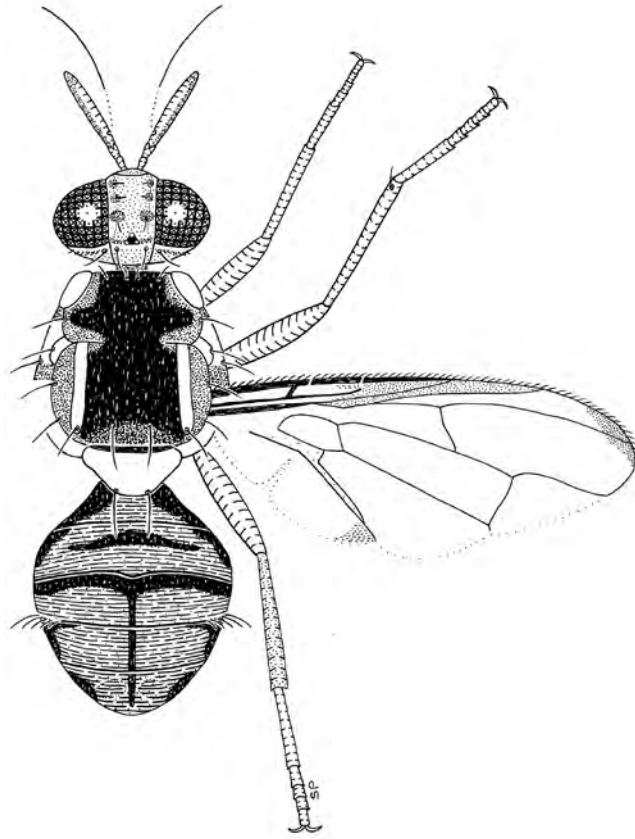**Figure 25.** *Bactrocera correcta*

Image courtesy of S. Phillips and the International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.8 *Bactrocera* (*Austrodacus*) *cucumis* (French)

TAXONOMIC INFORMATION

Common name: Cucumber fruit fly

Previous scientific names:

Dacus tryoni var. *cucumis*

Dacus cucumis

Austrodacus cucumis

Dacus (*Austrodacus*) *cucumis*

DIAGNOSIS

7.3.8.1.1   *Morphological - Adult*

Medium sized species; small fuscous to black facial spots present; postpronotal lobes and notopleura yellow; scutum orange-brown without dark markings, mesopleural stripe reaching almost to anterior *npl.* seta, lateral postsutural vittae beginning anterior to mesonotal suture, broad medial postsutural vitta present, scutellum yellow; wing with a narrow fuscous costal band and anal streak, cells bc and c pale fulvous (cell c slightly paler than cell bc), microtrichia in outer corner of cell c only; abdominal terga I and II orange-brown, terga III-V fulvous except for two broad lateral longitudinal orange-brown bands over all three terga and a narrow medial longitudinal band which is orange-brown on tergum III and orange-brown to dark fuscous on tergum IV and V (this band is broader on tergum V); posterior lobe of male surstylus long; female with aculeus tip blunt trilobed (Drew 1989; pers. comm. Drew 2010).

7.3.8.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.8.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 750 bp

*Bsr*I:	DNC		*Sna*BI:	DNC
*Hha*I:	550, 180		*Ssp*I:	DNC
*Hinf*I:	DNC		*Vsp*I:	DNC

*Sau*3AI DNC

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

While cucurbits are the major hosts of this species, it has been reared at moderate to high levels from several other species in different plant families, including pawpaw and tomato (PaDIL 2007).

The rare or incidental hosts (usually a single rearing) include mango, avocado, guava, carambola, apricot, some species of citrus, and capsicum. It is likely that most of these records could be attributed to fruit damage prior to oviposition. *B. cucumis* attacks wild cucurbits such as *Diplocyclos palmatus* and these may be reservoir hosts (CABI 2007).

Bactrocera cucumis has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Caricaceae, Combretaceae, Curcurbitaceae, Ebenaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae, Solanaceae and Vitaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

It is worth noting, however, that many of the host associations for this species in Hancock *et al.* (2000) are single records and are considered unusual. Hancock *et al.* (2000) concede that the publication may contain a variety of errors as only the records that could be confidently attributed to errors were removed. This does not rule out the possibility that many of the host association records contained within are still erroneous.

Further, the revision by Hancock *et al.* (2000) cites a large body of work conducted as early as 1951. Much of the work in those earlier publications may also contain a number of errors.

As such, the Hancock *et al.* (2000) publication should not be used as the sole basis for providing evidence of host association.

Major commercial hosts (Drew, 1989; Hancock *et al.* 2000):

Scientific name	Common name	Scientific name	Common name
Carica papaya	papaw	*Passiflora edulis*	passionfruit
Cucumis sativus	cucumber	*Solanum lycopersicum*	tomato
Cucurbita moschata	pumpkin	*Trichosanthes anguinea*	guada bean
Cucurbita pepo	squash and zucchini		

DISTRIBUTION

Eastern Queensland and northeast New South Wales although it has not been trapped as far south as Sydney. Hancock *et al.* (2000) list it as present in the Northern Territory and Torres Strait Islands but its presence there cannot be proven (pers. comm. Drew 2011). Although morphologically indistinguishable from Queensland specimens (Drew pers. comm. 2011), the Northern Territory strain does not infest commercial crops and in laboratory culture, failed to develop on undamaged cucurbit, solonaceous or other commercial hosts, but could be reared on sliced cucumber (Smith and Chin 1987).

REMARKS

Bactrocera cucumis is a pale orange-brown species with medial and lateral postsutural vittae present, a yellow scutellum, *prsc.* and *sa.* setae absent, 4 *sc.* seta present and a small elongate-oval black spot centrally on tergum V (Drew 1989).

Other remarks:

In common with most species in, or close to, subgenus *Zeugodacus*, the scutum has three yellow vittae (lateral and medial stripes), four setae on the margin of the scutellum, and the males lack a deep V-shaped notch in posterior margin of 5th sternite. This species is unusual in that it also lacks both anterior supra-alar setae and prescutellar acrostichal setae, and the males lack a pecten (comb of setae on each postero-lateral corner of tergite 3) (CABI 2007).

PEST STATUS

- Endemic
- Major pest species in Queensland. Regarded as a potential pest of fruit in the National Tropical Fruit IBP (page 26)

None known, but can be captured in traps emitting ammonia.

Figure 26. *Bactrocera cucumis*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
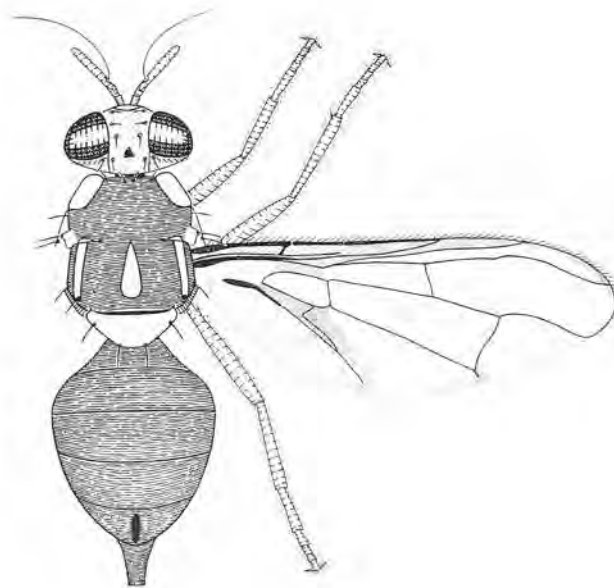
Figure 27. *Bactrocera cucumis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.9   *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett)

TAXONOMIC INFORMATION

Common name: Melon fly

Previous scientific names:

Dacus cucurbitae

Chaetodacus cucurbitae

Strumeta cucurbitae

Dacus (*Strumeta*) *cucurbitae*

Dacus (*Zeugodacus*) *cucurbitae*

Bactrocera cucurbitae

DIAGNOSIS

7.3.9.1.1   Morphological - Adult

Medium sized species; large black facial spots present; postpronotal lobes and notopleura yellow; scutum red-brown with or without fuscous markings, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae beginning anterior to mesonotal suture, narrow medial postsutural vitta present, scuttelum yellow; wing with a broad fuscous costal band expanding into a fuscous spot at wing apex, a broad fuscous anal streak, dark fuscous along dm-cu crossvein, pale infuscation along r-m crossvein, cells bc and c colourless, microtrichia in outer corner of cell c only; abdominal terga III-V orange-brown except for a narrow transverse black band across anterior margin of tergum III which expands over anterolateral corners, a narrow medial longitudinal dark fuscous to black band over all three terga and anterolateral corners of terga IV and V fuscous; posterior lobe of male surstylus long; female with aculeus needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.9.1.2   Morphological - Larvae

- Not available / included in this edition -

7.3.9.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 600 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	400, 180	*Ssp*I:	DNC
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI: DNC			

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera cucurbitae is primarily a pest of Cucurbitaceae, however it has also been recorded from eleven other families. These include: Agavaceae, Capparaceae, Fabaceae, Malvaceae, Moraceae,

Myrtaceae, Rhamnaceae, Rutaceae, Sapotaceae, Solanaceae and Vitaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Drew 1989, Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Coccinia grandis	ivy gourd	*Cucurbita pepo*	ornamental gourd
Cucumis melo	melon	*Momordica charantia*	
Cucumis satinus		*Trichosanthes cucumerina* var. *anguinea*	snakegourd
Cucurbita maxima	giant pumpkin		

DISTRIBUTION

Widely distributed over Southeast Asia, the Indian subcontinent, southern China, northern Africa and Papua New Guinea. Introduced into the Mariana Islands, the Hawaiian Islands and from Papua New Guinea to the Solomon Islands. Present in Indonesia and East Timor.

REMARKS

Bactrocera cucurbitae is similar to *B. emittens* in possessing only a slight widening of the costal band in wing apex, a narrow infuscation along dm-cu crossvein and abdominal terga with ground colour fulvous but differs in having the spot on apex of costal band not reaching M, cells bc and c colourless, abdominal tergum III with a narrow transverse black band across base and tip of piercer of ovipositor needle shaped. The most distinctive characteristic of the adult is the wing pattern (Drew 1989).

B. cucurbitae can appear similar to the endemic *B. chorista* and both are attracted to cue lure. *B. cucurbitae* has a narrower medial vitta and a larger marking at the distal end of the wing.

PEST STATUS

- Exotic
- High priority pest identified in the Tropical fruit and Vegetables IBPs
- *Bactrocera cucurbitae* is a very serious pest of cucurbit crops

ATTRACTANT

Cue lure or a mixture of methyl eugenol and cue lure (Dominiak *et al.* 2011).

FIGURES

Figure 28. *Bactrocera cucurbitae*

Image courtesy of Ken Walker, Museum Victoria, www.padil.gov.au (as of 22 August 2011)

Figure 29. *Bactrocera cucurbitae*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
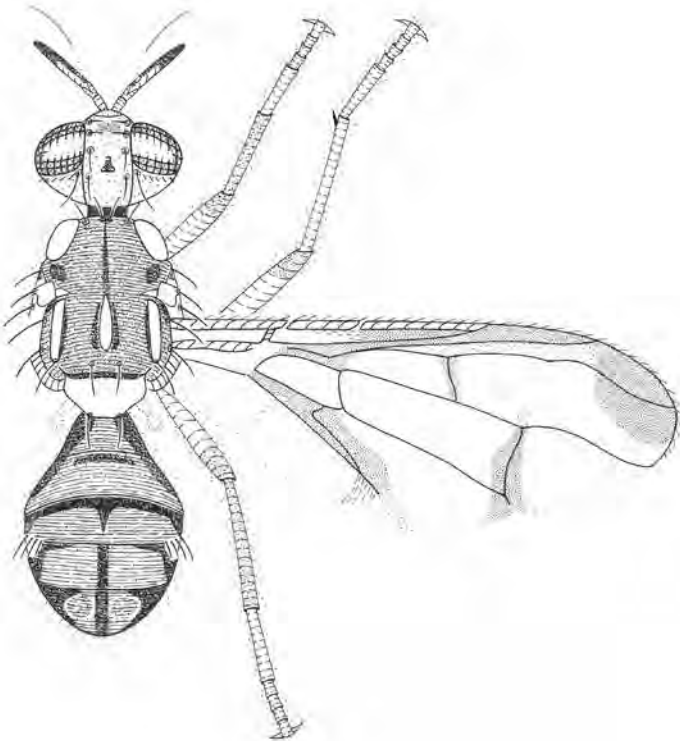
Figure 30. *Bactrocera cucurbitae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.10   *Bactrocera* (*Bactrocera*) *curvipennis* (Froggatt)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus curvipennis

Strumeta curvipennis

Dacus (*Strumeta*) *curvipennis*

DIAGNOSIS

7.3.10.1.1   *Morphological - Adult*

Small species; very small pale fuscous facial spots present; postpronotal lobes and notoluera yellow; scutum black, mesopleural stripe reaching midway between anterior margin of notoplueron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a broad fuscous costal band and anal streak, a broad fuscous band along r-m crossvein, cells bc and c pale fuscous, microtichia covering cell c and outer corner of cell bc; abdominal terga III-V orange-brown with a narrow transverse fuscous band along anterior margin of tergum III merging into broad lateral black margins and with anterolateral corners of terga IV and V fuscous; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.10.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.10.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*I:	570, 250	*Sna*BI: DNC
*Hha*I:	620, 170	*Ssp*I:	550, 200
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI: 420

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera curvipennis has been recorded on hosts from two families, Rutaceae and Anacardiaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Citrus reticulata	mandarin		

DISTRIBUTION

New Caledonia and one remote island in Vanuatu (Drew 1989).

REMARKS

Bactrocera curvipennis is distinct in having the mesopleural stripe not extending to the postpronotal lobes, microtrichia covering cell c and outer corner of cell bc, and abdominal terga III-V orange-brown with a very narrow transverse fuscous band across anterior margin of tergum III which merges into broad lateral black margins and the anterolateral corners of terga IV and V fuscous (Drew 1989).

PEST STATUS

- Exotic

ATTRACTANT

Cue lure, Willison's lure.

FIGURES

Figure 31. *Bactrocera curvipennis*

Image courtesy of Mr. S. Wilson and the International Centre for the Management of Pest Fruit Flies, Griffith University
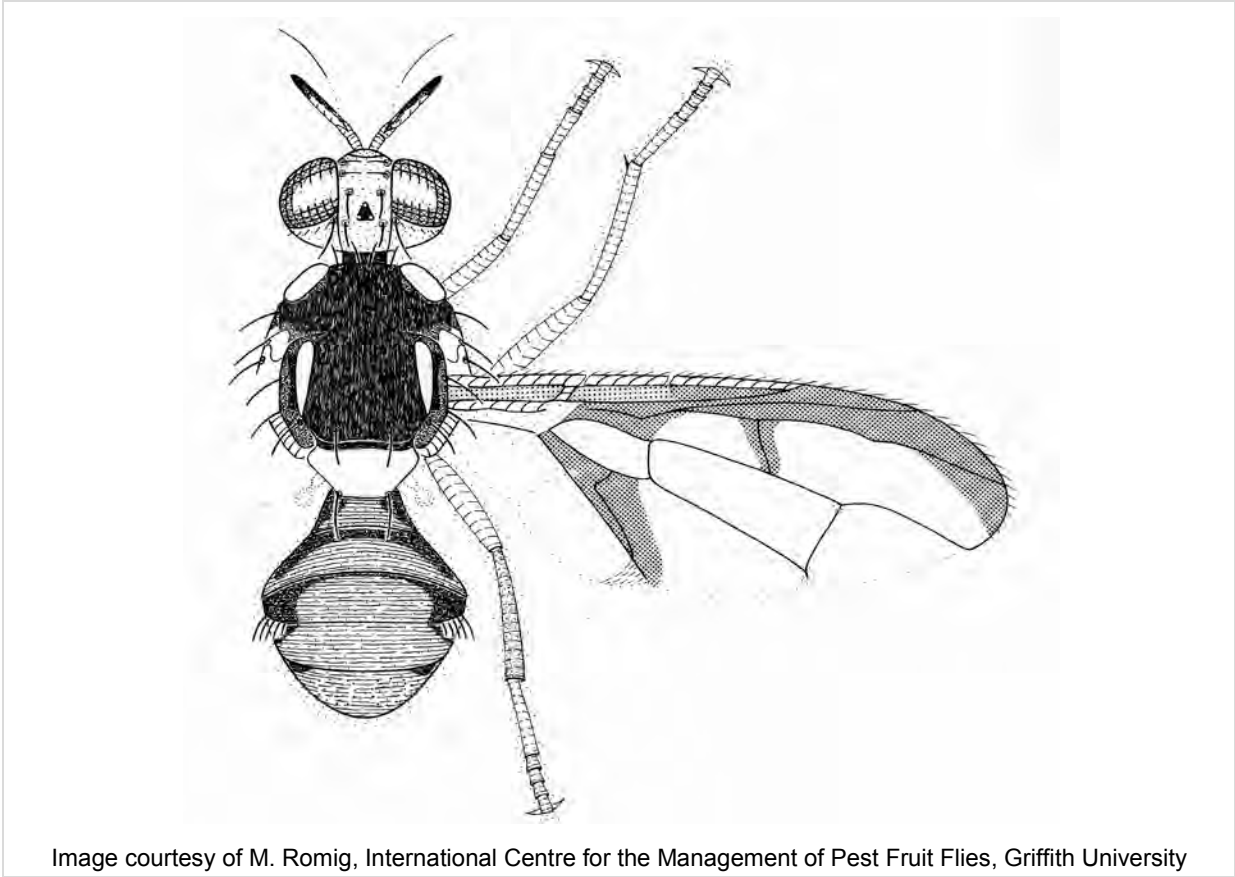
Figure 32. *Bactrocera curvipennis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.11   *Bactrocera* (*Paradacus*) *decipiens* (Drew)

TAXONOMIC INFORMATION

Common name: Pumpkin fruit fly

Previous scientific names:

Dacus (*Paradacus*) *decipiens*

DIAGNOSIS

7.3.11.1.1   *Morphological - Adult*

Large species; medium sized fuscous to black facial spots present; postpronotal lobes and notopluera yellow; scutum red-brown with two broad lateral longitudinal fuscous bands, mesoplural stripe reaching almost to anterior *npl.* seta, lateral postsutural vittae beginning anterior to mesonotal suture, broad medial postsutural vitta present, scutellum yellow: wing with a broad fucsous costal band and anal streak, an irregular recurved pale fuscous marking across wing, cells bc and c extremely pale fuscous (cell c paler in centre), microtrichia in outer 1/3 of cell c only; abdominal terga I-V fulvous except for broad lateral fuscous margins on tergum I and a narrow medial longitudinal fuscous band on tergum V; posterior lobe of male surstylus long; female with aculeus tip trilobed (Drew 1989; pers. comm. Drew 2010).

7.3.11.1.2   *Morphological - Larvae*

- Not available / included in this edition -

7.3.11.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 3.3.2.).

HOST RANGE

Pumpkin (*Cucurbita pepo*) is the only recorded host.

Major commercial hosts (Drew 1989):

Scientific name	Common name
Cucurbita pepo	pumpkin

DISTRIBUTION

Papua New Guinea (New Britain) (Drew 1989).

REMARKS

Bactrocera decipiens is similar to *B. perplexa* in possessing infuscation on wings in addition to costal band and anal streak but differs in having an irregular S-shaped pale fuscous marking across wing, microtrichia in outer 1/3 of cell c only, mesoplueral stripe not extending to postpronotal lobes, abdominal terga mostly fulvous with broad lateral fuscous margins on tergum I, a narrow medial longitudinal fuscous band on tergum V and apex of piercer of ovipositor with one pair of subapical lobes (Drew 1989).

PEST STATUS

- Exotic
- A major pest of pumpkins in New Britain

ATTRACTANT

No known record.

FIGURES

Figure 33. *Bactrocera decipiens*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
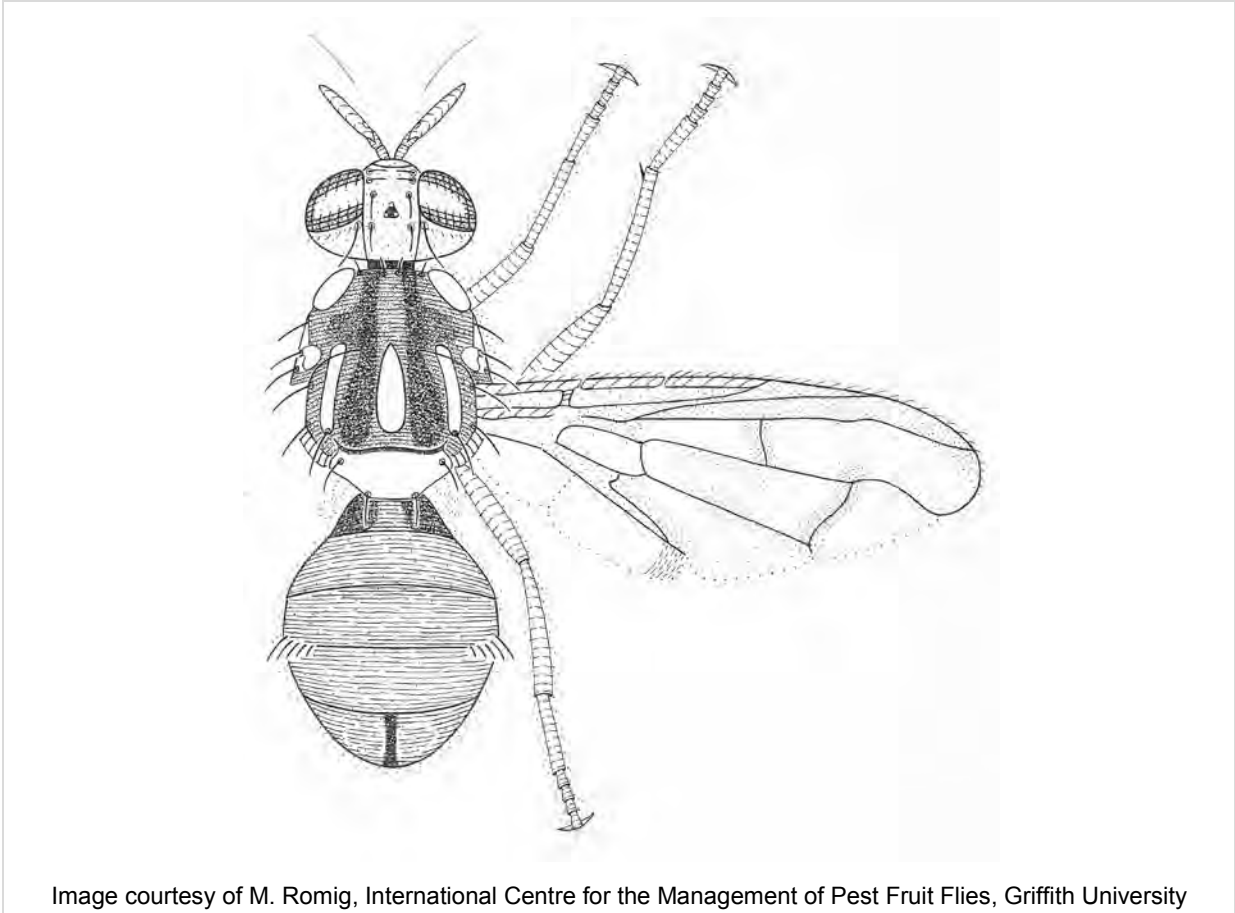
Figure 34. *Bactrocera decipiens*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.12   *Bactrocera* (*Bactrocera*) *dorsalis* (Hendel)

TAXONOMIC INFORMATION

Common name: Oriental fruit fly

Previous scientific names:

Dacus dorsalis

Dacus (*Strumeta*) *dorsalis*

Strumeta dorsalis

Dacus (*Bactrocera*) *dorsalis*

Bactrocera (*Bactrocera*) *dorsalis*

DIAGNOSIS

7.3.12.1.1   *Morphological - Adult*

Face fulvous with a pair of medium sized circular black spots; scutum black with extensive areas of red-brown to brown below and behind lateral postsutural vittae, around mesonotal suture, between postpronotal lobes and notopleura, inside postpronotal lobes; postpronotal lobes and notopleura yellow; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta dorsally; broad parallel sided lateral postsutural vittae ending behind *ia.* seta; medial postsutural vitta absent; scutellum yellow; legs with femora entirely fulvous, fore tibiae pale fuscous and hind tibiae fuscous; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, a narrow fuscous costal band confluent with R_{2+3} and remaining very narrow around apex of wing (occasionally there can be a very slight swelling around apex of R_{4+5}), a narrow pale fuscous anal streak; supernumerary lobe of medium development; abdominal terga III-V exhibits a range of colour patterns (see Drew and Hancock 1994) but possesses the basic pattern of a black 'T' consisting of a narrow transverse black band across anterior margin of tergum III, a narrow medial longitudinal black band over all three terga, narrow anterolateral fuscous to dark fuscous corners on terga IV and V; a pair of oval orange-brown to pale fuscous shining spots on tergum V; abdominal sterna dark coloured; posterior lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.12.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.12.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I: 650, 260   *Sna*BI: 326, 540

*Hha*I: 656, 192   *Ssp*I: DNC

*Hinf*I: 770   *Vsp*I: DNC

*Sau*3AI: DNC

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera dorsalis has been recorded on hosts from a wide range of families. These include: Alangiaceae, Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Burseraceae, Capparaceae, Caprifoliaceae, Caricaceae, Celastraceae, Chrysobalanaceae, Clusiaceae, Combretaceae, Convolvulaceae, Curcurbitaceae, Ebenaceae, Elaeocarpaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Lauraceae, Lecythidaceae, Malpighiaceae, Meliaceae, Moraceae, Musaceae, Myrtaceae, Olacaceae, Oleaceae, Oxalidaceae, Polygalaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Simaroubaceae and Solanaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew nut	*Mimusops elengi*	spanish cherry
Annona reticulata	bullock's heart	*Muntingia calabura*	Jamaican cherry
Annona squamosa	sugarapple	*Musa*	banana
Averrhoa carambola	carambola	*Prunus armeniaca*	apricot
Capsicum annuum	bell pepper	*Prunus avium*	sweet cherry
Carica papaya	papaw	*Prunus cerasus*	sour cherry
Chrysophyllum cainito	caimito	*Prunus domestica*	plum
Citrus reticulata	mandarin	*Prunus persica*	peach
Coffea arabica	arabica coffee	*Psidium guajava*	guava
Dimocarpus longan	longan tree	*Pyrus communis*	European pear
Diospyros kaki	persimmon	*Syzygium aqueum*	watery rose-apple
Malpighia glabra	acerola	*Syzygium cumini*	black plum
Malus domestica	apple	*Syzygium jambos*	rose apple
Mangifera foetida	bachang	*Syzygium malaccense*	malay-apple
Mangifera indica	mango	*Syzygium samarangense*	water apple
Manilkara zapota	sapodilla		

DISTRIBUTION

India, Sri Lanka, Nepal, Bhutan, Myanmar, southern China, Taiwan, Hong Kong, northern Thailand, Vietnam, Cambodia, Laos, Hawaii, Mariana Islands, Tahiti (pers. comm. Drew 2010).

REMARKS

Bactrocera dorsalis is similar to *B. carambolae*, *B. papayae* and *B. verbascifoliae* in possessing broad parallel sided lateral postsutural vittae, costal band confluent with or very slightly overlapping R_{2+3} and to *B. papayae* and *B. verbascifoliae* in having the costal band remaining very narrow beyond apex of R_{2+3}, femora entirely fulvous and abdominal terga III-V with a narrow medial longitudinal dark band.

It differs from *B. carambolae* in possessing a very narrow apical section of the costal band, narrow medial longitudinal dark band on abdominal terga III-V and triangular shaped anterolateral dark corners on abdominal terga IV and V (these markings are rectangular in *B. carambolae*).

It differs from *B. verbascifoliae* in possessing narrow lateral dark margins on abdominal terga IV and V and from *B. papayae* in having a short male aedeagus and female ovipositor.

Other *dorsalis* complex species that are similar to *B. dorsalis* are *B. hantanae*, *B. irvingiae*, *B. raiensis* and *B. syzygii* however, all of these species possess a broad medial longitudinal dark band on abdominal terga III-V and have not been recorded as having males responding to methyl eugenol. See Drew and Hancock (1994) for a full discussion of type specimens, relationships and synonymies.

Following the publication on the *dorsalis* complex by Drew and Hancock (1994), there has been considerable research to investigate the integrity of many of the morphologically close species in the *dorsalis* complex. The review of Clarke *et al.* (2005) summarised the bulk of this research and has demonstrated that most taxa within the complex can be satisfactorily resolved and that the complex is undergoing rapid morphological change.

PEST STATUS

- Exotic

- High priority pest identified in the Apple and Pear, Avocado, Banana, Citrus, Summerfruit, Tropical fruit and Vegetable IBPs

- *Bactrocera dorsalis* is a major economic pest and utilises a wide range of commercial, edible and rainforest fruits

ATTRACTANT

Methyl eugenol.

FIGURES

Figure 35. *Bactrocera dorsalis*
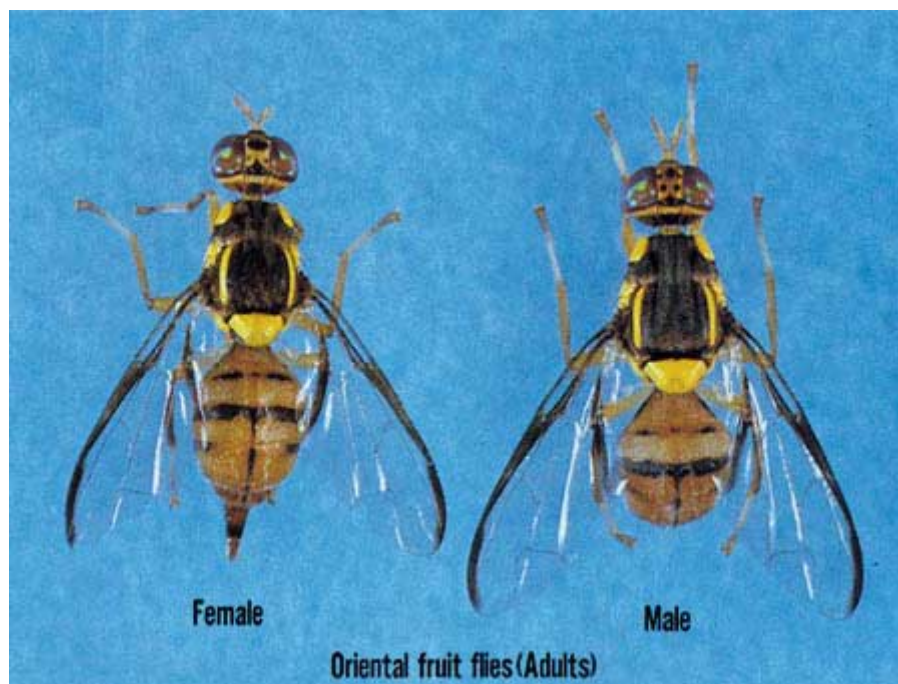
Image courtesy of the University of Florida and the Florida Department of Agriculture and Consumer Services
http://entomology.ifas.ufl.edu/creatures/index.htm (as of 22 August 2011)

Figure 36. *Bactrocera dorsalis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University
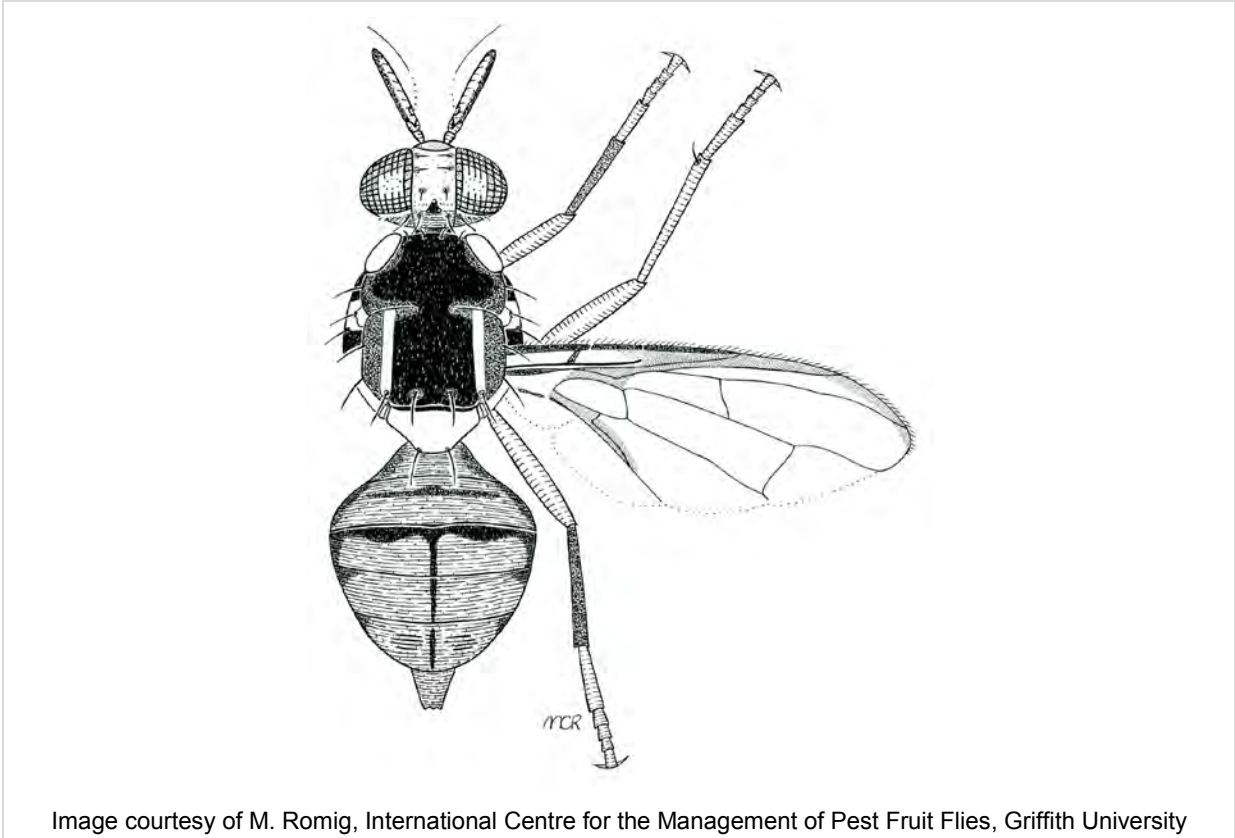
7.3.13   *Bactrocera* (*Bactrocera*) *facialis* (Coquillett)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus facialis

Chaetodacus facialis

Strumeta facialis

Dacus (*Strumeta*) *facialis*

DIAGNOSIS

7.3.13.1.1   Morphological - Adult

Small species; facial spots absent; postpronotal lobes and notopleura yellow; scutum dark fuscous to black, mesopleural stripe reaching almost to postpronotal lobes, narrow short lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and narrow pale fuscous anal streak, cells bc and c colourless with microtrichia in outer corner of cell c only; abdominal terga III-V orange-brown with a moderately broad medial longitudinal fuscous to black band over all three terga, broad lateral fuscous to black margins on tergum III and anterolateral corners of terga IV and V; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.13.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.13.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 750 bp

*Bsr*I: 580, 250   *Sna*BI: DNC

*Hha*I: 600, 180   *Ssp*I: DNC

*Hinf*I: DNC   *Vsp*I: DNC

*Sau*3AI: 400

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera facialis has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Combretaceae, Fabaceae, Lauraceae, Moraceae, Myrtaceae, Passifloraceae, Rosaceae, Rutaceae, Sapindaceae and Solanaceae(for a full list of recorded hosts see CABI 2007).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Capsicum annuum	bell pepper	*Psidium guajava*	guava
Mangifera indica	mango		

DISTRIBUTION

Known from the Tongatapu I. and the Ha'apai Group, Tonga (Drew 1989).

REMARKS

Bactrocera facialis is distinct in having broad black lateral margins on abdominal tergum III and anterolaterally on terga IV and V, a moderately broad medial longitudinal black band on terga III-V and lateral postsutural vittae very short and narrow ending at level of *sa*. setae (Drew 1989).

PEST STATUS

- Exotic

- *Bactrocera facialis* is a major pest, which causes up to 100% fruit loss in Capsicum species in Tonga

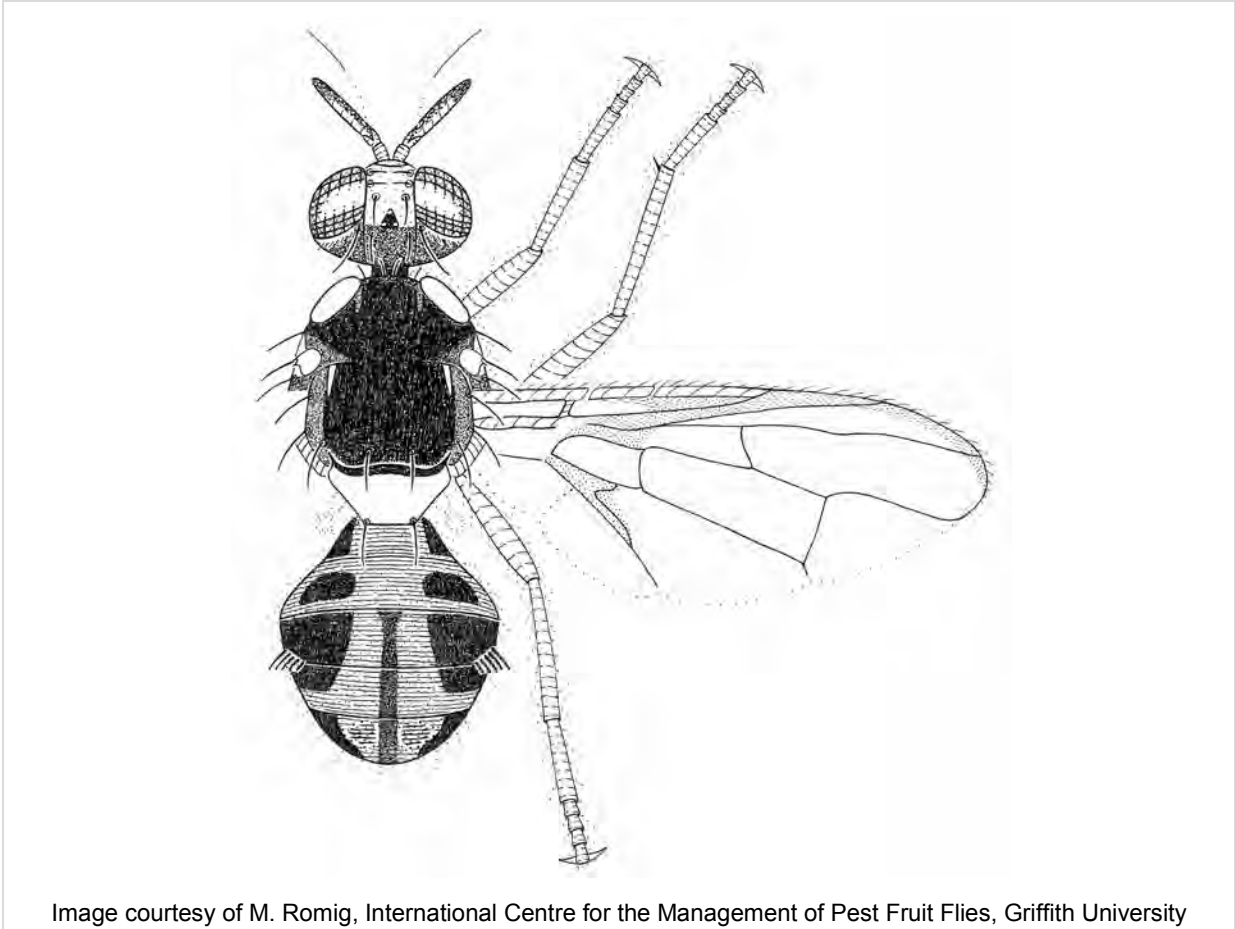## ATTRACTANT

Cue lure.

FIGURES

Figure 37. *Bactrocera facialis*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 38. *Bactrocera facialis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.14   *Bactrocera* (*Bactrocera*) *frauenfeldi* (Schiner)

TAXONOMIC INFORMATION

Common name: Mango fruit fly

Previous scientific names:

Dacus frauenfeldi

Strumeta frauenfeldi

Dacus (*Strumeta*) *frauenfeldi*

DIAGNOSIS

7.3.14.1.1   *Morphological - Adult*

Medium sized species; large black facial spots present; postpronotal lobes black; notopleura yellow; scutum glossy black, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with a black triangle on dorsal surface; wing with a narrow extremely pale fuscous costal band and broad fuscous anal streak, a narrow fuscous transverse band across wing, cells bc and c pale fuscous, microtrichia covering most of cell c; abdominal terga III-V orange-brown with a broad medial and 2 broad lateral longitudinal black bands; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.14.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.14.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 840 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	600, 200	*Ssp*I:	180, 620
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	400, 450		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera frauenfeldi has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Caricaceae, Clusiaceae, Combretaceae, Ebenaceae, Euphorbiaceae, Lecythidaceae, Loganiaceae, Malpighiaceae, Meliaceae, Moraceae, Musaceae, Myrtaceae, Olacaceae, Oxalidaceae, Passifloraceae, Rubiaceae, Rutaceae, Sapotaceae and Solanaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Mangifera indica	mango	*Psidium guajava*	guava
Manilkara kauki		*Syzygium malaccense*	malay-apple

DISTRIBUTION

Widely distributed in Papua New Guinea and across the Bismark Archipelago to the Solomon Islands, and established in the Torres Strait and northern Queensland as far south as Townsville (CABI 2007; Hancock *et al.* 2000).

REMARKS

Bactrocera frauenfeldi is similar to *B. parafrauenfeldi* and *B. trilineola* in having black postpronotal lobes and a black triangular marking on dorsal surface of scutellum extending to the apex but differs in possessing lateral postsutural vittae and with the black markings on the scutellum reaching the apex as a point. *Bactrocera albistrigata*, regarded as a synonym of *B. frauenfeldi* by Hardy and Adachi (1954), is a distinct species. It possesses yellow postpronotal lobes and is confined to South-east Asia (Drew 1989).

Other remarks:

This species can be separated from other members of the subgenus by the presence of a dark crossband from the pterostigma (cell sc), which also includes both the r-m and dm-cu crossvein. This runs roughly parallel to the anal stripe (diagonal mark across wing base). However, the costal band is very pale and often not visible at all beyond apex of R_{2+3}.

Bactrocera frauenfeldi can be identified by its entirely dark postpronotal lobes; the dark triangle shaped mark on the scutellum; and the short tapered lateral vittae on the scutum (CABI 2007).

PEST STATUS

- Established
- A major pest fruit fly species in Papua New Guinea, attacking most locally grown tropical fruits and nuts (with the exception of banana which is a rare host)

ATTRACTANT

Cue lure, Willison's lure.

FIGURES

Figure 39. *Bactrocera frauenfeldi*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University

Figure 40. *Bactrocera frauenfeldi*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.15　*Bactrocera* (*Afrodacus*) *jarvisi* (Tryon)

TAXONOMIC INFORMATION

Common name: Jarvis' fruit fly

Previous scientific names:

Chaetodacus jarvisi

Chaetodacus jarvisi var. *careya*

Dacus (*Afrodacus*) *jarvisi*

Afrodacus jarvisi

DIAGNOSIS

7.3.15.1.1　*Morphological - Adult*

Medium sized species; medium sized irregularly oval black facial spots present; postpronotal and notopluera yellow and connected by a broad yellow band; scutum red-brown, mesopleural stripe reaching almost to anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vittae absent, wing with a narrow fuscous costal band and broad fuscous anal streak, cells bc and c colourless with microtrichia in outer corner of cell c only; abdominal terga III-V orange-brown except for a fuscous to black transverse band across anterior margin of tergum III and fuscous to black medial longitudinal band generally over all three terga but often variable; posterior lobe of male surstylus long; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.15.1.2　*Morphological - Larvae*

- Not available/included in this edition -

7.3.15.1.3　*Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	600, 250	*Sna*BI:	DNC
*Hha*I:	650, 180	*Ssp*I:	700
*Hinf*I:	770	*Vsp*I:	DNC
*Sau*3AI:	420		

See also **PCR-DNA barcoding** (Section 6.3.2) and **Allozyme Electrophoresis** (Section 6.4.).

HOST RANGE

Bactrocera jarvisi has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Arecaceae, Cactaceae, Caricaceae, Celastraceae, Chrysobalanaceae, Clusiaceae, Combretaceae, Curcurbitaceae, Ebenaceae, Elaeocarpaceae, Lauraceae, Lecythidaceae, Malpighiaceae, Meliaceae, Moraceae, Musaceae, Myrtaceae, Oleaceae, Oxalidaceae, Passifloraceae, Punicaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae and Solanaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Mangifera indica	mango	*Prunus persica*	peach
Psidium guajava	guava	*Musa sp.*	banana

DISTRIBUTION

Northern Australia from Broome, Western Australia to eastern Arnhem Land, Northern Territory and northwest Queensland, Torres Strait islands and eastern Australia from Cape York to the Sydney district, New South Wales (Hancock *et al.* 2000). Has been recorded from Indonesia (Irian Jaya) by White and Elson-Harris on one occasion but is not established there and should not be regarded as a permanent record (pers. comm. Drew 2010).

REMARKS

Bactrocera jarvisi is similar to *B. ochracea* in having a pale coloured scutum, yellow notopleura and abdominal terga III-V without dark lateral margins. It is distinct from this species in having a broad yellow band connecting postpronotal lobes and notopluera, colourless cells bc and c with microtrichia in outer corner of cell c only, costal band expanded slightly at apex of wing and abdominal terga III-V with a fuscous to black narrow band across base of tergum III and a medial longitudinal fuscous to black band over all three terga (Drew 1989).

PEST STATUS

- Endemic
- A major pest in Queensland and the Northern Territory where it attacks a large number of fruit and vegetable crops

ATTRACTANT

Weakly attracted to cue lure in northwest Western Australia and Queensland (Drew 1989). Zingerone is a powerful selective male lure. A paper outlining research to this effect is currently in press (Fay 2011).

FIGURES

Figure 41. *Bactrocera jarvisi*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
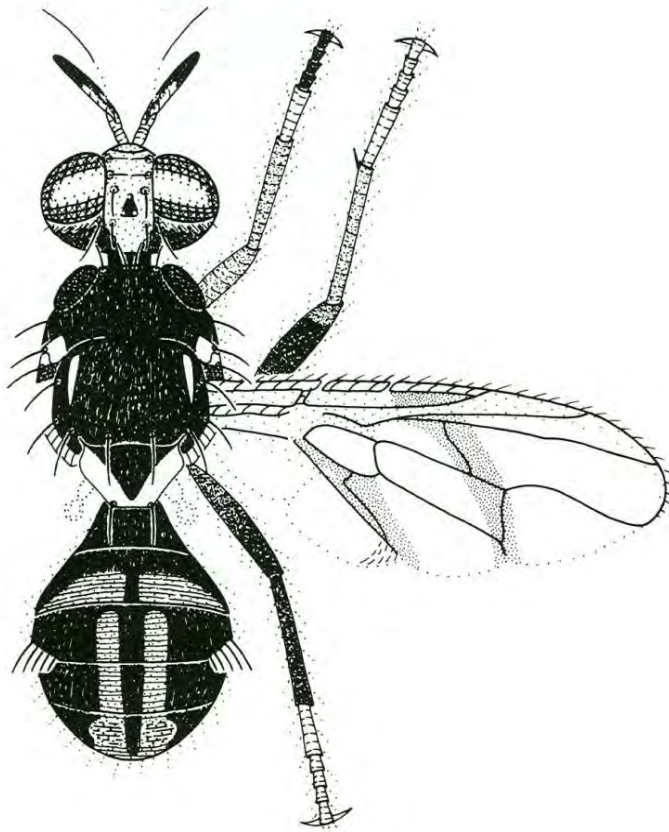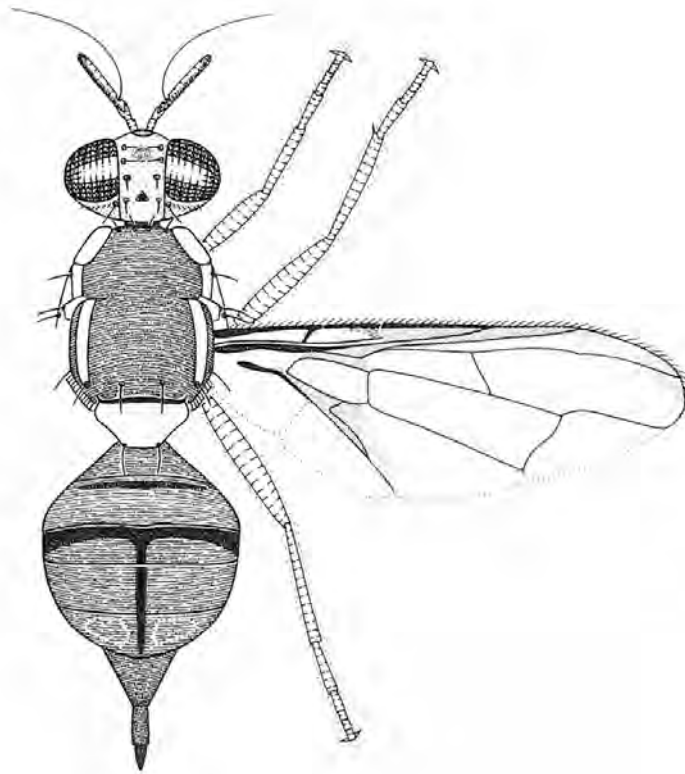
Figure 42. *Bactrocera jarvisi*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.16   *Bactrocera* (*Bactrocera*) *kandiensis* Drew and Hancock

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

DIAGNOSIS

7.3.16.1.1   *Morphological - Adult*

Face fulvous with a pair of large oval black spots; scutum black except brown below and behind lateral postsutural vittae, around mesonotal suture, inside postpronotal lobes, around *prsc.* setae and on anterocentral margin; postpronotal lobes yellow (anteromedial corners red-brown); notopleura yellow; mesopleural stripe slightly wider than notopleuron dorsally; narrow parallel sided lateral postsutural vittae ending at *ia.* seta; medial postsutural vitta absent; scutellum yellow with a moderately broad black basal band; legs with femora fulvous with dark fuscous on outer apical 2/3 of fore femora, inner apical 1/2 of mid and inner apical 1/3 of hind femora, fore tibiae fuscous, mid tibiae fulvous and hind tibiae dark fuscous; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, a narrow fuscous costal band confluent with R_{2+3} and remaining narrow around margin of wing to end between extremities of R_{4+5} and M, a narrow fuscous cubital streak; supernumerary lobe of medium development; abdominal terga III-V orange-brown with a narrow transverse black band across anterior margin of tergum III but not covering lateral margins, a very narrow medial longitudinal fuscous to dark fuscous band over all three terga (occasionally interrupted at intersegmental lines) and very narrow fuscous to dark fuscous anterolateral corners on terga IV and V, a pair of oval orange-brown shining spots on tergum V; abdominal sterna dark coloured; posterior lobe of male surstylus short; female with aculeus tip needle shaped (pers. comm. Drew 2010).

7.3.16.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.16.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera kandiensis has been recorded on hosts from six families, Anacardiaceae and Clusiaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al*. 1999; Tsuruta *et al*.1997):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew nut	*Psidium guajava*	guava
Annona glabra	pond apple	*Spondias cytherea*	jew plum
Citrus maxima	pummelo	*Syzygium aromaticum*	clove
Mangifera indica	mango	*Syzygium jambos*	rose apple
Averrhoa carambola	carambola	*Carica papaya*	papaya

DISTRIBUTION

Bactrocera kandiensis is confined to Sri Lanka (Drew and Hancock 1994).

Bactrocera kandiensis is similar to *B. caryeae* and *B. neoarecae* in possessing narrow parallel sided lateral postsutural vittae and dark patterns on the apices of all femora or, at least, on fore and mid femora.

It differs from *B. neoarecae* in possessing a single 'T' pattern over abdominal terga III-V (not on each of the three separate terga), a narrow black basal band on the scutellum and dark markings on the apices of all

femora and from *B. caryeae* in possessing a very narrow medial longitudinal dark band on abdominal terga III-V and narrow dark anterolateral corners on terga IV and V (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- *Bactrocera kandiensis* is a major pest of mangoes and is probably responsible for much of the damage generally attributed to *B. dorsalis* in Sri Lanka

ATTRACTANT

Methyl eugenol.

FIGURES
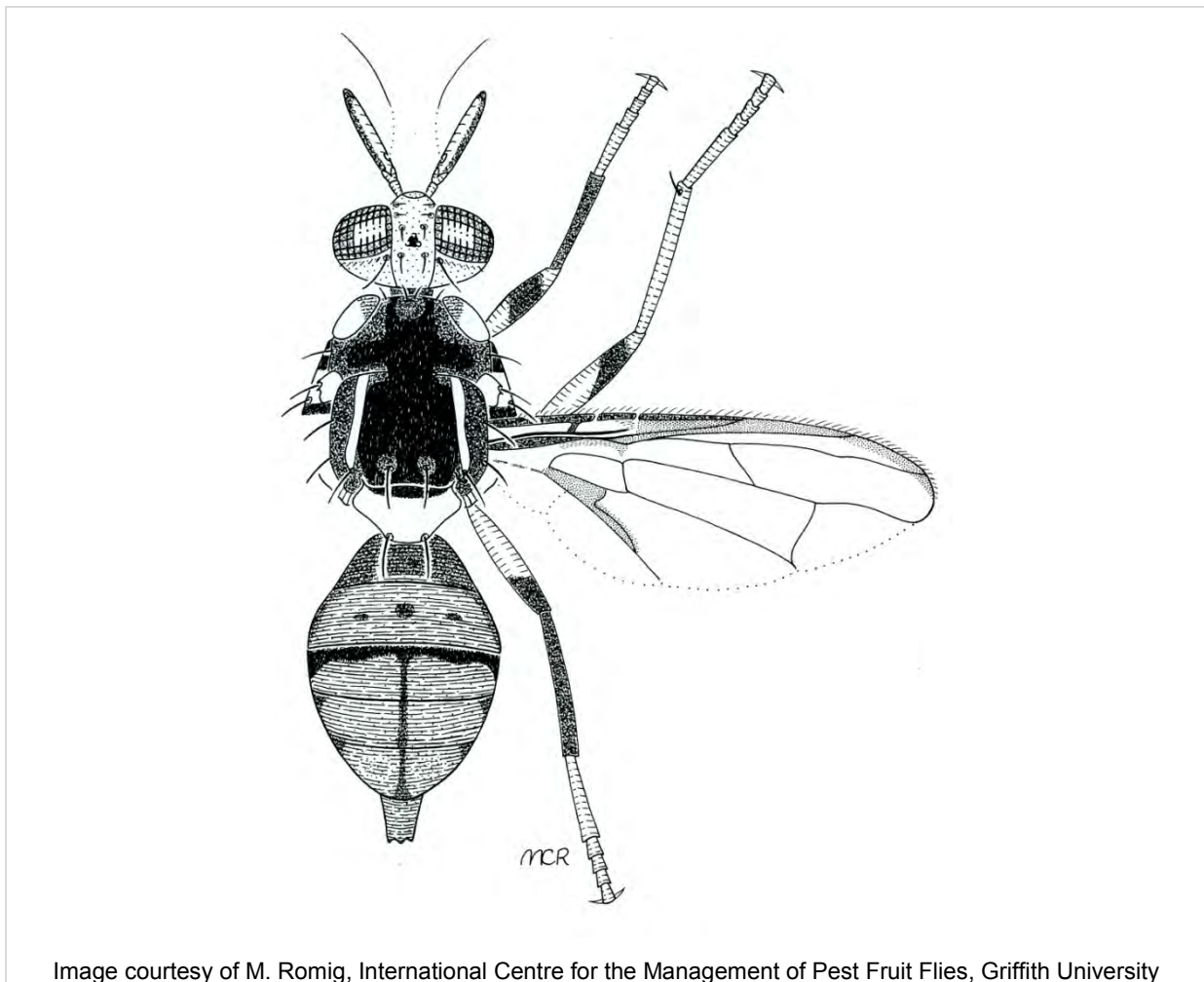
Figure 43. *Bactrocera kandiensis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.17   *Bactrocera* (*Bactrocera*) *kirki* (Froggatt)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus kirki

Strumeta kirki

Dacus (*Strumeta*) *kirki*

DIAGNOSIS

7.3.17.1.1   Morphological - Adult

Medium sized species; large black facial spots present; postpronotal lobes yellow (anterodorsal margins black); notopluera yellow; scutum glossy black, mesoplueral stripe slightly wider than notopleuron, lateral and medial postsutural vittae absent, scutellum glossy black with extreme lateral margins yellow; wing with a narrow pale fuscous costal band and narrow fuscous anal streak, a narrow pale fuscous tinge around r-m and dm-cu crossveins, cells bc and c with extremely pale fuscous tinge and microtrichia in outer ½ of cell c only; abdominal terga glossy black except for two longitudinal orange-brown bands over terga II-V either side of a broad medial longitudinal glossy black band; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.17.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.17.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	680, 190	*Ssp*I:	180, 620
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	400, 450		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera kirki has been recorded on hosts from a range of families. These include: Anacardiaceae, Bromeliaceae, Combretaceae, Fabaceae, Myrtaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rutaceae, Solanaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (Drew 1989):

Scientific name	Common name
Mangifera indica	mango
Psidium guajava	guava

DISTRIBUTION

Widespread in the South Pacific islands: Western Samoa, American Samoa, Tonga, Niue and Tahiti (Drew 1989).

REMARKS

Bactrocera kirki is similar to *B. setinervis* in having lateral and medial postsutural vittae absent, scutellum yellow with a black triangle on dorsal surface and postpronotal lobes yellow but differs in possessing facial spots and yellow notopleura. *Bactrocera kirki* is unusual in that it lacks yellow vittae on the scutum and the scutellum is largely black except for the pale margins (Drew 1989; pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Avocado IBP

- *Bactrocera kirki* is considered a major pest, and perhaps the most significant in the South Pacific region

ATTRACTANT
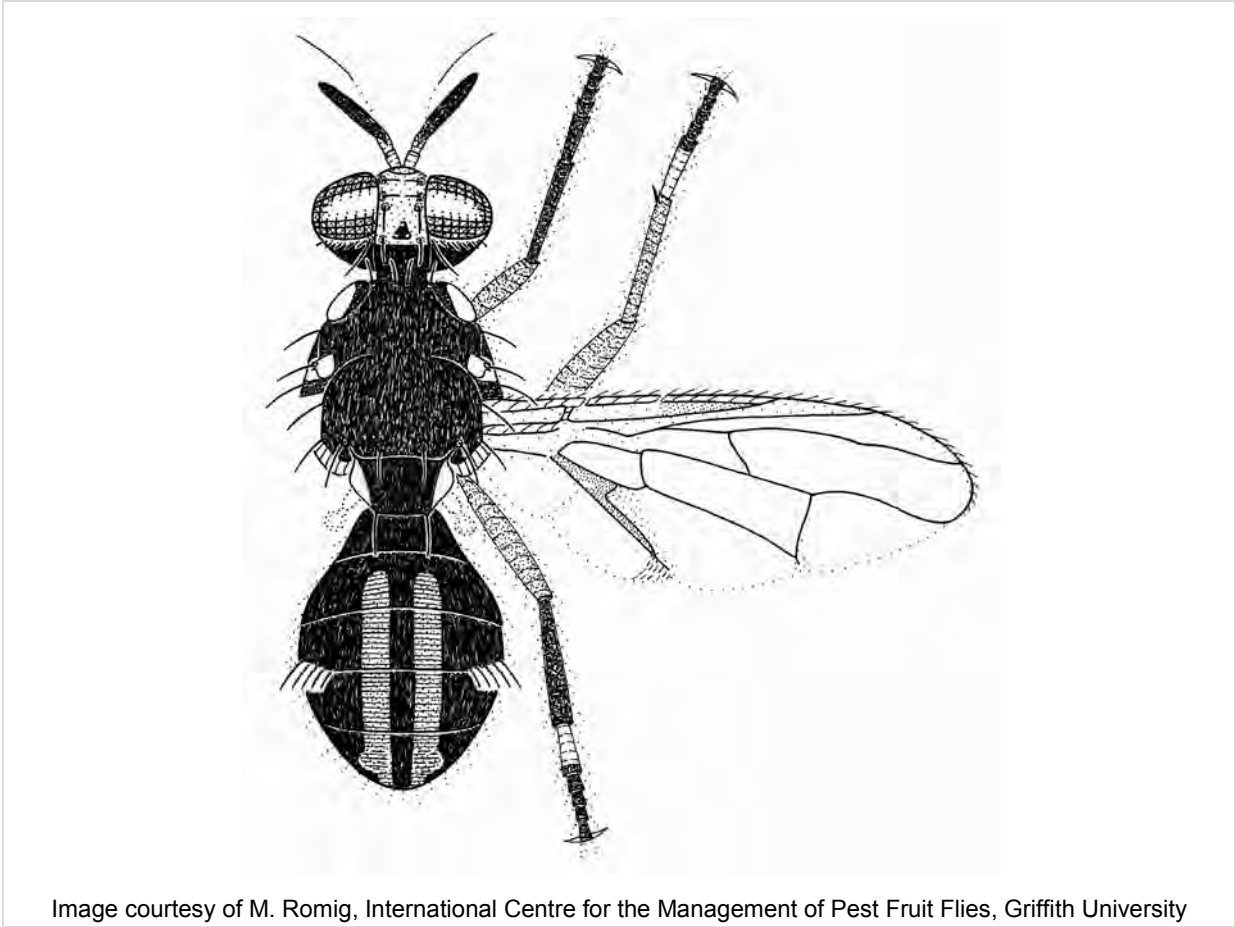
Cue lure, Willison's lure.

FIGURES

Figure 44. *Bactrocera kirki*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 45. *Bactrocera kirki*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.18   *Bactrocera* (*Bactrocera*) *kraussi* (Hardy)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus (*Strumeta*) *kraussi*

Strumeta kraussi

DIAGNOSIS

7.3.18.1.1   *Morphological - Adult*

Medium sized species; medium sized oval facial spots present; postpronotal lobes and notopleura yellow; scutum red-brown with irregularly shaped lateral longitudinal pale fuscous to fuscous bands, mesopleural stripe reaching midway between anterior margin of notopleron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with a broad red-brown to fuscous basal band; wing colourless or with a pale fulvous tint and a narrow fuscous costal band and broad fuscous anal streak, cells bc and c pale fulvous to fulvous with microtrichia in outer corner of cell c only, abdominal terga III and IV fuscous and tergum V fulvous except for broad lateral dark fuscous margins on terga III and IV and broad fuscous lateral margins on tergum V; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.18.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.18.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera kraussi has been recorded on hosts from a wide range of families. These include: Agavaceae, Anacardiaceae, Annonaceae, Apocynaceae, Clusiaceae, Combretaceae, Cunoniaceae, Davidsoniaceae, Elaeocarpaceae, Euphorbiaceae, Flacourtiaceae, Icacinaceae, Lauraceae, Lecythidaceae, Loganiaceae, Malpighiaceae, Meliaceae, Menispermaceae, Moraceae, Musaceae, Myrtaceae, Oleaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Solanaceae and Thymeliaceae (for a full list of recorded hosts see Hancock *et al.*, 2000).

Major commercial hosts (Drew 1989, Hancock *et al.* 2000):

Scientific name	Common name	Scientific name	Common name
Citrus sp.	Grapefruit, mandarin, orange	*Musa* sp.	banana
Mangifera indica	mango	*Psidium guajava*	guava

It should be noted that fruit flies are not known to attack hard green bananas (Hancock *et al.,* 2000).

DISTRIBUTION

Torres Strait Islands and northeast Queensland, as far south as Townsville (Hancock *et al.* 2000).

REMARKS

Bactrocera kraussi is similar to all other species in the *fagraea* complex being a general red-brown fly, scutellum with a broad dark basal band and cells bc and c not covered in dense microtichia. It differs from *B. rufescens* in lacking a medial dark band on abdomen, from *B. fagraea* and *B. russeola* in having lateral fuscous markings on abdominal terga III and IV and from *B. halfordiae* in having a red-brown scutum with or without fuscous markings, abdomen usually fuscous over terga III and IV and laterally on tergum V, mesopleural stripe 1 ½ times the width of notopleuron and lateral postsutural vittae parallel sided (Drew 1989).

PEST STATUS

- Endemic
- A moderate pest species in North Queensland

ATTRACTANT

Cue lure.

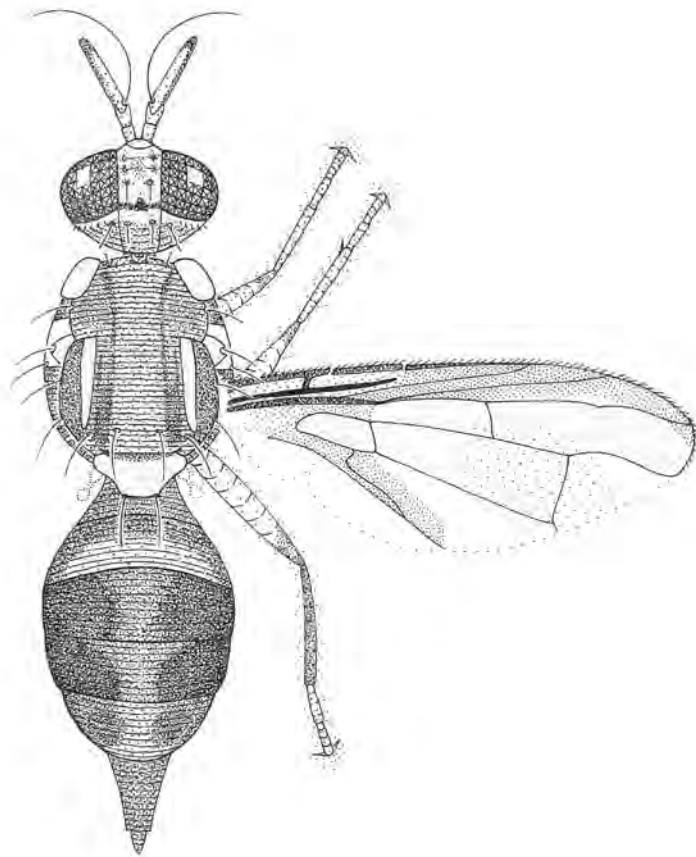## FIGURES

Figure 46. *Bactrocera kraussi*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.19   *Bactrocera* (*Bactrocera*) *latifrons* (Hendel)

TAXONOMIC INFORMATION

Common name: Solanum fruit fly

Previous scientific names:

Chaetodacus latifrons

Bactrocera (*Bactrocera*) *latifrons*

DIAGNOSIS

7.3.19.1.1   *Morphological - Adult*

A medium sized species; face fulvous with a pair of large oval black spots; postpronotal lobes and notopleura yellow; scutum dull black; lateral postsutural vittae present; medial postsutural vitta absent; mesopleural stripe extending to anterior *npl.* seta dorsally; scutellum yellow; wing with a narrow fuscous costal band overlapping R_{2+3} and expanding into a small spot around apex of R_{4+5}, a medium width fuscous anal streak; cells bc and c colourless; microtrichia in outer corner of cell c only; all abdominal terga entirely dark orange-brown, posterior lobe of male surstylus short; female with apex of aculeus trilobed (pers. comm. Drew 2010).

7.3.19.1.2   *Morphological - Larvae*

- Not available / included in this edition -

7.3.19.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 770 bp

Bsr1:	600, 200	SnaB1:	DNC
Hhal:	600, 190	Ssp1:	DNC
Hinfl:	DNC	Vspl:	DNC
Sau3a1:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera latifrons has been recorded on hosts from a wide range of families. These include: Lythraceae, Myrtaceae, Oleaceae, Passifloraceae, Punicaceae, Rhamnaceae, Rutaceae, Sapindaceae, Solanaceae and Verbenaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al*. 1999):

Scientific name	Common name	Scientific name	Common name
Capsicum sp.	peppers	*Solanum lycopersicum*	tomato
Capsicum annuum	bell pepper	*Solanum melongena*	eggplant

DISTRIBUTION

Sri Lanka, India, Pakistan through to Southern China, Japan, Taiwan, Thailand, Laos, Vietnam, Peninsular Malaysia, Indonesia, Hawaii, Tanzania (pers. comm. Drew 2010).

REMARKS

Bactrocera latifrons can be confused with species in the *B. musae* complex and the *B. dorsalis* complex in possessing similar body colour patterns. However it is distinct in having a trilobed apex on the aculeus and uniformly dark orange-brown abdominal terga. It is similar to *B. citima* in possessing a generally black scutum, costal band overlapping R_{2+3}, cells bc and c colourless and parallel sided lateral postsutural vittae but differs from this species in having red-brown around the lateral and posterior margins of the scutum, femora entirely fulvous and abdominal terga III-V entirely red-brown (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- This is a pest of solanaceous crops throughout its range

ATTRACTANT

No known record. Alpha-ionol, known as latilure is not a strong attractant but has been patented since 1989 (Flath *et al.* 1994). Latilure and cade oil were used in Jackson traps for surveys of *B. latifrons* in Tanzania (Flath *et al.*1994).

FIGURES
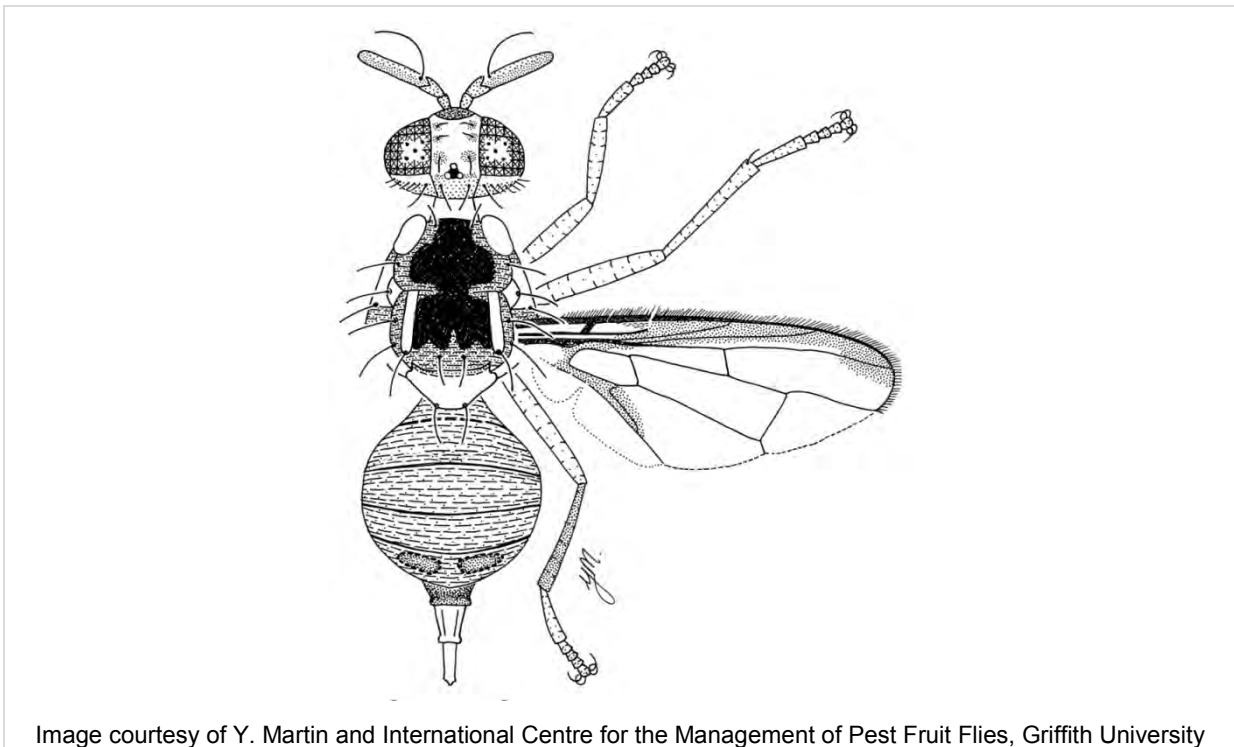
Figure 47. *Bactrocera latifrons*

Image courtesy of Y. Martin and International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.20   *Bactrocera* (*Bactrocera*) *melanotus* (Coquillett)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus melanotus

Chaetodacus melanotus

Strumeta melanotus

Dacus (*Strumeta*) *melanotus*

DIAGNOSIS

7.3.20.1.1   *Morphological - Adult*

Medium sized species; facial spots absent or small and pale; postpronotal lobes yellow (anterolateral corners black); notopleura glossy black; scutum glossy black, mesopleural stripe reaching to postpronotal lobe, lateral and medial postsutural vittae absent, scutellum glossy black; wing with a narrow pale fuscous costal band and narrow fuscous tint in anal cell, narrow pale fuscous markings along r-m and dm-cu crossveins, cells bc and c colourless or with a very pale fuscous tint, microtrichia in outer corner of cell c only; all abdominal terga entirely glossy black; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.20.1.2   *Morphological - Larvae*

- Not available / included in this edition -

7.3.20.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera melanotus has been recorded on hosts from seven families. These include: Anacardiaceae, Caricaceae, Combretaceae, Fabaceae, Myrtaceae, Rutaceae and Sapindaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Citrus sp.		*Psidium guajava*	guava
Mangifera indica	mango		

DISTRIBUTION

Restricted to Cook Is (Drew 1989).

REMARKS

Bactrocera melanotus is similar to *B. atra* and *B. perfuscain* possessing an entirely black scutellum, scutum black with medial and lateral postsutural vittae absent, abdominal terga black but differs from these species in having infuscation around r-m and dm-cu crossveins. In addition, it can be separated from *B. atra* in having postpronotal lobes mostly yellow, yellow mesopleural stripe and black femora (Drew 1989). *Bactrocera melanotus* is unusual in that its scutum, scutellum and abdomen are entirely dark coloured (black or very dark brown) (CABI 2007).

- Exotic

- High priority pest identified in the Avocado IBP

- *Bactrocera melanotus* is considered a major pest of papaw and citrus crops

ATTRACTANT

Cue lure.

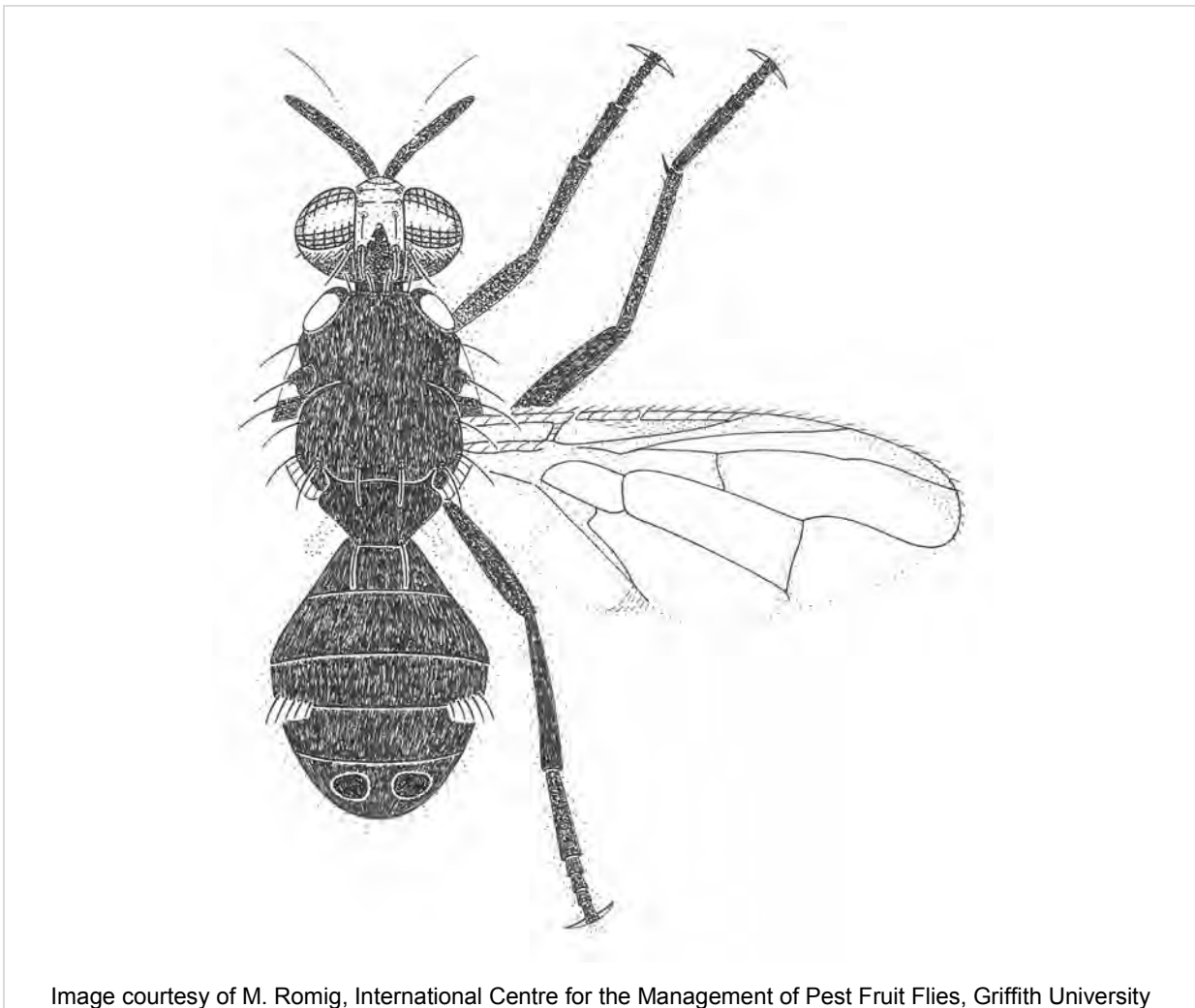*FIGURES*

Figure 48. *Bactrocera melanotus*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.21 *Bactrocera* (*Bactrocera*) *musae* (Tryon)

TAXONOMIC INFORMATION

Common name: Banana fruit fly

Previous scientific names:

Chaetodacus musae

Chaetodacus tryoni var. *musa*

Chaetodacus musae var. *dorsopicta*

Dacus (*Strumeta*) *musae*

Strumeta musae

Bactrocera (*Bactrocera*) *musae*

DIAGNOSIS

7.3.21.1.1 *Morphological - Adult*

Medium sized species; medium sized black facial spots present; postpronotal lobes and notopleura yellow; scutum dull black, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae present; medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and anal streak, cells bc and c colourless with microtrichia in outer corner of cell c only; abdominal terga III-V may vary from uniformly orange-brown to orange-brown with a fuscous to black medial longitudinal band and fuscous to black anterolateral corners on tergum III; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.21.1.2 *Morphological - Larvae*

- Not available / included in this edition -

7.3.21.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	600, 250	*Sna*BI:	320, 520
*Hha*I:	630, 220	*Ssp*I:	DNC
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera musae has been recorded on hosts from nine families. These include: Capparaceae, Caricaceae, Musaceae, Myrtaceae, Olacaceae, Passifloraceae, Rubiaceae, Rutaceae and Solanaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major commercial hosts (Drew, 1989; Hancock *et al.*, 2000):

Scientific name	Common name	Scientific name	Common name
Musa sp.	banana		

DISTRIBUTION

Torres Strait Islands and northeast Queensland, as far south as Townsville (Hancock *et al.* 2000), Papua New Guinea and associated islands, Bismark Archipelago and the Solomon Islands (Drew 1989).

REMARKS

There are a large number of species similar to *Bactrocera musae*, all placed in the *musae* complex. It is similar to *B. finitima* and *B. tinomiscii* in possessing a black scutum with lateral postsutural vittae present and ending at *ia.* setae and medial postsutural vittae absent, postpronotal lobes and notopleura yellow, scutellum yellow with a narrow dark basal band and cells bc and c colourless. It differs from *B. tinomiscii* in having the costal band dark and extending well below R_{2+3}, apex of piercer of ovipositor not curved upwards and subapical sensory setae on piercer of ovipositor consisting of two large and two small each side and from *B. finitma* in having the costal band not extending almost to R_{4+5}; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (pers. comm. Drew 2010).

B. musae has a considerable intraspecific variation and can appear similar to *B. endiandrae* (endemic rainforest species from Queensland) and *B. papaya*e which are also methyl eugenol attracted.

PEST STATUS

- Endemic
- Minor pest of commercial bananas.

ATTRACTANT

Methyl eugenol.

FIGURES

Figure 49. *Bactrocera musae*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 50. *Bactrocera musae*

Image courtesy of Ken Walker, Museum Victoria, **www.padil.gov.au** (as of 22 August 2011))
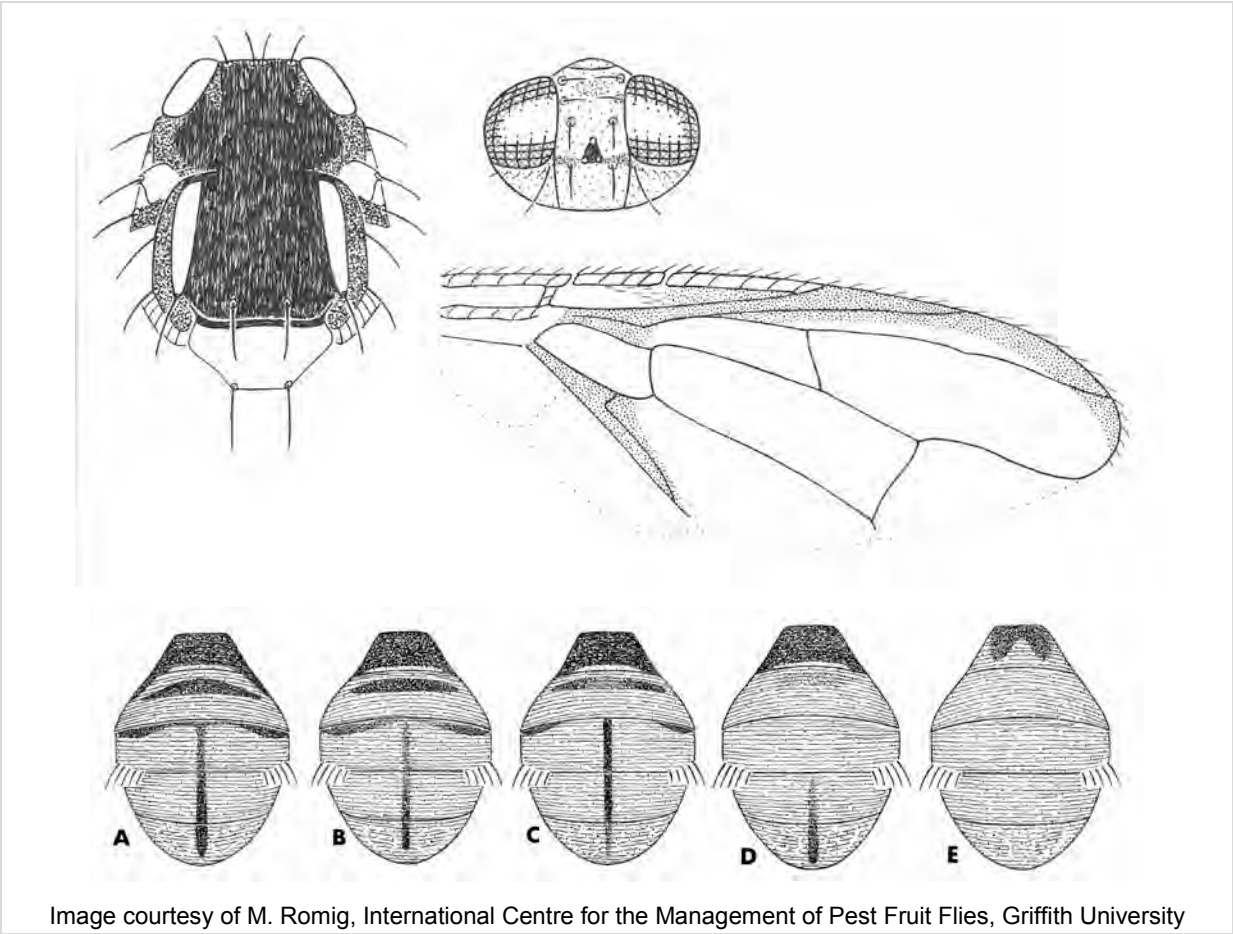
Figure 51. *Bactrocera musae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.22 *Bactrocera* (*Bactrocera*) *neohumeralis* (Hardy)

TAXONOMIC INFORMATION

Common name: Lesser Queensland fruit fly

Previous scientific names:

Chaetodacus humeralis

Strumeta humeralis

Dacus (*Strumeta*) *tryoni* var. *neohumeralis*

Dacus (*Strumeta*) *neohumeralis*

Dacus (*Bactrocera*) *neohumeralis*

DIAGNOSIS

7.3.22.1.1 *Morphological - Adult*

Medium sized species; medium sized black facial spots present; postpronotal lobes dark brown to fuscous; notopleura yellow; scutum dark red-brown with dark fuscous to black markings, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, cells bc and c fuscous, microtrichia covering cell c and outer ½ of cell bc; abdominal terga III-V generally dark fuscous to dull black and tending red-brown medially; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.22.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.22.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	200, 600	*Sna*BI:	DNC
*Hha*I:	640, 190	*Ssp*I:	180, 570
*Hinf*I:	770	*Vsp*I:	DNC
*Sau*3AI: 420			

PCR - Restriction Fragment Length Polymorphism (Test 2, Section 6.3.1):

(This species cannot be differentiated from *Bactrocera tryoni*)

*Alu*I	780-770, 240-230*, 170, 130 120 110
*Dde*I	1000-980*, 270, 220, 170-160
*Rsa*I	530-500*, 460-440*, 410, 290
*Ssp*I	1000, 550, 100

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera neohumeralis has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Basellaceae, Cactaceae, Capparaceae, Caricaceae, Celastraceae, Chrysobalanaceae, Clusiaceae, Combretaceae, Davidsoniaceae, Ebenaceae, Elaeocarpaceae, Euphorbiaceae, Flacourtiaceae, Hippocraterceae, Lauraceae, Leeaceae, Lecythidaceae, Malpighiaceae, Melastomataceae, Meliaceae, Moraceae, Musaceae, Myrtaceae, Olacaceae, Oleaceae, Oxalidaceae, Passifloraceae, Piperaceae, Rhamnaceae, Rhizophoraceae, Rosaceae, Rubiaceae, Rutaceae, Santalaceae, Sapindaceae, Sapotaceae, Smilacaceae, Solanaceae, Verbenaceae and Vitaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major commercial hosts:

A large number of important commercial/edible host fruits and vegetables (see Drew 1989; Hancock *et al.* 2000).

DISTRIBUTION

Common pest in Eastern Australia, south to Coffs Harbour, Torres Strait Islands and mainland Papua New Guinea (Drew 1989). It is not found in central and southern NSW (Osborne *et al.* 1997).

REMARKS

B. neohumeralis differs from *B. tryoni* in having dark postprotonotal lobes (this is a distinct character) in addition to being generally darker. Although these two species are very similar morphologically, their different daily mating periods (*B. tryoni* at dusk and *B. neohumeralis* during the middle of the day) are good reason to keep them separate (Drew 1989).

PEST STATUS

- Endemic
- *Bactrocera neohumeralis* is a major pest of commercial fruit crops in Queensland, Australia, and in some crops it occurs in equal abundance to *B. tryoni*

ATTRACTANT

Cue lure.

FIGURES

Figure 52. *Bactrocera neohumeralis*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
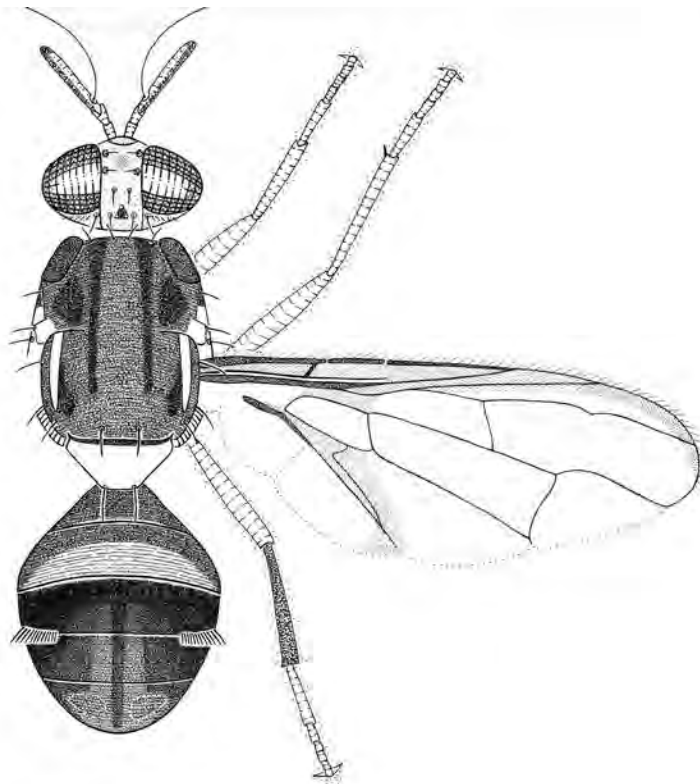
Figure 53. *Bactrocera neohumeralis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.23   *Bactrocera* (*Bactrocera*) *occipitalis* (Bezzi)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Chaetodacus ferrugineus var. *occipitalis*

Dacus (*Strumeta*) *dorsalis* var. *occipitalis*

Dacus (*Strumeta*) *occipitalis*

Dacus (*Bactrocera*) *occipitalis*

Bactrocera (*Bactrocera*) *occipitalis*

DIAGNOSIS

7.3.23.1.1   *Morphological - Adult*

Face fulvous with a pair of large oval black spots; scutum black except dark red-brown along posterior margin and enclosing *prsc.* setae, below and behind lateral postsutural vittae, around mesonotal suture, around anterior margin of notopleura and inside postpronotal lobes; postpronotal lobes and notopleura yellow; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta dorsally; broad parallel sided or subparallel lateral postsutural vittae ending at *ia.* seta (in some specimens the vittae end behind the *ia.* seta); medial postsutural vitta absent; scutellum yellow; legs with femora entirely fulvous, fore tibiae pale fuscous to fuscous, mid tibiae pale fuscous to fuscous basally tending paler apically, hind tibiae fuscous; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, a narrow fuscous costal band distinctly overlapping R_{2+3} and widening markedly across apex of wing, a narrow fuscous anal streak; supernumerary lobe of medium development; abdominal terga III-V with a narrow transverse black band across anterior margin of tergum III and expanding to cover lateral margins, dark fuscous to black rectangular markings anterolaterally on tergum IV which sometimes continue to cover posterolateral margins of this tergum, dark fuscous to black anterolateral corners on tergum V, a very broad medial longitudinal black band over all three terga, a pair of oval orange-brown shining spots on tergum V; abdominal sterna dark coloured; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (pers. comm. Drew 2010).

7.3.23.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.23.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Allwood *et al.* (1999) host records are incomplete due to a lack of field host survey work through the area of distribution of the species (pers. comm. Drew 2010a). *Bactrocera occipitalis* has been recorded on hosts from three families, Anacardiaceae, Myrtaceae and Rutaceae (Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Citrus microcarpa	musk lime	*Psidium guajava*	guava
Mangifera indica	mango		

DISTRIBUTION

Philippines, East Malaysia (Sabah), Brunei, Indonesia (Kalimantan) (pers. comm. Drew 2010).

REMARKS

Bactrocera occipitalis is similar to some specimens of *B. fuscitibia* in possessing broad parallel sided lateral postsutural vittae, costal band overlapping R_{2+3}, narrow to medium width dark patterns on lateral margins of abdominal terga III-V, shining spots on abdominal tergum V pale coloured, femora entirely fulvous or with a dark spot on outer apical surfaces of fore femora only and a broad medial longitudinal dark band on abdominal terga III-V.

It differs from *B. fuscitibia* in having the anterolateral bare area on the scutum broad and lateral dark markings on abdominal terga IV and V of medium width (not narrow). Some populations of fruit flies throughout South-East Asia have been misidentified as *B. occipitalis* in previous literature. See Drew & Hancock (1994) for a complete discussion on this species and previous misidentifications (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- High priority pest identified in the Tropical fruit IBP
- *Bactrocera occipitalis* is a major pest species within the *dorsalis* complex of South-east Asia

ATTRACTANT

Methyl eugenol.

Figure 54. *Bactrocera occipitalis*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
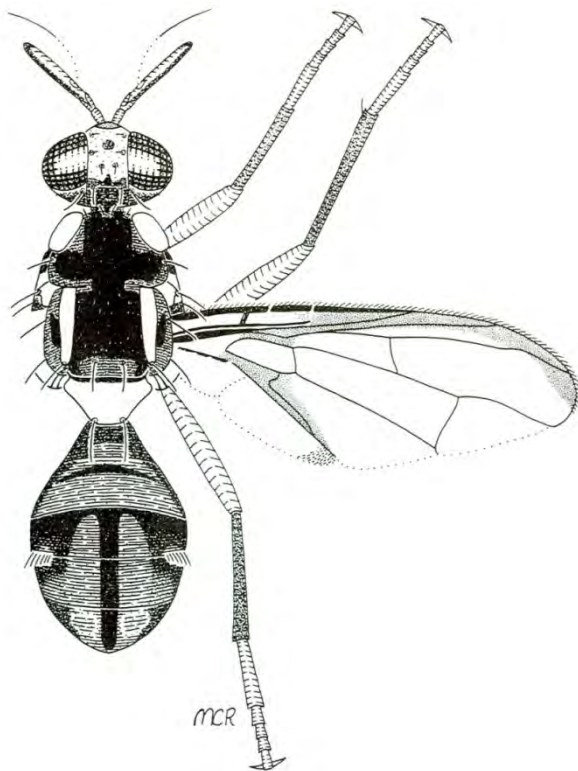
Figure 55. *Bactrocera occipitalis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.24   *Bactrocera* (*Bactrocera*) *papayae* Drew and Hancock

TAXONOMIC INFORMATION

Common name: Papaya fruit fly

Previous scientific names:

DIAGNOSIS

7.3.24.1.1   *Morphological - Adult*

Face fulvous with a pair of large oval black spots; scutum black with dark brown below and behind lateral postsutural vittae, around mesonotal suture and inside postpronotal lobes; postpronotal lobes and notopleura yellow; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta dorsally; broad parallel sided lateral postsutural vittae ending at or behind *ia.* seta; medial postsutural vitta absent; scutellum yellow; legs with femora entirely fulvous, fore and hind tibiae dark fuscous, mid tibiae fuscous basally and fulvous apically; wings with cells bc and c colourless, microtrichia in outer corner of cell c only, a narrow fuscous costal band confluent with R_{2+3} or just overlapping this vein where it becomes paler and remaining narrow around wing apex (in some specimens there is a slight expansion or a small fish-hook shape around apex of R_{4+5}), a narrow fuscous anal streak; supernumerary lobe of medium development in males and weak in females; abdominal terga III-V orange-brown with a 'T' pattern consisting of a narrow transverse black band across anterior margin of tergum III which expands laterally into narrow margins and a medium width medial longitudinal black band over all three terga, anterolateral corners of terga IV and V dark fuscous to black (in occasional specimens the transverse black band across anterior margin of tergum III is broken in the midline), a pair of oval orange-brown shining spots on tergum V; abdominal sterna dark coloured; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (pers. comm. Drew 2010).

7.3.24.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.24.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	650, 260	*Sna*BI:	320, 530
*Hha*I:	650, 190	*Ssp*I:	750
*Hinf*I:	770	*Vsp*I:	DNC

*Sau*3AI: DNC

See also **PCR-DNA barcoding** (Section 6.3.2) and **Allozyme Electrophoresis** (Section 6.4).

HOST RANGE

Bactrocera papayae has been recorded on hosts from a wide range of families. These include: Amaryllidaceae, Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Boraginaceae, Burseraceae, Cactaceae, Caricaceae, Clusiaceae, Combretaceae, Curcurbitaceae, Dilleniaceae, Ebenaceae, Elaeocarpaceae, Euphorbiaceae, Fagaceae, Flacourtiaceae, Flagellariaceae, Lauraceae, Lecythidaceae, Leguminosae, Loganiaceae, Malpighiaceae, Meliaceae, Menispermaceae, Moraceae, Musaceae, Myrusticaceae, Myrsinaceae, Myrtaceae, Oleaceae, Oxalidaceae, Passifloraceae, Punicaceae, Rhamnaceae, Rhizophoraceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Simaroubaceae, Solanaceae, Sterculiaceae, Tiliaceae, Ulmaceae, Verbenaceae, Vitaceae and Zingiberaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts:

A large number of important commercial/edible host fruits and vegetables (see Allwood *et al*. 1999; Hancock et al. 2000).

DISTRIBUTION

Irian Jaya, Papua New Guinea, Southern Thailand, Peninsular Malaysia, East Malaysia, Brunei, Singapore, Indonesia provinces, Christmas Island (pers. comm. Drew 2010).

Although *Bactrocera papaya* is not established in the Torres Strait Islands, occasional incursions do occur in the northern Torres Strait Islands. They are promptly eradicated.

REMARKS

Bactrocera papayae is similar to *B. carambolae*, *B. dorsalis*, *B. occipitalis* and *B. philippinensis* in possessing a black scutum with broad lateral postsutural vittae that are generally parallel sided and reaching to or behind *ia*. setae, a narrow costal band on the wing, abdominal terga III to V with a black 'T' pattern and dark lateral margins. It differs from *B. carambolae*, *B. dorsalis* and *B. occipitalis* in having a longer aculeus in the female ovipositor (1.77 to 2.12 mm) and the costal band mostly confluent with R_{2+3} and from *B. philippinensis* in having a shorter male aedeagus (mean 3.0 mm).

B. papayae and other *dorsalis* complex flies can appear similar to endemic fruit flies caught in methyl eugenol traps – namely *B. endiandrae* and *B. musae*, both of which can exhibit intraspecific variation that makes them appear more similar to *dorsalis* complex flies. The diagnostician should be familiar with this range of variation in the native species.

PEST STATUS

- Exotic
- High priority pest identified in the Apple and Pear, Avocado, Banana, Citrus, Mango, Summerfruit, Tropical fruit and Vegetable IBPs
- *Bactrocera papayae* is a major pest species within the *dorsalis* complex of South-east Asia

ATTRACTANT

Methyl eugenol.

Figure 56. *Bactrocera papayae*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 57. *Bactrocera papayae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.25　*Bactrocera* (*Bactrocera*) *passiflorae* (Froggatt)

TAXONOMIC INFORMATION

Common name: Fijian fruit fly

Previous scientific names:

Dacus passiflorae

Chaetodacus passiflorae

Strumeta passiflorae

Dacus (*Strumeta*) *passiflorae*

DIAGNOSIS

7.3.25.1.1　Morphological - Adult

Small species; facial spots absent; postpronotal lobes glossy black; notopleura yellow; scutum glossy black, mesopleural stripe reaching to or beyond anterior *npl.* seta, lateral and medial postsutural vittae absent, scutellum yellow; wing with a narrow fuscous costal band and narrow pale fuscous anal streak, cells bc and c colourless with microtrichia in outer corner of cell c only; abdominal terga I-IV glossy black and tergum V either glossy black with posterior margin dark fuscous or fuscous with a medial longitudinal black band; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.25.1.2　Morphological - Larvae

- Not available / included in this edition -

7.3.25.1.3　Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 830 bp

*Bsr*I:　　650, 270　　　*Sna*BI:　DNC

*Hha*I:　　650, 190　　　*Ssp*I:　　750

*Hinf*I:　　770　　　　　*Vsp*I:　　DNC

*Sau*3AI: DNC

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

The host list for *Bactrocera passiflorae* is large but unpublished. It is a major pest species and capable of attacking a wide range of commercial host plants.

Major commercial hosts (White and Elson-Harris 1992):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew nut	*Passiflora quadrangularis*	giant granadilla
Carica papaya	papaw	*Persea americana*	avocado
Citrus aurantiifolia	lime	*Psidium guajava*	guava
Citrus reticulata	mandarin	*Solanum melongena*	eggplant
Mangifera indica	mango	*Theobroma cacao*	cocoa
Passiflora edulis	passionfruit		

DISTRIBUTION

Fiji Islands, Niue, Wallis and Futuna. There is also a separate form of *B. passiflorae* with paler abdomen. This is probably an undescribed new species which occurs in Fiji, Tuvalu, Tokelau and possibly the Niuas group in Tonga. Its host range and potential pest status have not yet been well studied (SPC 2006).

REMARKS

Bactrocera passiflorae is similar to *B. thistleoni* in possessing black postpronotal lobes, scutellum entirely yellow, scutum black with lateral and medial postsutural vittae absent but differs in having facial spots absent and legs entirely fulvous (Drew 1989).

PEST STATUS

- Exotic
- High priority pest identified in the Avocado and Tropical Fruit IBPs

ATTRACTANT

Cue lure.

FIGURES

Figure 58. *Bactrocera passiflorae*

Image courtesy of the Secretariat of the Pacific Community Pacific Fruit Fly Web, www.spc.int/pacifly (as of 22 August 2011)
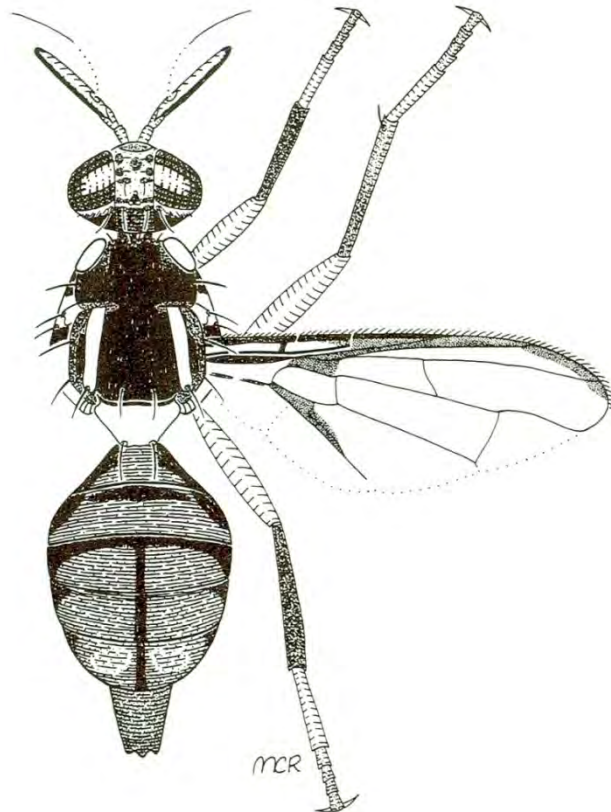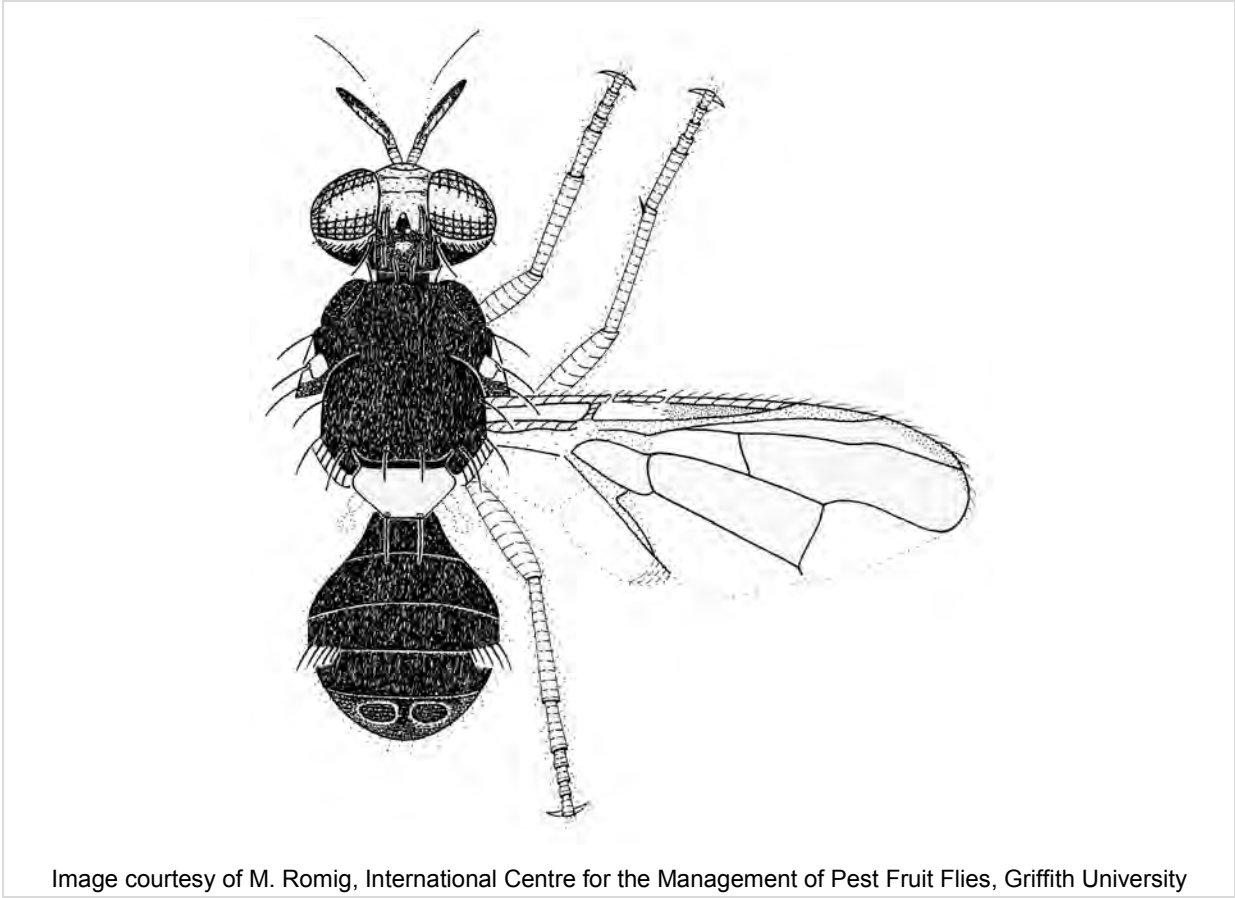
Figure 59. *Bactrocera passiflorae*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.26 *Bactrocera* (*Bactrocera*) *philippinensis* Drew and Hancock

TAXONOMIC INFORMATION

Common name: Philippine fruit fly

Previous scientific names:

DIAGNOSIS

7.3.26.1.1 *Morphological - Adult*

Face with a pair of large oval black spots; postpronotal lobes and notopleura yellow; scutum black; mesopleural stripes reaching midway between anterior margin of notopleura and anterior notopleural setae dorsally; two broad parallel sided lateral postsutural vittae ending at or behind *ia.* seate; scutellum yellow; legs with femora generally fulvous except for a small elongate dark fuscous spot on outer apical surfaces of fore femora in occasional specimens, all tibiae dark fuscous (mid tibiae paler apically); wings with cells bc and c colourless and microtrichia in outer corner of c only; costal band slightly overlapping R_{2+3} and usually expanding in a fish hook pattern on apex of R_{4+5}; cubital streak narrow; abdominal terga III-V with a black 'T' and small dark fuscous to black anterolateral corners on terga IV and V; the medial longitudinal black band is narrow to medium width; posterior lobe of male surstylus short; ovipositor with aculeus long (1.6 – 2.1mm) and needle shaped (pers. comm. Drew 2010).

7.3.26.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.26.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	630, 250	*Sna*BI:	530, 320
*Hha*I:	650, 190	*Ssp*I:	750
*Hinf*I:	770	*Vsp*I:	DNC

*Sau*3AI: DNC

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

The Philippines have not been the focus of a major fruit fly survey in the same manner as Malaysia and Thailand, and so the extent to which other fruit crops are attacked is uncertain (CABI 2007). *Bactrocera philippinensis* has been recorded on hosts from five families, Anacardiaceae, Caricaceae, Moraceae, Myrtaceae and Sapotaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood _et al._ 1999):

Scientific name	Common name	Scientific name	Common name
Carica papaya	papaw	_Mangifera indica_	mango
Citrus reticulata	mandarin	_Syzygium malaccense_	malay-apple

DISTRIBUTION

Bactrocera philippinensis has been recorded from the Philippines and Palau (pers. comm. Drew 2010).

REMARKS

Bactrocera philippinensis is similar to _B. carambolae_ and _B. papayae_ in possessing broad parallel sided lateral postsutural vittae, the costal band just overlapping R_{2+3}, some small areas of dark colour on lateral margins of abdominal terga III-V, femora mostly fulvous and tip of aculeus needle shaped.

It differs from _B. carambolae_ in having a narrower medial longitudinal band on abdominal terga III-V and a longer male aedeagus and female aculeus. It differs from _B. papayae_ in having a fish-hook barb pattern at the apex of the costal band and a longer male aedeagus and female aculeus (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Apple and Pear, Avocado, Banana, Citrus, Mango, Summerfruit, Tropical fruit and Vegetable IBPs

- _Bactrocera philippinensis_ is a very important pest of mango in the Philippines
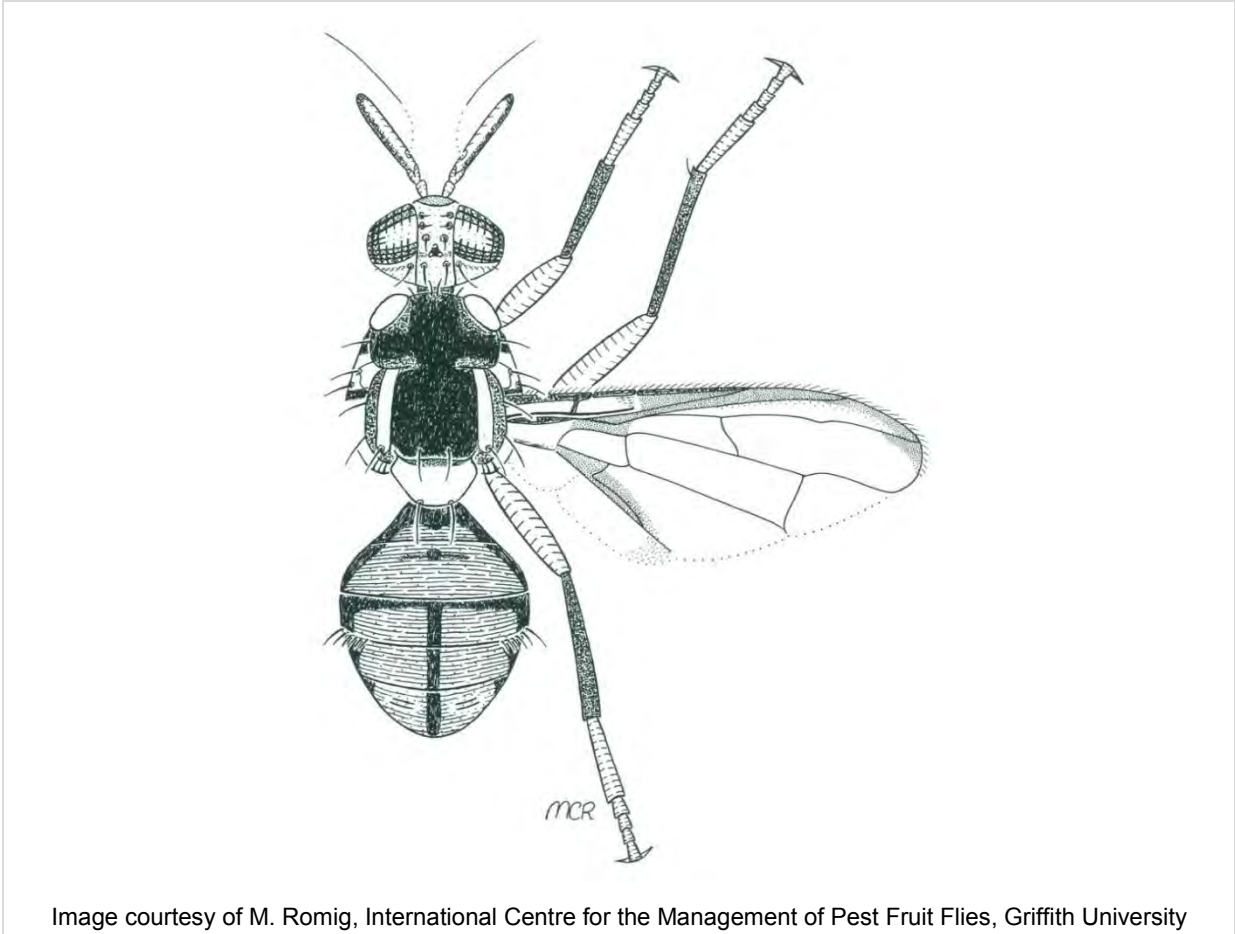
ATTRACTANT

Methyl eugenol.

FIGURES

Figure 60. _Bactrocera philippinensis_

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University

Figure 61. *Bactrocera philippinensis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.27   *Bactrocera* (*Bactrocera*) *psidii* (Froggatt)

TAXONOMIC INFORMATION

Common name: South sea guava fruit fly

Previous scientific names:

Tephritis psidii

Dacus psidii

Strumeta psidii

Dacus (*Strumeta*) *psidii*

DIAGNOSIS

7.3.27.1.1   Morphological - Adult

Medium sized species; generally small fuscous to dark fuscous facial spots present; postpronotal lobes yellow except anterodorsal corner black; notopleura yellow; scutum glossy black, mesopleural stripe equal in width to notopleuron, short lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow with a broad triangular black marking on dorsal surface; wing with a narrow tint of extremely pale fuscous colouration around costal margin and a narrow fulvous anal streak, a narrow tint of fuscous colouration around r-m and dm-cu crossveins, cells bc and c colourless to extremely pale fulvous with microtrichia in outer corner of cell c only; abdominal terga entirely glossy black; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.27.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.27.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 780 bp

*Bsr*1:	DNC	*Sna*BI:	DNC
*Hha*I:	640, 190	*Ssp*1:	200, 550
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera psidii has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Caricaceae, Combretaceae, Ebenaceae, Euphorbiaceae, Malpighiaceae, Moraceae, Musaceae, Myrtaceae, Oxalidaceae, Passifloraceae, Punicaceae, Rosaceae, Rutaceae and Vitaceae (for a full list of recorded hosts see SPC 2006).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Citrus sp.		*Psidium guajava*	guava
Mangifera indica	mango		

DISTRIBUTION

Restricted to New Caledonia (Drew 1989).

REMARKS

Bactrocera psidii is similar to *B. obliqua* in possessing infuscation on crossveins and the scutelum yellow with a broad black triangular marking on dorsal surface. It differs from this species in having the face fulvous with small pale spots in 75% of specimens, costal band narrow and not overlapping R_{2+3}, r-m crossvein shorter than dm-cu crossvein, infuscation around crossveins very narrow and pale, legs entirely fulvous, lateral postsutural vittae elongated and ending before *ia*. setae; posterior lobe of male surstylus short; female with apex of aculeus needle shaped. This species is unusual in having wing patterning very pale (including a mark along the r-m crossvein), scutellum marked with a large black triangle and the abdomen entirely dark (black or dark orange-brown) (Drew 1989; pers. comm. Drew 2010).

PEST STATUS

- Exotic
- *Bactrocera psidii* is a major pest

ATTRACTANT

Cue lure, Willison's lure.

Figure 62. *Bactrocera psidii*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
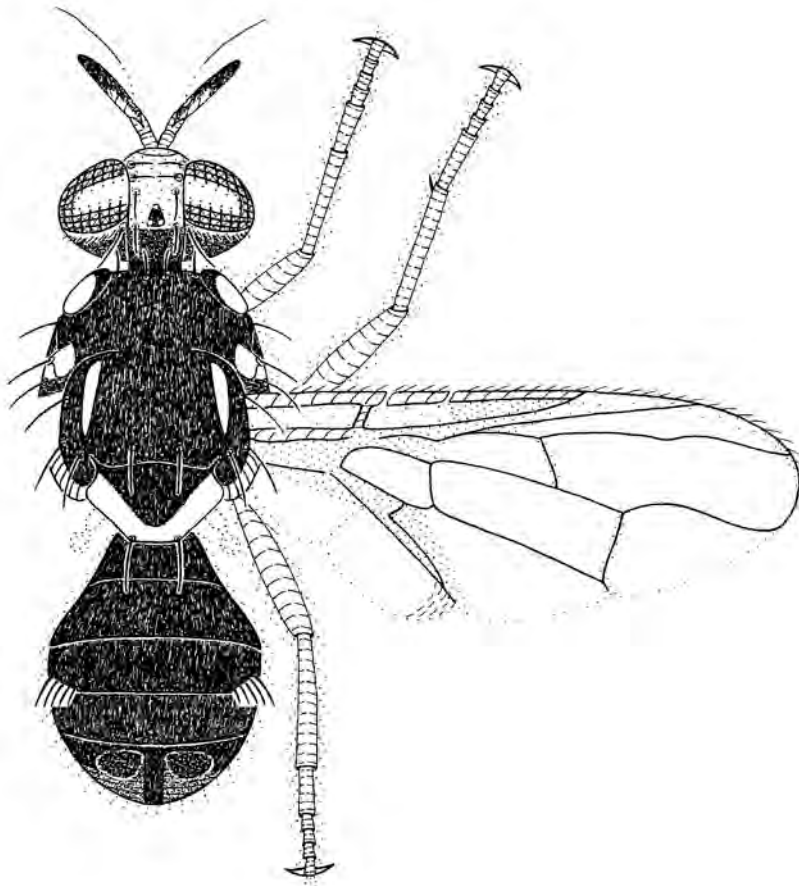
Figure 63. *Bactrocera psidii*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.28   *Bactrocera* (*Zeugodacus*) *tau* (Walker)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dasyneura tau

Dacus (*Zeugodacus*) *tau*

Bactrocera (*Zeugodacus*) *tau*

DIAGNOSIS

7.3.28.1.1   Morphological - Adult

A medium sized species; face fulvous with a pair of medium sized circular to oval black spots; postpronotal lobes and notopleura yellow; scutum black with large areas of red-brown centrally and anterocentrally; lateral and medial postsutural vittae present; yellow spot anterior to mesonotal suture in front of lateral postsutural vittae; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta; scutellum entirely yellow; wing with a narrow dark fuscous costal band overlapping R_{2+3} and expanding into a distinct apical spot and broad dark fuscous anal streak; cells bc and c colourless; microtrichia in outer corner of cell c only; abdominal terga III-V fulvous with a black 'T' pattern and anterolateral corners of terga IV and V with broad black markings; posterior lobe of male surstylus short; female with apex of aculeus trilobed (pers. comm. Drew 2010).

7.3.28.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.28.1.3   Molecular

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera tau has been recorded on hosts from nine families. These include: Arecaceae, Curcurbitaceae, Fabaceae, Loganiaceae, Moraceae, Myrtaceae, Oleaceae, Sapotaceae and Vitaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Cucumis melo	melon	*Manilkara zapota*	sapodilla
Cucumis sativus	cucumber	*Momordica charantia*	bitter gourd
Cucurbita maxima	giant pumpkin	*Psidium guajava*	guava
Luffa acutangula	angled luffa		

DISTRIBUTION

India, Sri Lanka, Bhutan, Vietnam, Southern China, Taiwan, Thailand, Peninsular Malaysia, Singapore, East Malaysia and Indonesian provinces (pers. comm. Drew 2010).

REMARKS

Bactrocera tau is a very common species throughout southeast Asia. It is an economic pest species, mainly in cucurbit crops, but can be misidentified as it belongs to a complex of closely related species. The *tau*-complex includes *Zeugodacus* species with a black scutum, wings colourless except for a costal band and cubital streak, cells bc and c colourless or with an extremely pale tint, costal band overlapping R_{2+3} and expanding into a distinct spot at apex. *Bactrocera tau* is distinct in having an entirely yellow scutellum, abdominal terga III-V with a distinct dark 'T' pattern and all femora with dark preapical spots (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- A major pest of cucurbit crops

ATTRACTANT

Cue lure.

FIGURES

Figure 64. *Bactrocera tau*

Image courtesy of Ken Walker, Museum Victoria, www.padil.gov.au (as of 22 August 2011)

Figure 65. *Bactrocera tau*
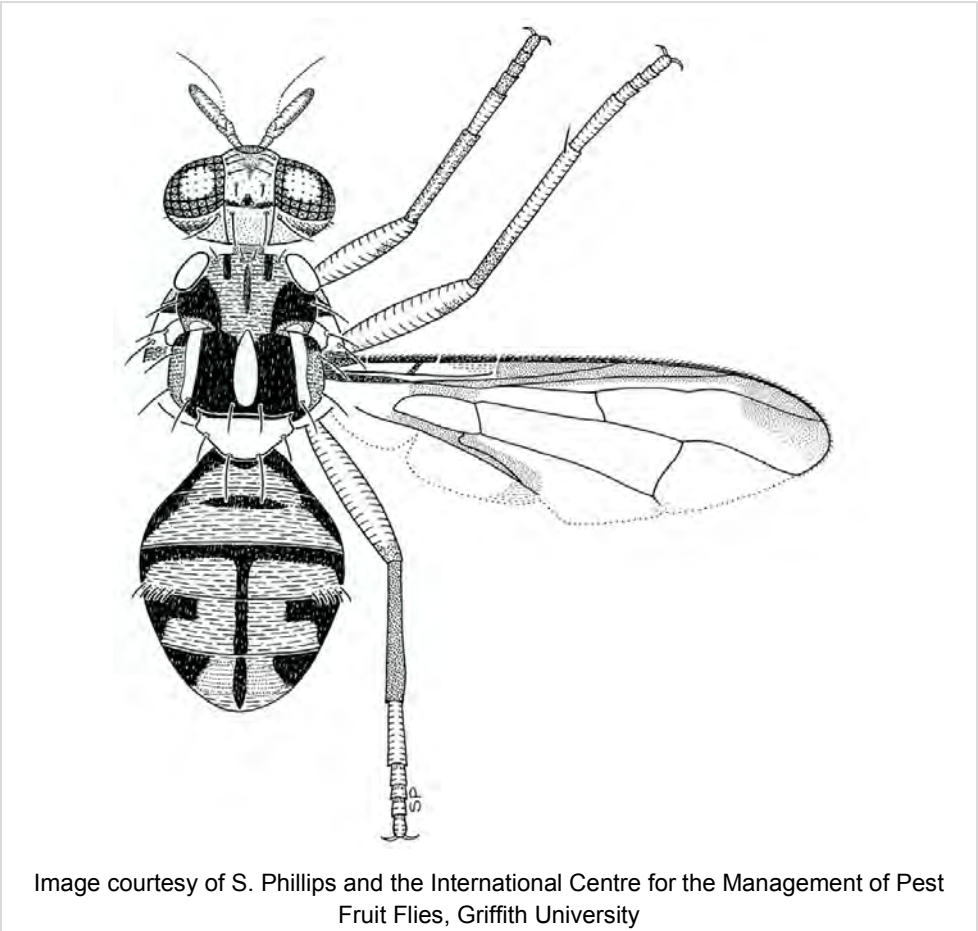

Figure 66. *Bactrocera tau*

Image courtesy of S. Phillips and the International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.29　*Bactrocera* (*Bactrocera*) *trilineola* Drew

Common name:

Previous scientific names:

Dacus (*Strumeta*) *triseriatus*

DIAGNOSIS

7.3.29.1.1　*Morphological - Adult*

Medium sized species; face entirely glossy black; postpronotal lobes fuscous to black; notopleura yellow; scutum glossy black; mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral and medial postsutural vittae absent, scutellum glossy black with lateral margins yellow; wing with a narrow extremely pale fuscous costal vein and broad fuscous anal streak, a narrow fuscous transverse band across wing, cells bc and c extremely pale fuscous, microtichia covering outer ½ of cell c only; abdominal terga mostly glossy black except for two broad longitudinal fulvous bands on terga II-V either side of a broad medial longitudinal glossy black band; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.29.1.2　*Morphological - Larvae*

- Not available/included in this edition -

7.3.29.1.3　*Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera trilineola has been recorded on hosts from a range of families. These include: Anacardiaceae, Annonaceae, Caricaceae, Caesalpinaceae, Combretaceae, Lauraceae, Moraceae, Musaceae, Myrtaceae, Oxalidaceae, Rutaceae and Sapindaceae (for a full list of recorded hosts see SPC 2006).

Major commercial hosts:

Scientific name	Common name
Mangifera indica	mango

DISTRIBUTION

Restricted to Vanuatu where it is common over nearly every island (Drew 1989).

REMARKS

Bactrocera trilineola belongs to the *frauenfeldi* complex. It differs from *B. caledoniesis* and *B. frauenfeldi* in possessing a glossy black face and in lacking lateral postsutural vittae and from *B. parafrauenfeldi* in having a glossy black face, cells bc and c extremely pale fuscous, microtrichia in outer ½ of cell c only, costal band present but very pale beyond subcostal cell and legs fulvous except apical 1/3 of hind femora and hind tibiae fuscous. The apex of piercer and the spicules on the middle segment of the ovipositor are similar in *B. frauenfeldi* and *B. trilineola*, however the apex of the aculeus is slightly more pointed in *B. trilineola*.

- Exotic

ATTRACTANT

Cue lure, Willison's lure.

FIGURES

Figure 67. *Bactrocera trilineola*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
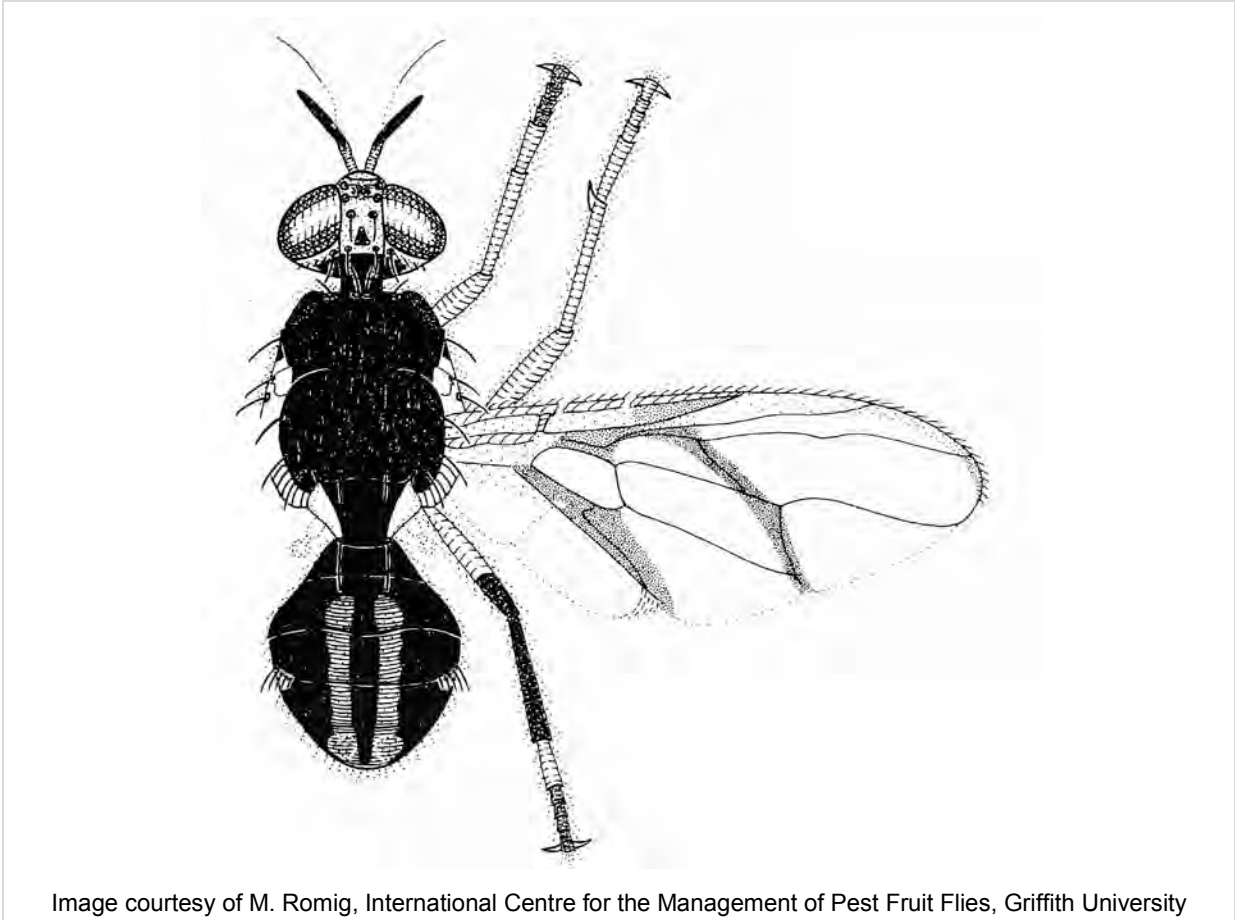
Figure 68. *Bactrocera trilineola*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.30   *Bactrocera* (*Bactrocera*) *trivialis* (Drew)

TAXONOMIC INFORMATION

Common name:

Previous scientific names:

Dacus (*Strumeta*) *trivialis*

DIAGNOSIS

7.3.30.1.1   *Morphological - Adult*

Medium sized species; medium sized pear shaped facial spots present; postpronotal lobes and notopleura yellow; scutum black, mesopleural stripe ending midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and anal streak, cells bc and c colourless, microtrichia in outer corner of cell c only; males with all leg segments fulvous except hind tibiae fuscous, females with dark colour patterns on femora and tibiae; abdominal terga III-V generally black with a medial longitudinal fulvous area from posterior margin of tergum III to tergum V; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.30.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.30.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera trivialis has been recorded on hosts from seven families. These include: Anacardiaceae, Combretaceae, Euphorbiaceae, Myrtaceae, Rosaceae, Rutaceae, Santalaceae and Solanaceae (for a full list of recorded hosts see SPC 2006).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Capsicum frutescens	chilli	*Prunus persica*	peach
Citrus x paradisi	grapefruit	*Psidium guajava*	guava

DISTRIBUTION

Mainland Papua New Guinea (less common in the Highlands than at low elevations), Indonesia (Irian Jaya) (Drew 1989).

Although *Bactrocera trivialis* is not established in the Torres Strait Islands, occasional incursions do occur. They are promptly eradicated.

REMARKS

A large collection of specimens reared from grapefruit at Mt. Hagen, 1980, 1981, show sexual dimorphism in leg colour patterns: females possess fore, mid and apical 1/3 of hind femora dark

fuscous, fore tibiae and apical four segments of fore tarsi fuscous, hind tibiae dark fuscous; males have all segments fulvous except hind tibiae fuscous.

It is similar to *B. cacuminata*, *B. nigrescens* and *B. opliae* (*dorsalis* complex) in having colourless cells bc and c and the mesopleural stripe reaching midway between the anterior margin of notoplueron and anterior *npl.* seta. It differs from *B. cacuminata* and *B. opiliae* in having an entirely black scutum and from *B. nigrescens* in having abdominal terga III-V mostly dark fuscous to black except orange-brown postercentrally on tergum III and centrally on terga IV and V; posterior lobe of male surstylus short; female with aculeus tip needle shaped (Drew 1989).

B. trivialis can appear similar to *B. rufofuscula,* an endemic north Queensland rainforest species, which is also trapped in cue traps. However *B. trivialis* has a black scutum.

Other remarks:

Bactrocera trivialis is similar to *B. laticosta* in having medium to broad lateral postsutural vittae, abdominal tergum III either entirely dark across tergum or with broad lateral bands, and terga IV and V with broad lateral longitudinal dark bands. It differs from this species in having a narrow medial longitudinal dark band (sometimes absent) and costal band confluent with R_{2+3} (Lawson *et al.* 2003).

PEST STATUS

- High priority pest identified in the Tropical fruit IBP

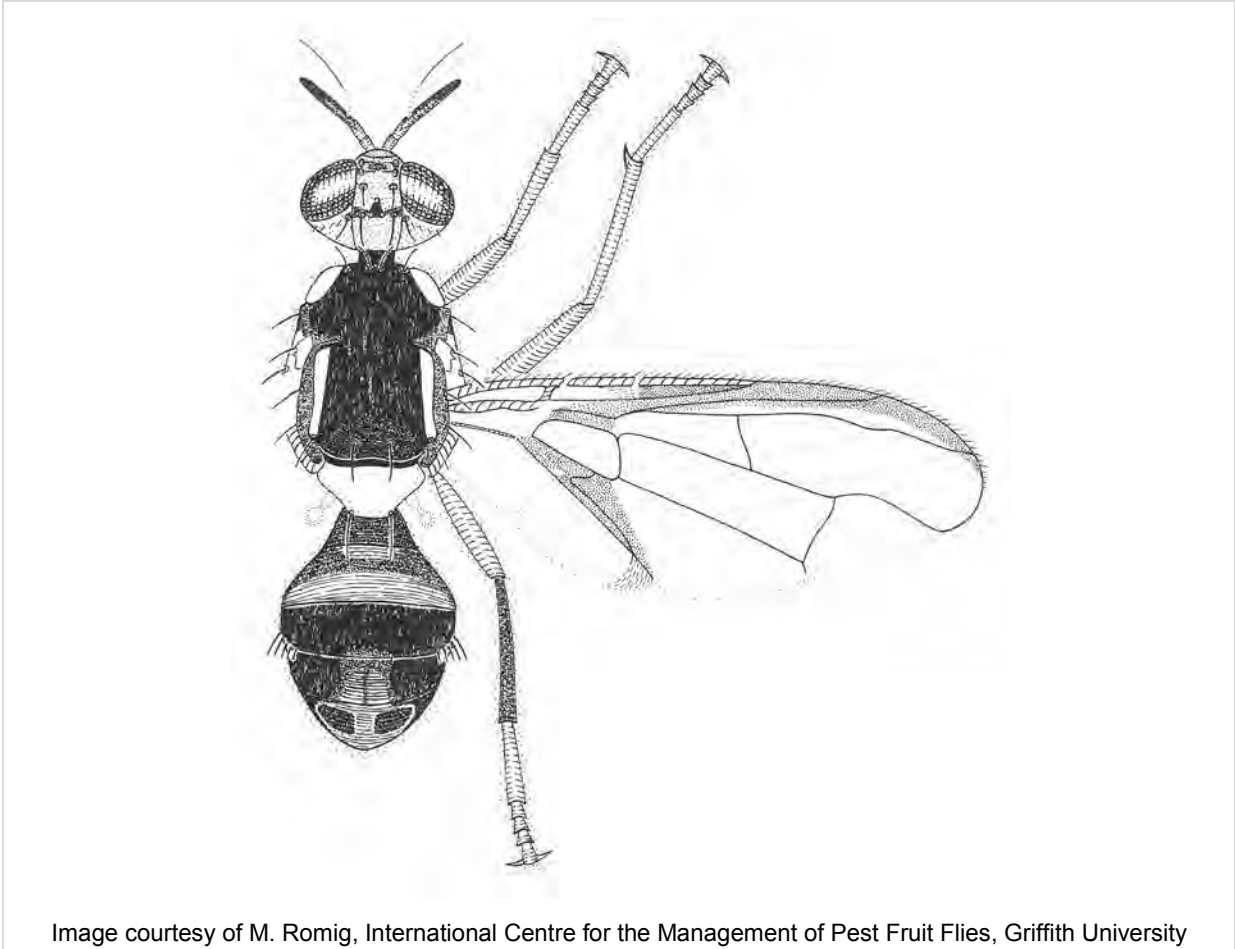## *ATTRACTANT*

Cue lure.

FIGURES

Figure 69. *Bactrocera trivialis*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 70. *Bactrocera trivialis*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.31   *Bactrocera* (*Bactrocera*) *tryoni* (Froggatt)

TAXONOMIC INFORMATION

Common name: Queensland fruit fly

Previous scientific names:

Tephritis tryoni

Dacus tryoni

Chaetodacus tryoni

Chaetodacus tryoni var. *juglandis*

Chaetodacus tryoni var. *sarcocephali*

Dacus (*Strumeta*) *tryoni*

Strumeta tryoni

Dacus (*Bactrocera*) *tryoni*

DIAGNOSIS

7.3.31.1.1   *Morphological - Adult*

Medium sized species; medium sized black facial spots present; postpronotal lobes and notopleura yellow; scutum red-brown with fuscous markings, mesopleural stripe reaching midway between anterior margin of notopleuron and anterior *npl.* seta, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a narrow fuscous costal band and broad fuscous anal streak, cells bc and c fuscous, microtrichia covering cell c and outer ½ of cell bc; abdominal terga III-V generally red-brown with a medial and two broad lateral longitudinal fuscous bands over all three terga and joined along anterior margin of tergum III; paler forms of the abdomen are often present; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.31.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.31.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Test 1, Section 6.3.1):

Approximate ITS1 Frag length - gel: 820 bp

*Bsr*I:	200, 600	*Sna*BI:	DNC
*Hha*I:	640, 190	*Ssp*I:	180, 570
*Hinf*I:	770	*Vsp*I:	DNC
*Sau*3AI:	420		

PCR - Restriction Fragment Length Polymorphism (Test 2, 6.3.1):

*Alu*I	780-770, 240-230*, 170, 130 120 110
*Dde*I	1000-980*, 270, 220, 170-160
*Rsa*I	530-500*, 460-440*, 410, 290
*Ssp*I	1000, 550, 100

See also **PCR-DNA barcoding** (Section 6.3.2) and **Allozyme Electrophoresis** (Section 6.4).

HOST RANGE

Bactrocera tryoni has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Cactaceae, Capparaceae, Caricaceae, Celastraceae, Clusiaceae, Combretaceae, Curcurbitaceae, Cunoniaceae, Davidsoniaceae, Ebenaceae, Elaeocarpaceae, Ericaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Goodeniaceae, Hippocraterceae, Juglandaceae, Lauraceae, Lecythidaceae, Loganiaceae, Malpighiaceae, Melastomataceae, Meliaceae, Moraceae, Musaceae, Myrtaceae, Oleaceae, Oxalidaceae, Passifloraceae, Punicaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Santalaceae, Sapindaceae, Sapotaceae, Smilacaceae, Solanaceae, Thymeliaceae, Tiliaceae, Verbenaceae, Vitaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major hosts (Hancock *et al.* 2000):

Scientific name	Common name	Scientific name	Common name
Anacardium occidentale	cashew	*Mangifera indica*	mango
Annona atemoya	atemoya	*Manikara zapota*	sapodilla
Annona glabra	pond apple	*Morus nigra*	mulberry
Annona muricata	soursop	*Passiflora edulis*	passionfruit
Annona reticula	bullock's heart	*Passiflora suberosa*	corky passionfruit
Averrhoa carambola	carambola	*Prunus persica*	peach
Capsicum annuum	capsicum	*Prunus persica* var. *nucipersia*	nectarine
Capsicum annuum	chilli	*Psidium cattleianum* (=*littorale*)	cherry guava
Carica papaya	papaya	*Psidium guajava*	guava
Casimiroa edulis	white sapote	*Solanum lycopersicum*	tomato
Chryosphyllum cainito	star apple	*Syzygium aqueum*	water apple
Coffea arabica	coffee	*Syzygium forte* ssp. *forte*	white apple
Eugenia uniflora	Brazilian cherry	*Syzygium jambos*	wax jambu
Eriobotrya japonica	loquat	*Syzygium malacense*	Malay apple
Fortunella japonica	kumquat	*Syzygium suborbiculare*	red bush apple
Malus sylvestris	apple	*Syzygium tierneyanum*	river cherry

DISTRIBUTION

Occurs in large populations throughout eastern Australia from Cape York (Queensland) to East Gippsland (Victoria). It is also established in New Caledonia, Austral Islands, many islands of the

society group, and has been eradicated from Easter Island (Drew *et al.* 1982). Despite three specimens being recorded from Papua New Guinea, it is most doubtful that this species is established there (Drew 1989). A review of the past and present distribution of *Bactrocera tryoni* in Australia is currently in press (Dominiak and Daniels 2011).

REMARKS

Bactrocera tryoni is similar to *B. aquilonis* (*tryoni* complex) in the general patterns of the wing, thorax and abdomen but *Bactrocera tryoni* differs in having dark fuscous patterns on the scutum and the abdomen. In *B. aquilonis* the scutum and abdomen are generally pale red-brown (pers. comm. Drew 2010). These species can also be separated on the differences on the ovipositors: apex of aculeus rounded and spicules with 7-10 uniform dentations in *B. tryoni* compared with the more pointed aculeus and uneven dentations in *B. aquilonis* (Drew 1989). However, these differences are not easily observed (Cameron *et al.* 2010).

PEST STATUS

- Endemic
- *Bactrocera tryoni* is the major fruit fly pest species in eastern Australia and is the target of major control and quarantine programmes

ATTRACTANT

Cue lure or a mixture of methyl eugenol and cue lure are effective at attracting *Bactrocera tryoni* (Dominiak *et al.* 2011). *Bactrocera tryoni* is also attracted to wet food lures such as protein and citrus juice although these lures are less effective (Dominiak *et al.* 2003; Dominiak and Nicol 2010).

FIGURES

Figure 71. *Bactrocera tryoni*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University

Figure 72. *Bactrocera tryoni*

Image courtesy of the International Centre for the Management of Pest Fruit Flies, Griffith University
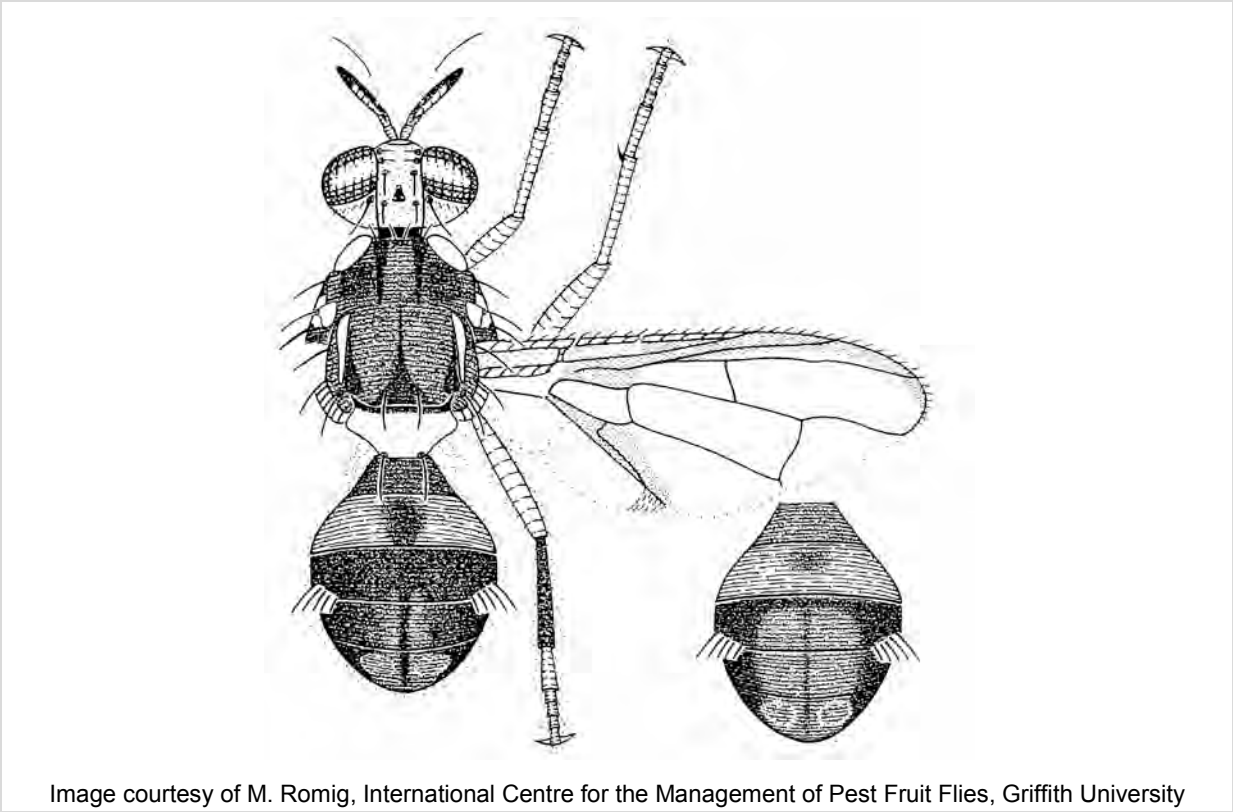
Figure 73. *Bactrocera tryoni*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.32   *Bactrocera* (*Bactrocera*) *umbrosa* (Fabricius)

TAXONOMIC INFORMATION

Common name: Breadfruit fruit fly

Previous scientific names:

Dacus umbrosus

Strumeta umbrosa

Dacus (*Strumeta*) *umbrosus*

Dacus (*Bactrocera*) *umbrosus*

DIAGNOSIS

7.3.32.1.1   Morphological - Adult

Medium sized species; medium sized black facial spots present; postpronotal lobes and notopleura yellow; scutum black, mesopleural stripe reaching to postpronotal lobe, lateral postsutural vittae present, medial postsutural vitta absent, scutellum yellow; wing with a broad fuscous costal band and anal streak, three transverse reddish-fuscous bands across wing with the basal one joining with the anal streak, cells bc and c fulvous with microtrichia in outer ½ of cell c only; abdominal terga varying from orange-brown with a medial longitudinal black stripe on terga IV and V to orange-brown with a broad medial and two broad longitudinal black bands over terga III-V; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.32.1.2   Morphological - Larvae

- Not available/included in this edition -

7.3.32.1.3   Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 780 bp

*Bsr*I: DNC   *Sna*BI: DNC

*Hha*I: 600, 190   *Ssp*I: 680

*Hinf*I: 730   *Vsp*I: DNC

*Sau*3AI: 380

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera umbrosa has been recorded on hosts from only the family Moraceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Artocarpus altilis	breadfruit	*Artocarpus heterophyllus*	jackfruit

DISTRIBUTION

Widespread and very common in Malaysia, southern Thailand, Philippines, Indonesia, Palau, Papua New Guinea (much less common in the Highlands), Solomon Islands, Vanuatu and New Caledonia (pers. comm. Drew 2010).

REMARKS

Bactrocera umbrosa bears no close resemblance to other species. It is easily recognised by the three broad transverse bands across the wings which are red-brown, not the usual fuscous colour (Drew 1989).

PEST STATUS

- Exotic
- Major pest of *Artocarpus* species
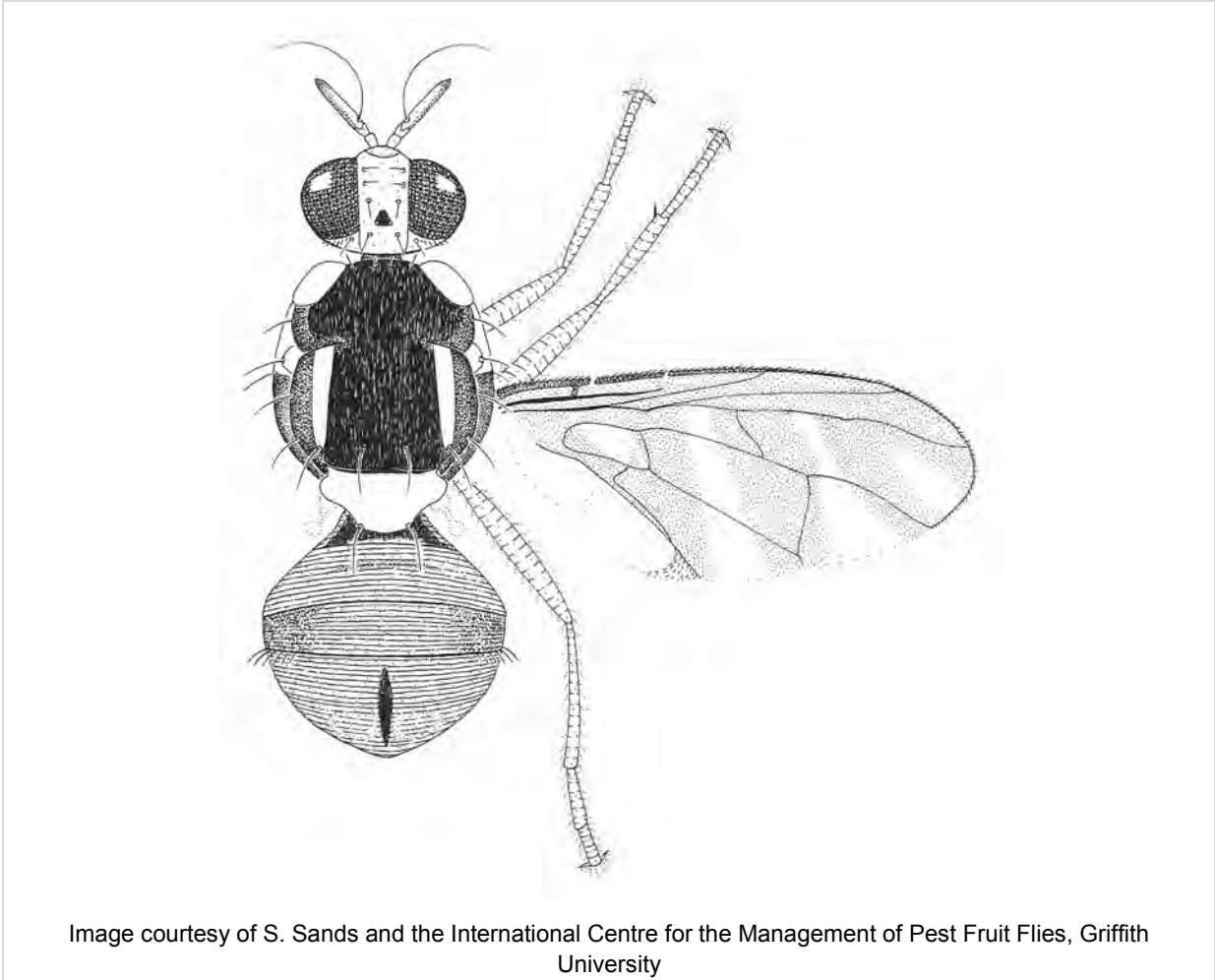
ATTRACTANT

Methyl eugenol.

FIGURES

Figure 74. *Bactrocera umbrosa*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum

Figure 75. *Bactrocera umbrosa*

Image courtesy of S. Sands and the International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.33   *Bactrocera* (*Notodacus*) *xanthodes* (Broun)

TAXONOMIC INFORMATION

Common name: Pacific fruit fly

Previous scientific names:

Tephrites (*Dacus*) *xanthodes*

Dacus (*Tephrites*) *xanthodes*

Chaetodacus xanthodes

Dacus xanthodes

Notodacus xanthodes

Dacus (*Notodacus*) *xanthodes*

DIAGNOSIS

7.3.33.1.1   *Morphological - Adult*

Medium sized species; small black facial spots present; postpronotal lobes fulvous except for a broad yellow band on posterior 2/3; notopleura orange-brown; scutum transparent with a shining orange-brown colouration and with irregular dark markings, broad lateral yellow band running from postpronotal lobe to end just before anterior end of lateral postsutural vitta, large yellow spot on pleural region in place of the normal mesopleural stripe, lateral postsutural vittae present and beginning anterior to mesonotal suture, medial postsutural vitta present, scutellum orange-brown with lateral yellow margins, wing with a narrow fuscous costal band and a broad fulvous anal streak, cells bc and c extremely pale fulvous with microtrichia in outer corner of cell c only, abdominal terga transparent and shining orange-brown with no dark markings; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (Drew 1989; pers. comm. Drew 2010).

7.3.33.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.3.33.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 860 bp

*Bsr*I:	DNC	*Sna*BI: DNC	
*Hha*I:	670, 200	*Ssp*I:	380, 250
*Hinf*I:	680	*Vsp*I:	DNC
*Sau*3AI: DNC			

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera xanthodes has been recorded on hosts from a range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Caricaceae, Combretaceae, Euphorbiaceae, Lauraceae, Lecythidaceae, Moraceae, Passifloraceae, Rutaceae and Sapotaceae (for a full list of recorded hosts see SPC 2006).

Major commercial hosts (Drew 1989):

Scientific name	Common name	Scientific name	Common name
Artocarpus altilis	breadfruit	*Carica papaya*	pawpaw

DISTRIBUTION

Fiji Islands, Tonga, Niue, Samoa, American Samoa, Southern group of Cook Islands, Wallis and Futuna. Introduced on Nauru (first detected in 1992) but subsequently eradicated by male annihilation. Detected in April 1998 on Raivavae (French Polynesia) but subsequently eradicated by male annihilation (Drew 1989).

REMARKS

Bactrocera xanthodes is a unique species having a pair of well-developed postpronotal lobe setae, the transparent integument on the head, thorax and abdomen, a soft integument particularly noticeable on the abdomen where the terga fold ventrally in dead specimens (Drew 1989).

Other remarks:

Bactrocera xanthodes belongs to subgenus *Notodacus*, an unusual feature of which is the presence of a seta on each postpronotal lobe (i.e. shoulder). It has a very distinct V-shaped notch in the apex of its scutellum. *Bactrocera paraxanthodes* has this to a lesser extent. Another unusual feature of *B. xanthodes* is that the lateral stripes (vittae) on the scutum extend forward to the postpronotal lobes and back down the sides of the scutellum. There is also a medial yellow stripe that extends to the posterior edge of the scutum (immediately before the scutellum); this stripe is shorter in *B. paraxanthodes*. The most obvious difference between the closely related *B. paraxanthodes and B. xanthodes* is that *B. xanthodes* has yellow lateral margins to the scutellum while *B. paraxanthodes* has dark margins (CABI 2007).

PEST STATUS

- Exotic
- High priority pest identified in the Avocado and Tropical fruit IBPs

ATTRACTANT

Methyl eugenol.

Figure 76. *Bactrocera xanthodes*

Image courtesy of Mr. S. Wilson, the International Centre for the Management of Pest Fruit Flies, Griffith University and Queensland Museum
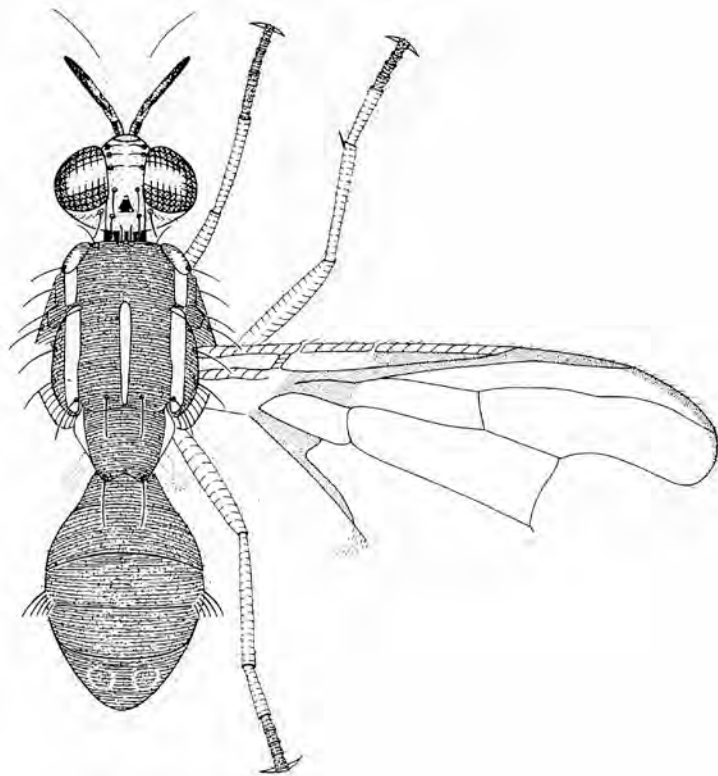
Figure 77. *Bactrocera xanthodes*

Image courtesy of M. Romig, International Centre for the Management of Pest Fruit Flies, Griffith University

7.3.34 *Bactrocera* (*Bactrocera*) *zonata* (Saunders)

TAXONOMIC INFORMATION

Common name: Peach fruit fly

Previous scientific names:

Dasyneura zonatus

Dacus (*Strumeta*) *zonatus*

Bactrocera (*Bactrocera*) *zonata*

DIAGNOSIS

7.3.34.1.1 *Morphological - Adult*

Face fulvous with a pair of medium sized oval black spots; scutum red-brown with pale fuscous patterning posteriorly; postpronotal lobes and notopleura yellow; mesopleural stripe reaching to or almost to anterior *npl.* seta dorsally; medium width parallel sided lateral postsutural vittae ending at or just behind *ia.* seta; medial postsutural vitta absent; scutellum yellow; legs with all segments entirely fulvous except apices of femora red-brown and hind tibiae pale fuscous to fuscous; wings with cells bc and c colourless and entirely devoid of microtrichia, a narrow fuscous costal band confluent with R_{2+3} and ending at apex of this vein, a small oval fuscous spot across apex of R_{4+5}, anal streak reduced to a pale tint within cell cup; supernumerary lobe of medium development; abdominal terga III-V red-brown with a 'T' pattern consisting of a narrow transverse black band across anterior margin of tergum III (this band is often broken in the central region) and a narrow medial longitudinal black band over all three terga (this band is often reduced to a stripe over parts of terga IV and V), narrow anterolateral fuscous corners on terga IV and V, a pair of oval red-brown shining spots on tergum V; posterior lobe of male surstylus short; female with apex of aculeus needle shaped (pers. comm. Drew 2010).

7.3.34.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.3.34.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 850 bp

*Bsr*I:	600, 200	*Sna*BI:	535, 330
*Hha*I:	680, 190	*Ssp*I:	750, 120
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Bactrocera zonata has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Arecaceae, Caricaceae, Combretaceae, Curcurbitaceae, Fabaceae, Lecythidaceae, Malpighiaceae, Malvaceae, Myrtaceae, Punicaceae, Rosaceae, Rutaceae and Tiliaceae (for a full list of recorded hosts see Allwood *et al.* 1999).

Major commercial hosts (Allwood *et al.* 1999):

Scientific name	Common name	Scientific name	Common name
Mangifera indica	mango	*Psidium guajava*	guava
Prunus persica	peach		

DISTRIBUTION

Sri Lanka, India, Pakistan, Thailand, Vietnam, Mauritius and Egypt (pers. comm. Drew 2010).

REMARKS

Bactrocera zonata is a red brown species that is similar in general appearance to *B. tryoni*. It is easily distinguished from *B. tryoni* in having the costal band interrupted beyond apex of R_{2+3}. *Bactrocera correcta* possess a similar costal band but has a black scutum and a black 'T' pattern on abdominal terga III-V (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Tropical fruit IBP

- In India, Pakistan and now Egypt, it is an important fruit fly pest and causes severe damage to peach, guava and mango

ATTRACTANT
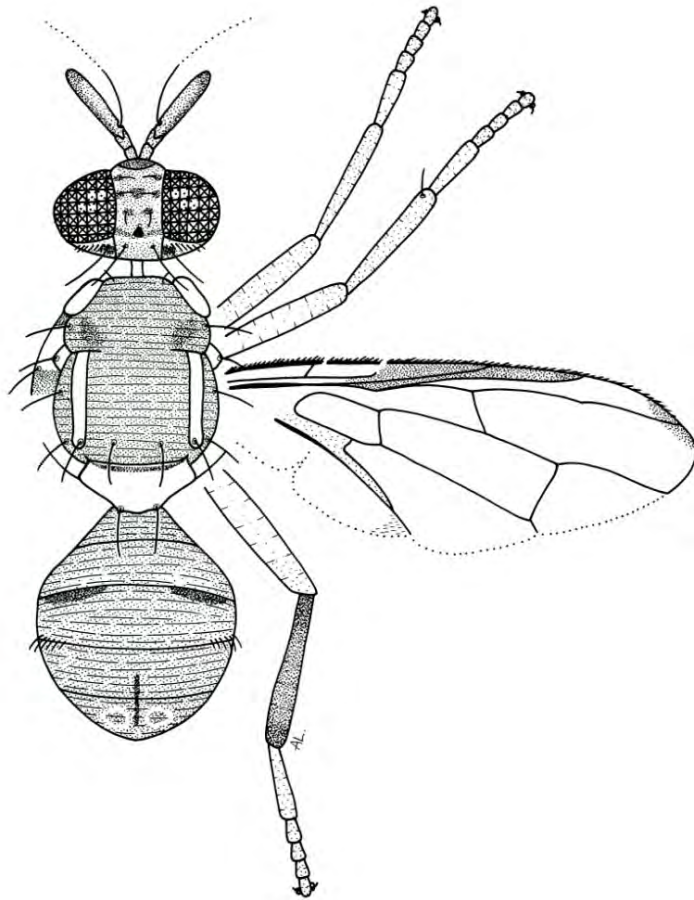
Methyl eugenol.

Figure 78. *Bactrocera zonata*

Image courtesy of A. Carmichael and the International Centre for the Management of Pest Fruit Flies, Griffith University

7.4 *Ceratitis*

7.4.1 *Ceratitis capitata* (Wiedemann)

TAXONOMIC INFORMATION

Common name: Mediterranean fruit fly

Previous scientific names:

Trypeta capitata

DIAGNOSIS

7.4.1.1.1 *Morphological - Adult*

In Australia, there are no species of *Ceratitis* that look similar to *C. capitata*. Consequently, the following characters can be used to distinguish *Ceratitis capitata* from all other species of Tephritidae occurring in Australia. Small to medium-sized, brightly coloured flies; scutellum swollen, rounded above, shiny black with a thin sinuate yellow streak near base dorsally; scutum yellowish with numerous black areas in a characteristic pattern; abdomen yellowish with two narrow transverse light-coloured bands; wing relatively broad in comparison with its length, cloudy yellow, with three brown bands on apical two-thirds, all separated from each other, and smaller dark irregular-shaped streaks within the cells in the proximal half; cell cu*p* with its apical extension short; males with a black diamond-shaped expansion of the apex of the anterior orbital seta.

These characters also distinguish *C. capitata* from all other species in the genus wherever they may occur worldwide. Several species of the subgenus *Ceratitis* closely resemble *C. capitata* in the thoracic pattern, the apical expansion of cell cu*p*, the presence of dark markings in the basal half of the wing, and in having the anterior orbital bristle of the male modified in some way. In *C. capitata*, it is black and resembles a diamond apically rather than some other shape (Foote, Blanc and Norrbom 1993).

7.4.1.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.4.1.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Test 1, Section 6.3.1):

Approximate ITS1 Frag length - gel: 900 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	520, 160
*Hinf*I:	DNC	*Vsp*I:	650, 200
*Sau*3AI:	DNC		

PCR - Restriction Fragment Length Polymorphism (Test 2, Section 6.3.1):

*Alu*I	1300, 130, 120, 110
*Dde*I	1150, 270, 220,130
*Rsa*I	450, 380, 290, 260, 240, 210
*Ssp*I	1020, 520, 100

See also **PCR-DNA barcoding** (Section 6.3.2) and **Allozyme Electrophoresis** (Section 6.4).

HOST RANGE

Ceratitis capitata is a highly polyphagous species and its pattern of host relationships from region to region appears to relate largely to what fruits are available (CABI 2007). It has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Cactaceae, Caricaceae, Clusiaceae, Combretaceae, Ebenaceae, Juglandaceae, Lauraceae, Lythraceae, Malpighiaceae, Malvaceae, Muntingiceae, Myrtaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae, Santalaceae, Sapindaceae, Sapotaceae, Solanaceae and Vitaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name	Scientific name	Common name
Annona cherimola	cherimoya	*Malus domestica*	apple
Capsicum annuum	bell pepper	*Prunus* spp.	stone fruit
Citrus spp.	citrus	*Prunus salicina*	Japanese plum
Coffea spp.	coffee	*Psidium guajava*	guava
Ficus carica	fig	*Theobroma cacao*	cocoa

DISTRIBUTION

Native to Africa, has spread to the Mediterranean region, southern Europe and Middle east, Western Australia, Central and South America and Hawaii (PaDIL 2007). A review of the past and present distribution of *Ceratitis capitata* in Australia is currently in press (Dominiak and Daniels 2011).

REMARKS

The males of *Ceratitis capitata* are easily separated from all other members of the family by the black pointed expansion at the apex of the anterior pair of orbital setae. The females can be separated from most other species by the characteristic yellow wing pattern and the apical half of the scutellum being entirely black (White and Elson-Harris 1992).

PEST STATUS

- Endemic

- High priority pest identified in the Mango IBP

- *Ceratitis capitata* is an important pest in Africa and has spread to almost every other continent to become the single most important pest species in the family Tephritidae

- It is ecologically adapted to regions of Mediterranean climate and less of a problem in subtropical and tropical areas although it can still be damaging in elevated tropical regions.

ATTRACTANT

Trimedlure/capilure and terpinyl acetate.

Figure 79. *Ceratitis capitata*

Image courtesy of Scott Bauer, USDA Agricultural Research Service, Bugwood.org

Figure 80. *Ceratitis capitata*

Image courtesy of Ken Walker, Museum Victoria, www.padil.gov.au (as of 22 August 2011)

7.4.2　Ceratitis (*Pterandrus*) *rosa* Karsch

TAXONOMIC INFORMATION

Common name: Natal fruit fly

Previous scientific names:

Pterandrus rosa

DIAGNOSIS

7.4.2.1.1　Morphological - Adult

Head: Anterior pair of orbital setae not modified in any way.

Thorax: Scutellum marked black and yellow, with yellow lines or areas meeting margin, such that each apical scutellar seta is based in or adjacent to a yellow stripe; male mid-femora without stout ventral setae; mid-tibiae with rows of stout setae along the distal half of both the anterior and posterior edges giving a feathered appearance. Wing length 4-6 mm.

The males of most species of subgenus Pterandrus have rows of stout setae on both the anterior and posterior edges of each mid-tibia, giving a feathered appearance. *Ceratitis rosa* can be separated from most other members of this subgenus by having this feathering confined to the distal half of the tibia and by lacking stout setae on the underside of the mid-femur. The males also lack the spatulate head appendages of subgenus *Ceratitis*. Unfortunately there is no simple method of recognizing females, except that *Pterandrus* species tend to have brown wing bands and a generally brown body colour, which contrasts with the yellow markings of *C. capitata*.

7.4.2.1.2　Morphological - Larvae

- Not available/included in this edition -

7.4.2.1.3　Molecular

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 1020 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	570, 480
*Hinf*I:	800, 200	*Vsp*I:	600, 300
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Ceratitis rosa has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Apocynaceae, Caricaceae, Clusiaceae, Combretaceae, Lauraceae, Malvaceae, Moraceae, Myrtaceae, Oxalidaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Solanaceae, and Vitaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (UF & FDACS 2009; CABI 2007):

Scientific name	Common name	Scientific name	Common name
Coffea arabica	coffee	*Prunus persica*	peach
Citrus spp.	citrus	*Psidium* spp.	guava

DISTRIBUTION

Angola, Ethiopia, Kenya, Malawi, Mali, Mozambique, Nigeria, Republic of South Africa (KwaZulu Natal), Rwanda, Rhodesia, Swaziland, Tanzania, Uganda, Zaire, and the islands of Mauritius and Reunion (UF & FDACS 2009).

REMARKS

Ceratitis rosa is best recognised by its characteristic pattern of brown wing bands, the three black areas in the apical half of the scutellum, and by the male having feathering on the mid tibiae, but no feathering on the mid femora (White and Elson-Harris 1992). This fruit fly closely resembles the Mediterranean fruit fly in appearance. It averages slightly larger and has characteristic picture wings and dark black spots on the thorax. The arista of the antenna is plumose, while that of the *C. capitata* bears only short pubescence. The front of the male lacks the pair of conspicuous spatulate setae which is found on the male *C. capitata*. The mesothoracic tibiae of the males are clothed with dorsal and ventral brushes of elongated bluish-black scales, lacking in the *C. capitata*. The ovipositor sheath of the female is shorter than the width at its base. Length of the fly 4 to 5 mm (UF & FDACS 2009).

PEST STATUS

- Exotic

- High priority pest identified in the Mango IBP

- *Ceratitis rosa* is highly polyphagous and causes damage to a very wide range of unrelated fruit crops

ATTRACTANT

Trimedlure and terpinyl acetate.

Figure 81. *Ceratitis rosa*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

Figure 82. *Ceratitis rosa*
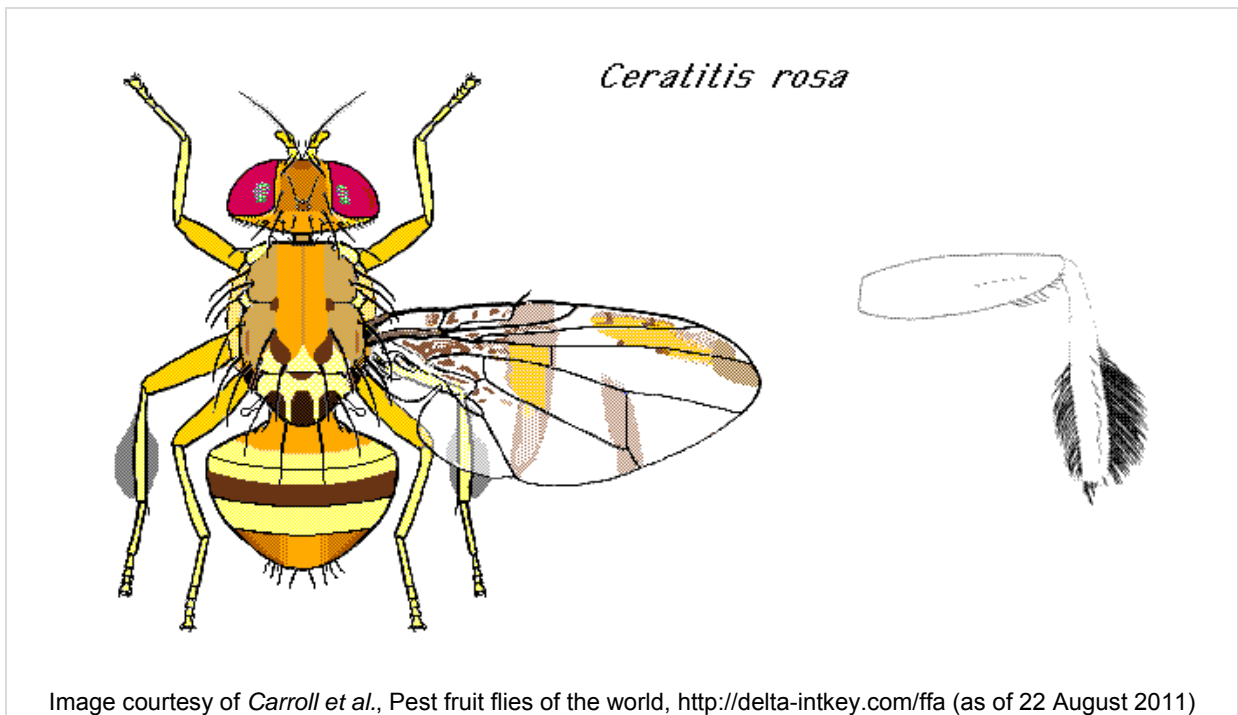
Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.5 *Dirioxa*

7.5.1 *Dirioxa pornia* (Walker)

TAXONOMIC INFORMATION

Common name: Island fly

Previous scientific names:

Trypeta pornia

DIAGNOSIS

7.5.1.1.1 *Morphological - Adult*

Head with arista plumose on dorsal surface, bare on ventral surface; thorax with scutum mostly red-brown, 6 scutellar setae; scutellum flat and bare of microsetae; legs with one strong apical spine on mid tibiae; wing pattern as per **Figure 83**; abdominal terga fulvous with transverse black patterns on terga III to V; male surstylus short and thick; female aculeus rounded and blunt at apex (pers. comm. Drew 2010).

7.5.1.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.5.1.1.3 *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 530 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	300, 220
*Hinf*I:	DNC	*Vsp*I:	DNC
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2) and **Allozyme Electrophoresis** (Section 6.4).

HOST RANGE

Dirioxa pornia attacks ripe, damaged and fallen fruit. It has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Araucariaceae, Capparaceae, Caricaceae, Clusiaceae, Combretaceae, Curcurbitaceae, Ebenaceae, Euphorbiaceae, Fabaceae, Lauraceae, Lecythidaceae, Loganiaceae, Moraceae, Musaceae, Myrtaceae, Oleaceae, Oxalidaceae, Passifloraceae, Proteaceae, Rosaceae, Rubiaceae, Rutaceae, Sapindaceae, Sapotaceae, Solanaceae and Xanthophyllaceae (for a full list of recorded hosts see Hancock *et al.* 2000).

Major hosts: No major host fruits have been identified but has created occasional quarantine problems.

DISTRIBUTION

Eastern Australia, from Iron Range, Cape York Peninsula, to southern New South Wales. Introduced to Perth, Western Australia. (Hancock *et al.* 2000). Also in Northern Victoria.

REMARKS

Dirioxa spp. are the only tephritids with six setae on the scutellar margin, that are likely to be found in fruit crops; the wing pattern is characteristic (White and Elson-Harris 1992).

PEST STATUS

- Endemic

ATTRACTANT

Protein and citrus juice (Dominiak *et al.* 2003).

FIGURES

Figure 83. *Dirioxa pornia*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6   *Anastrepha*

7.6.1   *Anastrepha fraterculus* (Wiedemann)

TAXONOMIC INFORMATION

Common name: South American fruit fly

Previous scientific names:

Dacus fraterculus

Trypeta fraterculus

Acrotoxa fraterculus

Trypeta (*Acrotoxa*) *fraterculus*

DIAGNOSIS

7.6.1.1.1   *Morphological - Adult*

Among all *Anastrepha* species found in the Americas, *A. fraterculus*, *A. obliqua* and *A. suspensa* present the most difficult identification problems in the genus; these three species are likely to be confused because of the similarity of their external features. Critical differences between *A. fraterculus* and *A. obliqua* are:

A.   *A. frateculus*:

   a.   Aculeus usually longer than the distance on vein M from the junction of MP and M to vein r-m.

   b.   Subscutellum darkened laterally

B.   *A. obliqua*:

   a.   Aculeus always shorter than the distance on vein M from the junction of MP and M to vein r-m.

   b.   Subscutellum not darkened laterally.

The apical arm of the S band of *A. fraterculus* is narrow compared with that of *A. suspensa*. There is frequently a distinct scutoscutellar black spot, but it is usually smaller than in *A. suspensa*. One of the most important distinguishing features is the nature of the aculeus tip, which has serrations only on its apical third in contrast to that of *A. obliqua* (Foote, Blanc and Norrbom 1993).

7.6.1.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.6.1.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Anastrepha fraterculus has been recorded on hosts from a wide range of families. These include: Actinidiaceae, Anacardiaceae, Annonaceae, Combretaceae, Ebenaceae, Fabaceae, Juglandaceae, Lauraceae, Lythraceae, Malvaceae, Moraceae, Myrtaceae, Oleaceae, Oxalidaceae, Rosaceae, Rubiaceae, Rutaceae, Sapotaceae and Vitaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (UF & FDACS 2009; CABI 2007):

Scientific name	Common name	Scientific name	Common name
Annona cherimola	cherimoya	*Prunus persica*	peach
Citrus spp.	citrus	*Psidium guajava*	guava
Eugenia uniflora	Surinam cherry	*Syzygium jambos*	rose apple

DISTRIBUTION

Northern Mexico south to northern Argentina, Trinidad; introduced to Galapagos Is.; occasionally trapped in USA (southern Texas), but not currently established (Carroll *et al.* 2002).

REMARKS

Anastrepha fraterculus is believed to belong to a group of closely related sibling species which, to date, have not been identified and described. In addition, it is very close to *A. obliqua* and *A. suspensa*. Consequently, *A. fraterculus* is difficult to diagnose and its exact area of distribution uncertain. It is regarded as a species of major economic importance (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Citrus IBP

- *Anastrepha fraterculus* is an important pest of guavas and mangoes, and also to some extent of *Citrus* spp. and *Prunus* spp.

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.

Figure 84. *Anastrepha fraterculus*

scutellum
subscutellum
mediotergite

Image courtesy of *Carroll et al.,* Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)
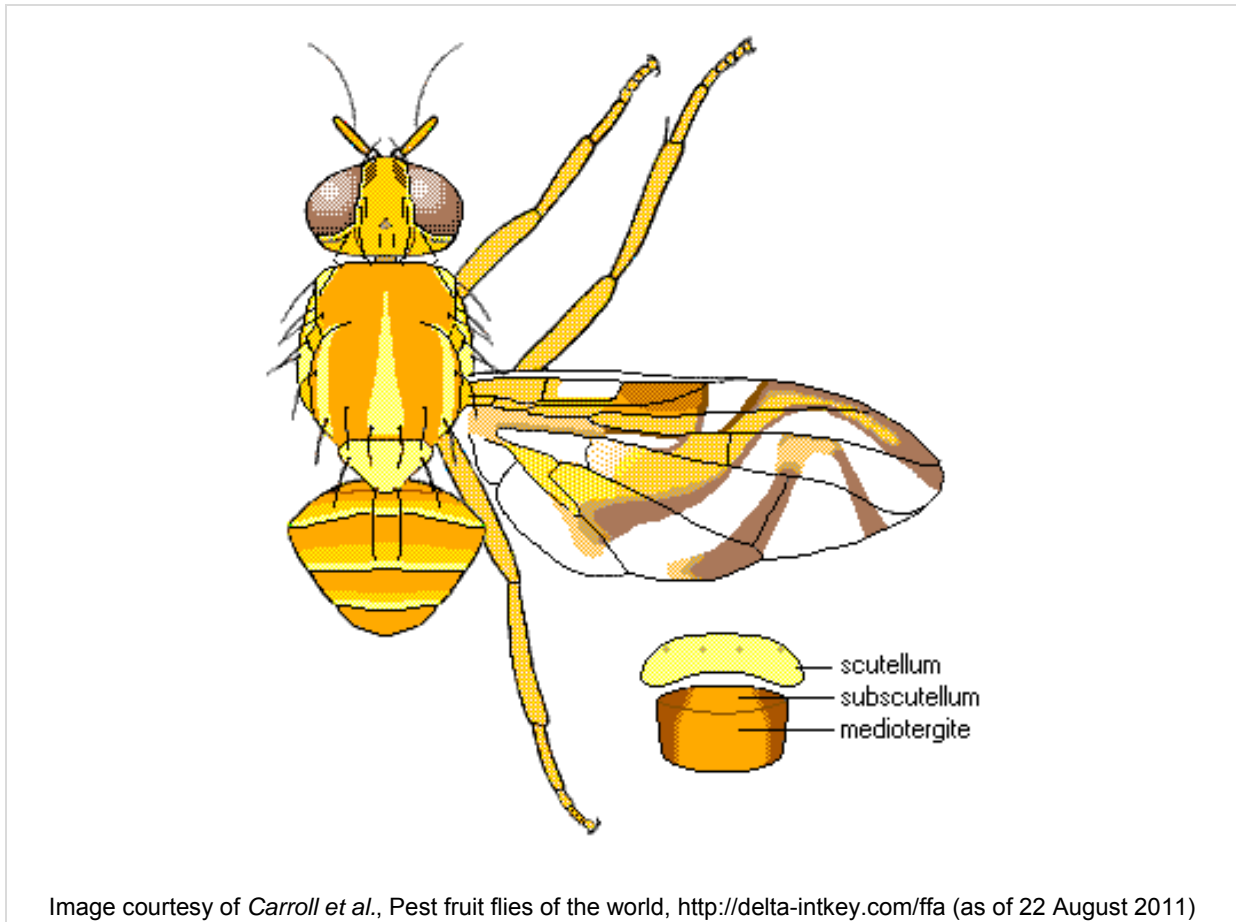
Figure 85. *Anastrepha fraterculus*

Image courtesy of *Carroll et al.,* Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6.2   *Anastrepha ludens* (Loew)

TAXONOMIC INFORMATION

Common name: Mexican fruit fly

Previous scientific names:

Trypeta ludens

Acrotoxa ludens

Trypeta (*Acrotoxa*) *ludens*

DIAGNOSIS

7.6.2.1.1   *Morphological - Adult*

Anastrepha ludens is characterized by a relatively long aculeus and oviscape, the former 3.4 - 4.7mm long and the latter correspondingly long and tapering in its apical third. This external character alone will alert the identifier to the possibility of *A. ludens*. The apical third of the aculeus tip is slightly expanded in the area of the lateral serrations, which are relatively few and not prominent. *Anastrepha suspensa* and *A. fraterculus* differ in having a much shorter aculeus and aculeus tip with more prominent lateral serrations and by other characters as well. *Anastrepha ludens* usually has a pair of lateral dark spots on the subcutellum which typically extend ventrally onto the mediotergite. The V band is usually not connected to the S band and is faint anteriorly in most specimens (Foote, Blanc and Norrbom 1993).

7.6.2.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.6.2.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 650 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	DNC
*Hinf*I:	550	*Vsp*I:	550
*Sau*3AI:	DNC		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Anastrepha ludens has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Caricaceae, Clusiaceae, Ebenaceae, Lauraceae, Lythraceae, Myrtaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae and Sapotaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (UF & FDACS 2009; CABI 2007):

Scientific name	Common name	Scientific name	Common name
Annona cherimola	cherimoya	*Mangifera indica*	mango
Citrus spp.	citrus	*Prunus persica*	peach

DISTRIBUTION

Texas, United States, south through Mexico to Costa Rica (Foote, Blanc and Norrbom 1993).

REMARKS

Anastrepha ludens is a well-defined and clearly distinct species, although there is a possibility of a separate but nearly indistinguishable form in the extreme southern part of its distribution in Costa Rica (UF & FDACS 2009).

PEST STATUS

- Exotic

- High priority pest identified in Citrus IBP

- *Anastrepha ludens* is serious pest of *Citrus* spp. and mangoes

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.

FIGURES

Figure 86. *Anastrepha ludens*
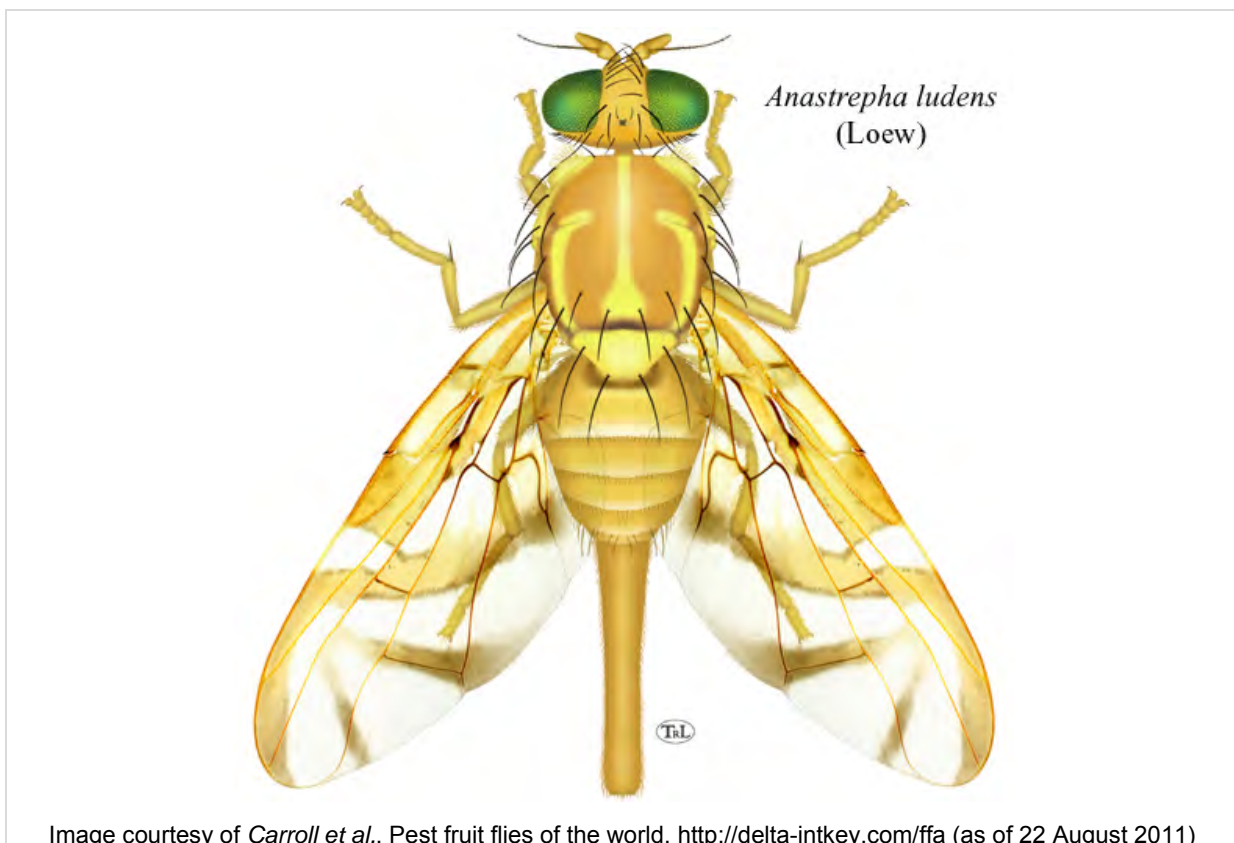
Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

Figure 87. *Anastrepha ludens*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6.3   *Anastrepha obliqua* (Macquart)

TAXONOMIC INFORMATION

Common name: West Indian fruit fly

Previous scientific names:

Tephritis obliqua

Anastrepha obliqua

DIAGNOSIS

7.6.3.1.1   *Morphological - Adult*

Externally, *Anastrepha obliqua* quite closely resembles *A. fraterculus* and *A. suspensa*, thereby presenting problems in their separation. However, a number of characters exist that appear to be critical in separating *A. obliqua* from *A. fraterculus*. The aculeus is subtly different from those of *A. fraterculus* and *A. suspensa*, having lateral serrations on more than two-thirds of the tip in contrast to those of the other species, where they are limited to the apical two-fifths to three-fifths of the tip. In *A. obliqua*, the tip also is relatively wider at the base of the serrations compared with the width at the genital opening. The white medial vitta on the scutum is wider in *A. obliqua* than in *A. suspensa* and *A. fraterculus*, and no scutoscutellar black spot or lateral dark marks on the subscutellum are present, although the mediotergite usually has a lateral dark stripe (Foote, Blanc and Norrbom 1993).

7.6.3.1.2   *Morphological - Larvae*

- Not available / included in this edition -

7.6.3.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 670 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	150, 550
*Hinf*I:	270, 450	*Vsp*I:	550
*Sau*3AI:	200, 450		

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Anastrepha obliqua has been recorded on hosts from a range of families. These include: Anacardiaceae, Ebenaceae, Malpighiaceae, Moraceae, Myrtaceae, Oxalidaceae, Passifloraceae, Rosaceae, Rubiaceae, Rutaceae and Sapotaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name	Scientific name	Common name
Mangifera indica	mango	*Spondias purpurea*	purple mombin

DISTRIBUTION

Throughout the greater and lesser Antilles, Jamaica, Trinidad, the Rio Grande Valley of Texas, Mexico to Panama, Venezuela, Ecuador, and the vicinity of Rio de Janeiro, Brazil (UF & FDACS 2009).

REMARKS

Anastrepha obliqua, along with *A. fraterculus* and *A. suspensa*, is best recognised by the wing colour pattern (**Figure 89**). It is one of the most widely distributed *Anastrepha* species, having been recorded from Florida (USA), Southern and Central America and the West Indian islands. It is an important pest of mangoes, guava, rose apple and *Spondias* (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- *Anastrepha obliqua* is one of the most important fruit fly pests of mango in Central and Southern America

ATTRACTANT

No known record, but can be captured in traps emitting ammonia..

FIGURES

Figure 88. *Anastrepha obliqua*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)
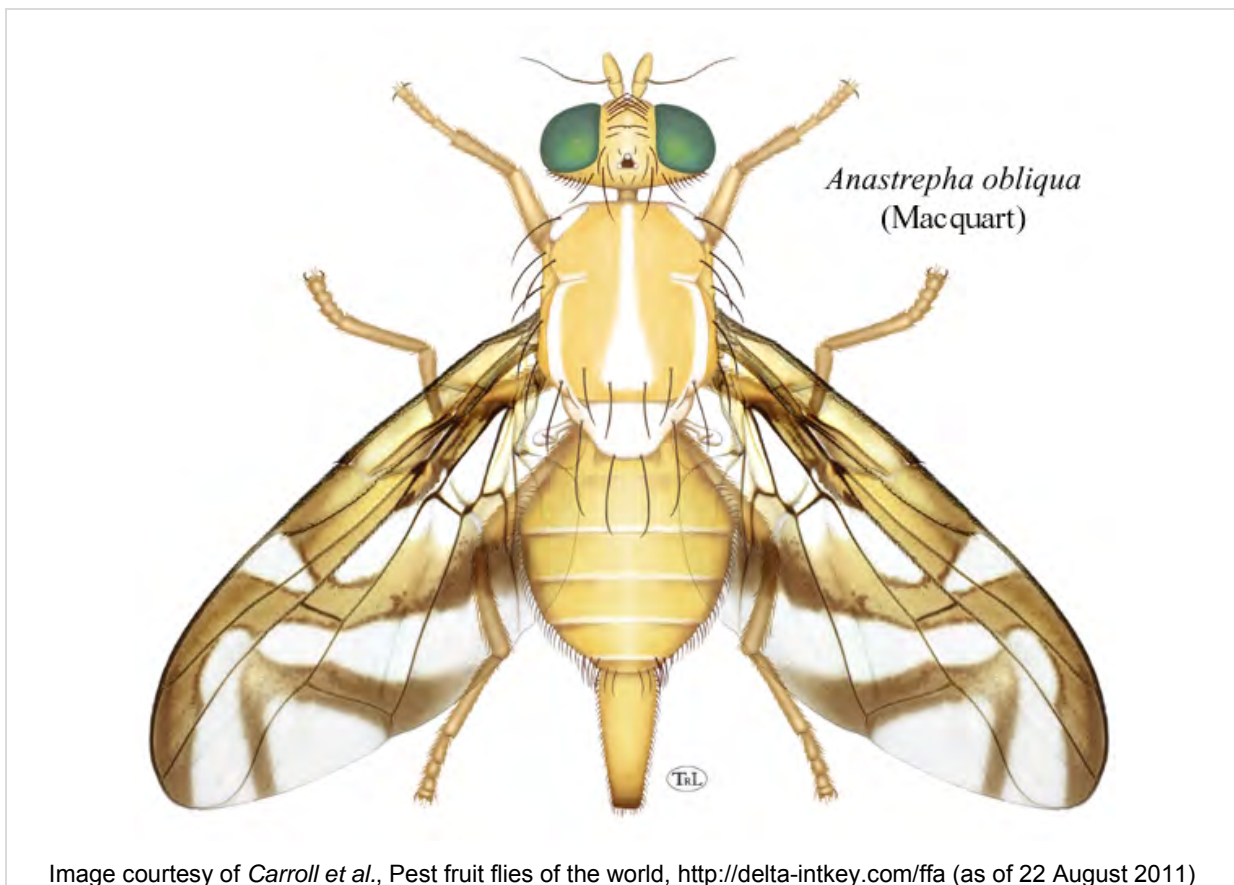
Figure 89. *Anastrepha obliqua*
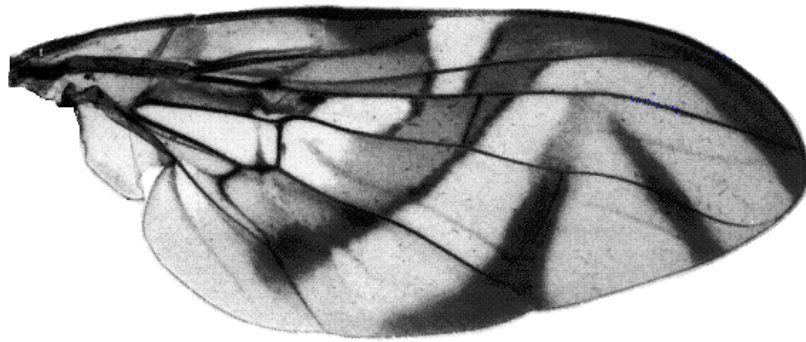
Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6.4   *Anastrepha serpentina* (Wiedemann)

TAXONOMIC INFORMATION

Common name: Sapote fruit fly

Previous scientific names:

Dacus serpentinus

Acrotoxa serpentinus

Anastrepha serpentina

DIAGNOSIS

7.6.4.1.1   *Morphological - Adult*

As in *Anastrepha ocresia* and a few non-U.S. *Anastrepha* species, the very dark wing markings of *A. serpentina* contrast strongly with the light hyaline areas of the wing. The S band is quite slender and is not connected to the proximal area of the V band, the apical arm of which is absent in all specimens. *Anastrepha serpentina* and *A. ocresia* are the only species of *Anastrepha* occurring in the United States that have a distinct pale yellow to hyaline area in cell r_1 immediately posterior to the pterostigma, but the former may be distinguished from the latter by the complete absence of the distal arm of the V band and the difference in abdominal markings. The scutum of the species is characterised by contrasting light and dark markings; the subscutellum and mediotergite are very dark, with a lighter brownish or yellowish spot or stripe dorsally (Foote *et al.* 1993).

7.6.4.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.6.4.1.3   *Molecular*

PCR - Restriction Fragment Length Polymorphism (Section 6.3.1):

Approximate ITS1 Frag length - gel: 750 bp

*Bsr*I:	DNC	*Sna*BI:	DNC
*Hha*I:	DNC	*Ssp*I:	DNC
*Hinf*I:	DNC	*Vsp*I:	250, 420

*Sau*3AI: 200, 530

See also **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Anastrepha serpentina has been recorded on hosts from eight families. These include: Anacardiaceae, Annonaceae, Clusiaceae, Lauraceae, Myrtaceae, Rosaceae, Rutaceae and Sapotaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name	Scientific name	Common name
Chrysophyllum cainito	cainito	*Manilkara zapota*	sapodilla
Citrus spp.	citrus		

DISTRIBUTION

Northern Mexico south to Peru and northern Argentina. Also known from Trinidad & Tobago and Curaçao (Norrbom 2003).

REMARKS

Anastrepha serpentina is distinguished by its very dark wing patterns (Figure 91. **Anastrepha serpentina** It is most prevalent in Mexico, Southern and Central America, as far south as Brazil. It has a wide host range but is not considered to be of significant economic importance (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- Not considered to be of significant economic importance

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.

.

FIGURES

Figure 90. *Anastrepha serpentina*

Anastrepha serpentina
(Wiedemann)
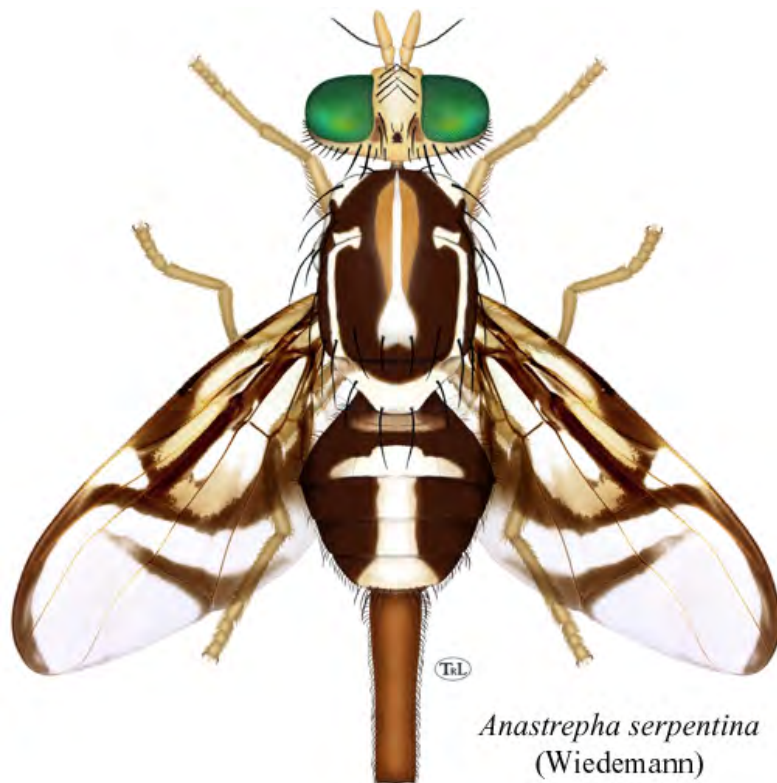
Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

Figure 91. *Anastrepha serpentina*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6.5   *Anastrepha striata* Schiner

TAXONOMIC INFORMATION

Common name: Guava fruit fly

Previous scientific names:

DIAGNOSIS

7.6.5.1.1   Morphological - Adult

A small to medium-sized species with a "normal" *Anastrepha* wing pattern, *A. striata* is one of the few species occurring in the United States that has distinct dark scutal markings in addition to darkening along the scutoscutellar suture. On the sublateral dark scutal areas, a pair of dense patches of short, brownish black setae is present, as well as some hoary pile visible only when viewed from in front, but the lateral half of the scutal brown stripe is denuded. *Anastrepha striata* is the only U.S. species having such scutal characters. The aculeus tip is distinctly broad and wedge-shaped with a very blunt apex and extremely fine lateral serrations. The size of the hyaline mark at the apex of vein R_1 varies considerably (Foote *et al*. 1993).

7.6.5.1.2   Morphological - Larvae

- Not available/included in this edition -

7.6.5.1.3   Molecular

See **PCR-DNA barcoding** (Section).

HOST RANGE

Anastrepha striata has been recorded on hosts from a range of families. These include: Anacardiaceae, Annonaceae, Combretaceae, Ebenaceae, Euphorbiaceae, Lauraceae, Myrtaceae, Rosaceae, Rutaceae and Sapotaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name
Psidium guajava	guava

DISTRIBUTION

Southern Texas, Mexico, Central America, south to Peru, Bolivia and Brazil. Also found in Trinidad, West Indies (UF & FDACS 2009).

REMARKS

Anastrepha striata is a smaller species of *Anastrepha* and best diagnosed by the distinct dark colour markings on the scutum, composed of a U-shaped black pattern. It is present in Mexico, Central America and most of Southern America. It is primarily a pest of guava (pers. comm. Drew 2010).

PEST STATUS

- Exotic

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.
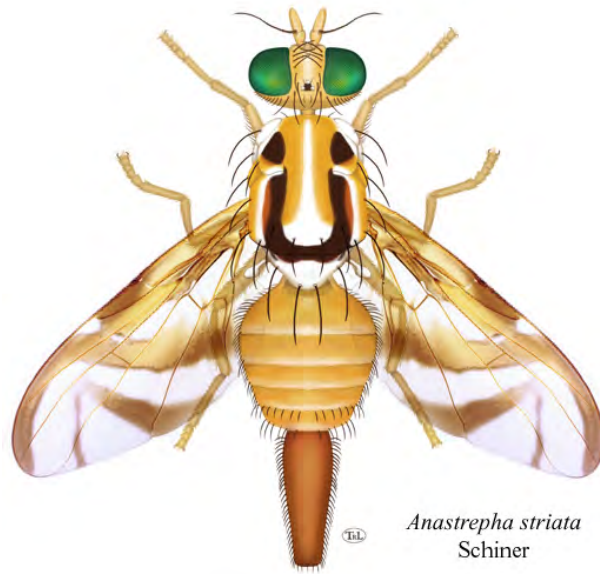
FIGURES

Figure 92. *Anastrepha striata*

Anastrepha striata
Schiner

Carroll et al., Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.6.6 *Anastrepha suspensa* (Loew)

TAXONOMIC INFORMATION

Common name: Caribbean fruit fly

Previous scientific names:

Trypeta suspensa

Acrotoxa suspensa

Trypeta (*Acrotoxa*) *suspensa*

DIAGNOSIS

7.6.6.1.1 *Morphological - Adult*

Anastrepha suspensa possesses external characters that closely resemble those of *A. fraterculus* and *A. obliqua*; therefore, the separation of these three species is sometimes difficult. One of the most obvious distinguishing marks in *A. suspensa* is the presence (except in some specimens from Jamaica) of a dark spot at the junction of the scutum and scutellum. This spot is sometimes present in *A. fraterculus* but is usually smaller, and it is absent in *A. obliqua*. The apical part of the S band in *A. suspensa* is relatively wide compared with that in the other two species, and its inner margin is less concave. It covers the apex of vein M or ends immediately anterior to it, whereas in the other two species it normally ends well anterior to the apex of vein M, or its inner margin is strongly concave. As in the identification of other species of *Anastrepha*, the shape of the aculeus tip is important. In *A. suspensa*, as in *A. fracterculus*, the serrations occupy no more than three-fifths of the tip, whereas those in *A. obliqua* occupy at least two-thirds; this character is variable and should be used with care (Foote *et al.* 1993).

7.6.6.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.6.6.1.3 *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Anastrepha suspensa has been recorded on hosts from a wide range of families. These include: Anacardiaceae, Annonaceae, Arecaceae, Canellaceae, Caricaceae, Chrysobalanaceae, Clusiaceae, Combretaceae, Curcurbitaceae, Ebenaceae, Lauraceae, Lythraceae, Malpighiaceae, Moraceae, Myrtaceae, Oxalidaceae, Polygonaceae, Rosaceae, Rutaceae, Salicaceae, Sapindaceae, Sapotaceae and Solanaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007; UF & FDACS 2009):

Scientific name	Common name	Scientific name	Common name
Annona reticulata	bullock's heart	*Prunus persica*	peach
Eugenia uniflora	Surinam cherry	*Psidium guajava*	guava
Fortunella margarita	oval kumquat	*Syzygium jambos*	rose apple
Manilkara zapota	sapodilla	*Terminalia catappa*	Singapore almond

DISTRIBUTION

Cuba, Jamaica, Hispaniola, Puerto Rico, southern Florida,(United States) (UF & FDACS 2009).

REMARKS

Along with *Anastrepha obliqua* and *A. fraterculus*, *A. suspensa* is very difficult to identify. These species all have similar wing colour patterns and *A. fraterculus* is suspected of belonging to a complex of closely related species. Generally, *A. suspensa* possesses a dark spot on the posterocentral area of the scutum where it joins the scutellum. *A. suspensa* is distributed in Florida (USA), the Bahamas and the West Indies, has a wide host range and is considered to be of major economic importance (pers. comm. Drew 2010).

PEST STATUS

- Exotic
- Major economic importance

ATTRACTANT

No known record, but can be captured in traps emitting ammonia...

FIGURES

Figure 93. *Anastrepha suspensa*

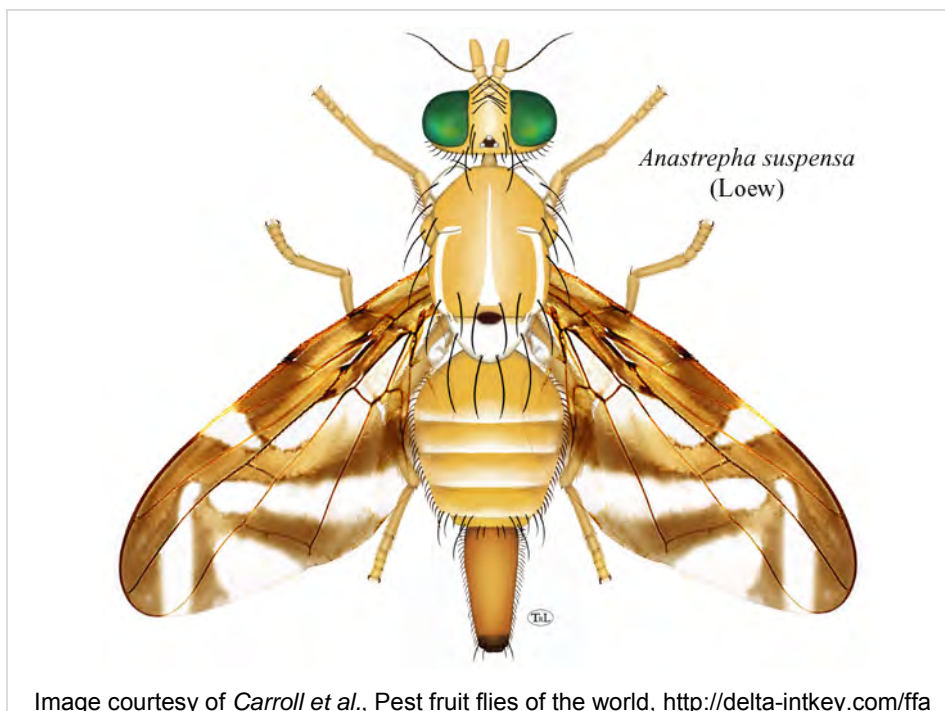Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa

Figure 94. *Anastrepha suspensa*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.7   *Rhagoletis*

7.7.1   *Rhagoletis completa* Cresson

TAXONOMIC INFORMATION

Common name: Walnut husk fly

Previous scientific names:

Rhagoletis suavis completa

Rhagoletis suavis var. *completa*

DIAGNOSIS

7.7.1.1.1   *Morphological - Adult*

The four transverse wing bands are present and are usually distinctly separated by hyaline areas, except in occasional specimens in which the discal and subapical bands are connected posteriorly. In the former case, the pattern closely resembles that of *R. berberis*, but the host relationships of these two species are quite different. The thorax and abdomen of *R. completa* are golden yellow (completely black in *R. berbeis*) and the scutellum is concolorous yellow (black with a distinct yellow spot in *R. berberis*) (Foote *et al.* 1993).

7.7.1.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.7.1.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Rhagoletis completa has been recorded on hosts from two families, Juglandaceae and Rosaceae (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name	Scientific name	Common name
Juglans californica	California walnut	*Juglans nigra*	black walnut
Juglans hindsii	Californian black walnut	*Juglans regia*	walnut

DISTRIBUTION

Southern and Central USA including Mexico; adventive in Western USA since the 1920s. Also established in Southern Europe since the early 1990s (CABI 2007).

REMARKS

Rhagoletis completa is an unusual economic tephritid species in that it is a major pest of walnuts, in contrast to the soft fleshy fruit hosts of other fruit fly species. It is best diagnosed by the distinctive wing colour patterns (**Figure 95**) and a red-brown thorax. It is widely distributed over Central and Western mainland USA (pers. comm. Drew 2010).

- Exotic

- High priority pest identified in the Nuts IBP

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.

FIGURES

Figure 95. *Rhagoletis completa*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.7.2   *Rhagoletis fausta* (Osten-Sacken)

TAXONOMIC INFORMATION

Common name: Black cherry fruit fly

Previous scientific names:

Trypeta (*Acidia*) *fausta*

Trypeta fausta

Rhagoletis fausta

Acidia fausta

DIAGNOSIS

7.7.2.1.1   Morphological - Adult

Rhagoletis fausta is very close to *R. striatella* in that the posterior apical band in both species arises from the subapical band in the vicinity of vein r-m, making an F-shaped pattern in the apical half of the wing similar to that in the *pomonella* group (however, in the latter, note that the subapical band is missing and the apical bands are connected to the discal band). In wing pattern alone, *R. fausta* is unique among North American *Rhagoletis* in combining a very broad connection between the discal and subapical bands in cell dm with the presence of both an anterior and posterior apical band, the latter arising in much the same location as in *R. striatella*. In *R. striatella*, the discal and subapical bands are separate or connected only along the posterior wing margin. In many respects, the wing pattern of *R. fausta* resembles that of *R. suavis*, but *R. fausta* has both anterior and posterior apical bands and an isolated hyaline spot in the distal half of cell cua_1. *Rhagoletis suavis* has a yellowish body; that of *R. fausta* is black and without yellowish bands at the posterior margins of the abdominal terga (Foote *et al.* 1993).

7.7.2.1.2   Morphological - Larvae

- Not available/included in this edition -

7.7.2.1.3   Molecular

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Rhagoletis fausta has been recorded on hosts the Rosaceae family (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name	Scientific name	Common name
Prunus avium	sweet cherry	*Prunus cerasus*	sour cherry

DISTRIBUTION

Widespread occurrence in western and eastern North America (United States and Canada) (CABI 2007).

REMARKS

Rhagoletis fausta is a dark coloured species with the scutum and abdominal tergites primarily black. It also has a unique wing colour pattern (Figure 96). It is widely distributed over mainland USA where it infests cherry varieties in the plant genus *Prunus* (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Cherry IBP

- *Rhagoletis fausta* is an important pest of cherries in North America

ATTRACTANT

 No known record, but can be captured in traps emitting ammonia.

FIGURES

Figure 96. *Rhagoletis fausta*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.7.3   *Rhagoletis indifferens* Curran

TAXONOMIC INFORMATION

Common name: Western cherry fruit fly

Previous scientific names:

Rhagoletis cingulata

Rhagoletis cingulata indifferens

DIAGNOSIS

7.7.3.1.1   *Morphological - Adult*

Rhagoletis indifferens is similar to *R. cingulata* in wing pattern but the anterior arm of *R. indifferens* is broken to produce an apical spot in only about 5% of individuals in contrast to *R. cingulata*, in which this spot is much more commonly encountered. In addition, other characters that distinguish *R. indifferens* from *R. cingulata* are as follows:

Rhagoletis indifferens:

    A. Apical yellow shading on posterior margin of tergite 5 of male lacking.

    B. Black shading always present on posterior surface of fore coxa

    C. Epandrium dark-coloured

Rhagoletis cingulata:

    A. Apical yellow shading on posterior margin of tergite 5 of male present

    B. Fore coxae concolorous yellow

    C. Epandrium light-coloured

Most individuals of *R. indifferens* may be distinguished from those of *R. chionanthi* and *R. osmanthi* by the differences in geographical distribution and hosts and by the generally smaller size and lesser development of the wing bands. (Foote *et al.* 1993).

7.7.3.1.2   *Morphological - Larvae*

- Not available/included in this edition -

7.7.3.1.3   *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Rhagoletis indifferens has been recorded on hosts the Rosaceae family (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name
Prunus avium	sweet cherry

DISTRIBUTION

Rhagoletis indifferens is a western North American species (Canada and United States). Adventive populations have been found in southern Switzerland since the early 1990s (CABI 2007).

REMARKS

Rhagoletis indifferens may not be a distinct species but a colour variety of *Rhagoletis cingulata*. It is distributed primarily over western regions of mainland USA where it infests wild and cultivated cherries of the plant genus *Prunus* (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Cherry IBP

- *Rhagoletis indifferens* is an important pest of cherries in North America

ATTRACTANT

No known record, but can be captured in traps emitting ammonia.

FIGURES

Figure 97. *Rhagoletis indifferens*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

7.7.4 *Rhagoletis pomonella* (Walsh)

TAXONOMIC INFORMATION

Common name: Apple maggot

Previous scientific names:

Trypeta pomonella

Trypeta (*Rhagoletis*) *pomonella*

DIAGNOSIS

7.7.4.1.1 *Morphological - Adult*

Rhagoletis pomonella, together with *R. zephyria*, *R. mendax* and *R. cornivora* are among the most readily recognised species of *Rhagoletis* by virture of their wing pattern, which consists of a slightly oblique discal band to which the anterior and posterior apical bands are connected, forming a characteristic F-shaped pattern in the apical half of the wing. The absence of the subapical band distinguishes the species of the *pomonella* group from all other species of *Rhagoletis*. *Rhagoletis striatella*, which also has an F-shaped apical wing pattern, is distinguished from *R. pomonella* by the colour pattern of the scutellum and the additional characters given in the key to species. *Rhagoletis pomonella* is separable from the other three species of the *pomonella* group by the presence in most specimens of heavy black shading on the posterior surface of the fore femur, and, in specimens from the northern part of its range, by a generally larger body size and by the longer aculeus (0.90-1.49mm). In the southern part of its range, specimens of *R. pomonella* generally are smaller than in the north. For that reason and because of a consequently shorter aculeus, females are not separable from those of *R. mendax* and *R. cornivora* by the use of morphological characters. Mexican specimens of *R. pomonella* resemble those that occur in the United States and Canada but generally are larger and possess a light spot near the base of the apical wing band (Foote, Blanc and Norrbom 1993).

7.7.4.1.2 *Morphological - Larvae*

- Not available/included in this edition -

7.7.4.1.3 *Molecular*

See **PCR-DNA barcoding** (Section 6.3.2).

HOST RANGE

Rhagoletis pomonella has been recorded on hosts from the Rosaceae family (for a full list of recorded hosts see CABI 2007).

Major commercial hosts (CABI 2007):

Scientific name	Common name
Malus domestica	apple

DISTRIBUTION

Canada, United States and Mexico (Carroll *et al.* 2002).

REMARKS

Rhagoletis pomonella possesses primarily a black scutum and abdomen and a distinctive wing colour pattern (**Figure 98**). It has been the subject of extensive taxonomic, ecological and pest management

research in the USA and is considered the major economic pest species within the genus *Rhagoletis*. It is a major pest of cultivated apples. It is distributed over the central and north-eastern regions of mainland USA and extreme southern Canada. In 1979 it was introduced into the western coastline of the USA and is now widespread in that region (pers. comm. Drew 2010).

PEST STATUS

- Exotic

- High priority pest identified in the Apple and Pear, and Cherry IBPs

- *Rhagoletis pomonella*, which primarily attacks apples, is the most serious fruit fly pest in North America

ATTRACTANT

No known record, but can be captured in traps emitting ammonia..

FIGURES

Figure 98. *Rhagoletis pomonella*

Image courtesy of *Carroll et al.*, Pest fruit flies of the world, http://delta-intkey.com/ffa (as of 22 August 2011)

8 Diagnostic resources

8.1 Key contacts and facilities

Contact	Facility
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Dr. David Yeates Curator of Diptera **David.Yeates@csiro.au** **Dr. Paul De Barro** **Paul.DeBarro@csiro.au**	**CSIRO Entomology** GPO Box 1700, Canberra, ACT 2601 **Phone: (02) 6246 4001** **Fax: (02) 6246 4177**
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For international fruit fly authorities, see **www.sel.barc.usda.gov/Diptera/tephriti/TephWork.htm**

8.2   Reference collections

Collection	Location
Victorian Agricultural Insect Collection, DPI Vic.	AgriBio Building, DPI Bundoora Campus, Victoria.
Queensland Primary Industries Insect Collection, DEEDI.	Biosecurity Queensland, DEEDI Ecosciences Precinct, GPO Box 46, Brisbane QLD 4001, Australia.
DEEDI Biosecurity Insect Collection.	21 Redden Street, Cairns QLD 4870, Australia.
NAQS Insect Collection.	Airport Business Park, Cairns Airport Building 114, Catalina Cresent, Cairns QLD 4870, Australia.
The Northern Territory Economic Insect Reference Collection.	Department of Resources, Primary Industry, Berrimah Farm, Makagon Road, Berrimah NT 0828, Australia.
Museum and Art Gallery of the Northern Territory, NRETAS.	Conacher Street, Fannie Bay, Darwin NT 0820, Australia.

8.3 Printed and electronic resources

8.3.1 Morphological keys

In practice in Australia the two paper keys that are most commonly used are:

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

- Drew, R.A.I. and Hancock, D.L. (1994) The Bactrocera dorsalis complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research*. Supplementary Series 2: 1-68.

Other paper-based keys include:

- Drew, R.A.I. and Raghu, S. (2002) The fruit fly fauna (Diptera: Tephritidae: Dacinae) of the rainforest habitat of the Western Ghats, India. *Raffles Bulletin of Zoology*. **50**, 327-352.

- Drew, R.A.I. and Hancock, D.L. (1994) Revision of the tropical fruit flies (Diptera: Tephritidae: Dacinae) of South East Asia. I. *Ichneumonopsis* Hardy and *Monacrostichus* Bezzi. *Invertebrate Taxonomy*, 8, 829-838.

- Drew, R.A.I., Hancock, D.L. and White, I.M. (1998) Revision of the tropical fruit flies (Diptera: Tephritidae: Dacinae) of South-east Asia. II. *Dacus* Fabricius. *Invertebrate Taxonomy*, 12, 567-654.

- Drew, R.A.I. and Romig, M.C. (2001) The fruit fly fauna (Diptera: Tephritidae: Dacinae) of Bougainville, the Solomon Islands and Vanuatu. *Australian Journal of Entomology*, 40, 113-150.

- Drew, R.A.I., Hooper, G.H.S. and Bateman, M.A. (1982) *Economic fruit flies of the South Pacific region*. Queensland Department of Primary Industries, Brisbane, Queensland. 139 pp.

- White, I.M. and Elson-Harris, M.M. (1992) *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International. Oxon, UK. 601 p.

- Rohani, I. (1987) Identification of larvae of common fruit fly pest species in West Malaysia. *Journal of Plant Protection in the Tropics*, 4 (2), 135-137.

- Hardy, E.D. (1986) Fruit flies of the subtribe Acanthonevrina of Indonesia, New Guinea, and the Bismarck and Solomon Islands (Diptera: Tephritidae: Trypetinae: Acanthonevrina). *Pacific Insect Monographs,* No. 42. Honolulu, Hawaii. 191 p.

- Hardy, E.D. (1974) The fruit flies of the Philippines (Diptera - Tephritidae). *Pacific Insect Monographs*, No. 32. Honolulu, Hawaii. 266 p.

- Significant information on the larvae of many Australian fruit flies, including ones not of economic importance but that might turn up during sampling, was given in the PhD thesis of Dr Marlene Elson-Harris lodged at the University of Queensland.

Electronic keys available include:

- White, I.M. and Hancock, D.L. (2003) Fauna Malesiana [electronic key to fruit flies]. ISBN 9075000359.

- White, I.M. and Hancock, D.L. (1997) Indo-Australasian Dacini Fruit Flies (CABIKEY) International Institute of Entomology, London. CD-ROM.

- Lawson, A.E., McGuire, D.J., Yeates, D.K., Drew, R.A.I. and Clarke, A.R. (2003) *Dorsalis: an interactive identification tool to fruit flies of the Bactrocera dorsalis complex*. Griffith University. Brisbane, Australia. [CD-ROM] [Out of print]

- An interactive key is also available on the Fruit Flies of the World website: **http://delta-intkey.com/ffa**

8.3.2   Electronic resources

- **Tephritid Barcoding Initiative (TBI): www.barcodeoflife.org**. The TBI aims to barcode 10,000 specimens representing 2,000 species of fruit flies, including all taxa (about 350 species) of major and minor economic importance.

- **The Diptera Site: www.sel.barc.usda.gov/Diptera/tephriti/tephriti.htm**. Contains a large amount of biological and other information about fruit flies.

- **Pest Fruit Flies of the World**: **http://delta-intkey.com/ffa**. Contains comprehensive information and keys on fruit flies of all regions.

- **ANIC Anatomical atlas of flies**: **www.csiro.au/resources/ps252.html**. Great for illustrations of every feature of acalyptrate flies.

- **On the fly: interactive atlas and key to Australian fly families**: **www.csiro.au/resources/ps236.html**.

- **Australian Pest and Diseases Image Library (PaDIL): www.padil.gov.au**. Contains species information as well as photos for a number of fruit fly species (endemic and exotic).

- **NSW government fruit fly resource**: **www.agric.nsw.gov.au/Hort/ascu/fruitfly/fflyinde.htm**. List of fruit fly species found in New South Wales or believed to be present there, with links to summary information on each and key.

- **International Centre for Management of Pest Fruit Flies (Griffith University and Malaysia)**: **http://www.icmpff.org**

- **South Pacific fruit fly website (Pacifly): http://www.pacifly.org**. Contains profiles of all species found in the South Pacific.

- **Featured Creatures: http://entomology.ifas.ufl.edu/creatures/index.htm**. Contains profiles for a limited number of fruit fly species.

- **The Australian Plant Pest Database (APPD)**: **http://pha.vpac.org**. A national, online database of pests and diseases of Australia's economically important plants.

8.4   Supplier details

Supplier	Address	Contact details
Applied Biosystems *(for PCR)*	1270 Ferntree Gully Road Scoresby, VIC 3179	Ph: (03) 9212 8500 Fax: (03) 9212 8502 **www.appliedbiosystems.com.au**
Astral Scientific	PO Box 232 Gymea, NSW 2227	Ph: 1800 221 280 Fax: (02) 9540 2051
Bio-Rad Laboratories Pty. Ltd.	Level 5, 446 Victoria Road Gladesville, NSW 2111	Ph: (02) 9914 2800 or 1800 224 354
GENESEARCH *(agents for New England Biolabs)*	14 Technology Drive Arundel, QLD 4214	Ph: 1800 074 278 or (07) 5594 0562 **www.genesearch.com.au**
Interpath services	1/46 Sheehan Rd Heidelberg West, VIC 3081	Ph: (03) 9457 6277 or 1800 626 369 Fax: (03) 9458 4010
Invitrogen *(for primer synthesis)*	PO Box 4296 Mulgrave, VIC 3170	Ph: 1800 331 627 Fax: (03) 9562 7773 **www.invitrogen.com**
Mirella Research Pty. Ltd.	PO Box 365 Brunswick, VIC 3056	Ph: (03) 9388 1088 or 1800 640 444 Fax: (03) 9388 0456
Promega Corporation *(for Molecular weight marker)*	PO Box 168 Annandale, NSW 2038	Ph: (02) 9565 1100 Fax: (02) 9550 4454 **www.promega.com**
Qiagen Pty Ltd *(for DNA extraction)*	PO Box 25 Clifton Hill, VIC 3068	Ph: (03) 9489 3666 Fax: (03) 9489 3888 **www.qiagen.com**
Roche Diagnostics Australia Pty. Ltd.	31 Victoria Avenue Castle Hill, NSW 2154	Ph: (02) 9899 7999 Fax: (02) 9634 2949
Sigma-Aldrich Pty. Ltd. *(for chemicals)*	PO Box 970 Castle Hill, NSW 1765	Ph: 1800 800 097 Fax: 1800 800 096 **www.sigmaaldrich.com**

9   References

Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hamacek, E.L. and Hancock, D.L. (1999). Host plant records for fruit flies (Diptera: Tephritidae) in South East Asia. *Raffles Bull. Zool.* Supplement No. 7: 1-92.

Armstrong K.F. and Cameron,C.M., 1998. Molecular Kit for Species Identification Fruit Flies (Tephritidae), Lincoln University

Armstrong, K.F. and Ball, S.L. (2005). DNA barcodes for biosecurity: invasive species identification. *Philos. Trans. R. Soc. Lond., B, Biol. Sci*. 360: 1813-1823

Blacket M.J., Semeraro L., and Malipatil M.B. (2012). Barcoding Queensland Fruit Flies (*Bactrocera tryoni*): impediments and improvements. *Mol. Ecol. Resour.* (*In press).*

CABI (2007) *Crop Protection Compendium, 2007 Edition*. CAB International, Wallingford, UK. Online version: http://www.cabi.org/compendia/cpc/ [accessed May 2010]

Cameron, E.C., Sved, J.A., and Gilchrist, A.S. (2010). Pest fruit fly (Diptera: Tephritidae) in northwestern Australia: one species or two? Bulletin of Entomological Research. 100(2):197-206.

Carroll, L.E., I.M. White, A. Freidberg, A.L. Norrbom, M.J. Dallwitz, and F.C. Thompson. (2002 onwards). Pest fruit flies of the world. Version: 8th December 2006. http://delta-intkey.com [accessed May 2010].

Clarke, A.R., Armstrong, K.F., Carmichael, A.E., Milne, J.R., Raghu, S., Roderick, G.K. and Yeates, D.K. (2005) Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology.* 50: 293-319.

Department of Agriculture Western Australia. Exotic Fruit Fly http://agspsrv34.agric.wa.gov.au/ento/Surveillance/Fruit%20fly.html#The%20Lynfield%20lure%20trap [accessed January 2011].

Dominiak, B. C. and Daniels, D. (2011) Review of the past and present distribution of Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) and Queensland fruit fly (*Bactrocera tryoni* Froggatt) in Australia. *Australian Journal of Entomology*. (In press)

Dominiak, B.C., Gilmour, A.R., Kerruish, B., and Whitehead, D. (2003) Detecting low populations of Queensland fruit fly *Bactrocera tryoni* (Froggatt) with McPhail and Lynefield traps. *General and Applied Entomology* Vol.32 49-54.

Dominiak, B.C., Kerruish, B., Barchia, I., Pradhan, U., Gilchrist, A.S., and Nicol, H.I. (2011) The influence of mixtures of parapheromone lures on trapping of fruit fly in New South Wales, Australia. *Plant Protection Quarterly* Vol.26(4) 136-140.

Dominiak, B.C. and Nicol, H.I. (2010) Field performance of McPhail and Lynefield traps for monitoring male and female sterile *Bactrocera tryoni* (Froggatt) and wild *Dacus newmani* (Perkins). *Pest Management Science* Vol.66 741-744.

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

Drew, R.A.I. and Hancock, D.L. (1994) The Bactrocera dorsalis complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research.* Supplementary Series 2: 1-68.

Drew, R.A.I. and Lambert, D.M. (1986) On the specific status of *Dacus aquilonis* and *Dacus tryoni* Diptera Tephritidae. *Annals of the Entomological Society of America*. 79(6): 870-878.

Drew, R.A.I., G.H.S. Hooper and M.A. Bateman. (1982). Economic Fruit Flies of the South Pacific Region. Queensland Department of Primary Industries.

Fay, H.A.C. (2011). A highly effective and selective male lure for *Bactrocera jarvisi* (Tryon) (Diptera: Tephritidae). *Australian Journal of Entomology* (In press)

Flath, R.A., Cunningham, R.T., Liquido, N.J. and McGovern, T.P. (1994). Alpha-Ionol as Attractant for Trapping *Bactrocera latifrons* (Diptera: Tephritidae). Journal of Economic Entomology 87(6):1470-1476.

Floyd, R., Lima, J., deWaard, J., Humble, L., and Hanner, R. (2010). Common goals: policy implications of DNA barcoding as a protocol for identification of arthropod pests. *Biological Invasions*. 12: 2947-2954.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**: 294-299.

Foote, R.H., Blanc, F.L. and Norrbom, A.L. (1993) *Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico*. Comstock, Ithaca, N.Y.

Gilbert, M.T.P., Moore, W., Melchior, L., and Worobey, M. (2007). DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS ONE* 2: e272.

Hancock, D.L., Hamacek, E.L., Lloyd, A.C. and Elson-Harris, M.M. (2000). *The distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia.* Queensland Department of Primary Industry. Information series Q199067. Brisbane, Queensland. 75pp.

Lawson, A.E., McGuire, D.J., Yeates, D.K., Drew, R.A.I. and Clarke, A.R. (2003). *Dorsalis: an interactive identification tool to fruit flies of the Bactrocera dorsalis complex*. Griffith University. Brisbane, Australia. [CD-ROM]

Manchenko, G.P. (1994). *Handbook of detection of enzymes on electrophoretic gels.* CRC Press, Boca Raton, Florida.

McKenzie, C., Gillispie, P. and Hailstones, D. (2004). *Draft molecular diagnostic standard for fruit flies of economic significance to Australia.* Plant Health Australia and NSW Department of Primary Industries.

McKenzie, L., Dransfield, L., Driver, F. and Curran, J. (1999). *Molecular protocols for species identification of tephritid fruit flies*. Report to AQIS. CSIRO, Canberra.

Metcalf, R.L., Mitchell, W.C., and Metcalf, E.R. (1983). Olfactory receptors in the melon fly *Dacus cucurbitae* and the oriental fruit fly *Dacus dorsalis. Proc. Natl. Acad. Sci. USA.* 80: 3143-3147.

Norrbom, A.L. (2003). The Diptera site. Systematic Entomology Laboratory, ARS, USDA and Department of Entomology, NMNH, SI. www.sel.barc.usda.gov/diptera/tephriti/tephriti.htm [accessed May 2010].

Osborne, R., Meats, A., Frommer, M., Sved, J.A., Drew, R.A.I., and Robson, M.K. (1997) Australian Distribution of 17 Species of Fruit Flies (Diptera: Tephritidae) Caught in Cue Lure Traps in February 1994. *Australian Journal of Entomology* 36: 45-50.

PaDIL (2007). Pest and disease image library. An Australian Government initiative. www.PaDIL.gov.au [accessed May 2010].

Queensland Department of Primary Industries and Fisheries (2002). *OP-03: Fruit fly identification guidelines*. Operational Procedures. August, 2002.

Richardson, B. J., Baverstock, P. R., and Adams, M. (1986). *Allozyme electrophoresis. A handbook for animal systematics and population studies*. Academic Press, Sydney, Australia.

Secretariat of the Pacific Community (2006) Pacific Fruit Fly Web. www.spc.int/pacifly [accessed May 2010]

Semeraro,L. and Malipatil,M.B., (2005), Molecular diagnostic test for Queensland fruit fly larvae (*Bactrocera tryoni* Froggatt).

Smith, E.S.C. and Chin D. (1987). Current fruit fly research in the Northern Territory. Proceedings of the 4th Workshop in Tropical Entomology. Darwin, NT 10-15 May 1987, pp. 128-135.

Smith, E.S.C., Chin, D., Allwood, A.J., and Collins, S.G. (1988) A revised host list of fruit flies (Diptera: Tephritidae) from the Northern Territory of Australia. *Queensland Journal of Agricultural and Animal Sciences.* 45(1): 19-28.

Tsuruta, K., White, I.M., Bandara, H.M.J., Rajapakse, H., Sundaraperuma, S.A.H., Kahawatta, S.B.M.U.C., and Rajapakse, G.B.J.P. (1997) A preliminary notes on the host plants of fruit flies of the tribe Dacini (Diptera: Tephritidae) in Sri Lanka. *Esakia.* 37: 149-160.

White, I.M. and Elson-Harris, M.M. (1992). *Fruit Flies of Economic Significance: Their Identification and Bionomics*. CAB International. Oxon, UK. 601 p.

University of Florida and the Florida Department of Agriculture and Consumer Services (2009) Featured Creatures. http://entomology.ifas.ufl.edu/creatures/index.htm [accessed May 2010].

10 Appendices

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APPENDIX 1

Review of the outbreak threshold for Queensland fruit fly (*Bactrocera tryoni* Froggatt)

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Abstract

Fruit flies cause losses in horticultural produce across the world and are a major quarantine concern for most countries. Queensland fruit fly (Qfly) is a native to Australia and is also present in a small number of Pacific Island countries. The detection of Qfly in recognized pest free areas triggers quarantine restrictions from domestic and international markets. In Australia, the detection of five male flies has been taken to indicate an outbreak (i.e. unacceptable risk). Matching the domestic standard, many countries have accepted the 5-fly limit as a quarantine threshold. But some other countries have set the detection of two male flies, or even a single fly, as the threshold for an outbreak. This different standard creates an administrative complexity for exporters and trade regulators.

In this paper, we review the published science covering the impediments to pest establishment. Outbreak data from Victoria and New South Wales during 2007 and 2009 are reviewed in relation to the 2-fly and 5-fly thresholds. Large volumes of fruit have been traded within Australia and internationally based on the 5-fly threshold without incident and there is no evidence that the 2-fly threshold is more appropriate. While Qfly is recognized as being capable of longer distance dispersal than some other fruit fly species, it is also recognized as a poor colonizer. The 5-fly threshold is proposed as the most appropriate threshold for imposition of quarantine restrictions and is recommended as a universal standard for harmonization of quarantine regulations.

Introduction

There are about 4500 species of fruit flies worldwide. In the Pacific area alone, there are 350 species of which at least 25 species are regarded as being of major economic importance (Allwood 2000). The genus *Bactrocera* contains over 400 species, distributed primarily though the Asia-Pacific area including Australia (Drew 1974).

Tephritid fruit flies cause direct losses to many fresh fruit and some vegetable industries, resulting in adverse impacts on trade and economies of many countries (Li *et al*. 2010, Stephenson *et al*. 2003). With the increasing globalization of trade (Stanaway *et al*. 2001, Plant Health Australia 2010), fruit flies pose a major quarantine concern that is currently monitored through regional surveillance programs (International Atomic Energy Agency 2003, Stephenson *et al*. 2003, Oliver 2007).

The Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) (Qfly) is a major fruit fly pest of Australian horticulture, attacking most fruit and many vegetable crops (e.g. stone fruit, citrus, coffee, tomato, capsicum, pome fruit) (Bateman 1991, Anon. 1996, Hancock *et al*. 2000). Qfly is an Australian native and is currently only found in Australia and on some Pacific islands (Drew 1989, White and Elson-Harris 1992). Given its pest status within Australia, Qfly is also a significant quarantine concern for many trading partners. Markets trading in commodities that may be subject to Qfly infestation require assurance of reliable monitoring grids, evidence-based outbreak thresholds and appropriate quarantine measures (Bateman 1991, Anon. 1996, Clarke *et al*. 2011).

In the early 1990s, Bateman (1991) reviewed existing domestic trade conditions and recommended a uniform agreement among the Australian states for the management of and trade in Qfly host commodities. In response, the Code of Practice for the Management for Queensland Fruit Fly (Anon. 1996) was published, with particular emphasis on managing the Tri-State Fruit Fly Exclusion Zone (FFEZ) so that fruit could be traded domestically with increased efficiency. The FFEZ production area is managed as a pest free area and is recognized by all Australian states as being free from economic fruit flies. Strict quarantine measures are in place to prevent entry of fruit flies and any incursions invoke a rapid and thorough eradication response. Within the FFEZ, four separate pest free areas have been established to facilitate trade into international markets. These include the Riverina area of New South Wales, the Sunraysia region of Victoria/New South Wales, the Riverland area of South Australia, and the Shepparton Irrigation Region of Victoria. Under some circumstances, Qfly do enter the FFEZ and are detected in monitoring traps (Dominiak *et al.* 2003a, Dominiak and Coombes 2009). Single-fly detections are almost always isolated incursions that do not indicate breeding populations (Meats *et al.* 2003).

For domestic trade (Anon. 1996), an outbreak is declared following one (or more) of three thresholds. These thresholds are the detection of:

(1) five male flies within 1 km within 14 days, or
(2) one mated female, or
(3) one or more larvae in fruit grown in the area.

The quarantine distance around any outbreak is 15 km. This domestic trade agreement (Anon. 1996) was broadly adopted in principle by 19 countries as the basis of international trade. However some key components of this agreement, such as the outbreak threshold, have not been accepted by some importing countries. In 1996, the outbreak threshold varied from 1, 2 and 5 male flies for 1, 14, and 3 countries respectively (Robert McGahy personal communication). The threshold of two male flies and five flies (hereafter referred to as 2-fly and 5-fly thresholds) are the most commonly used quarantine thresholds. The 2-fly threshold is based on detections within 400 m while the 5-fly threshold is based on detections within 1 km. By 2009, with increased international acceptance of the 5-fly threshold, this position had changed with 1, 11 and 9 countries accepting 1, 2 and 5 male flies respectively as outbreak thresholds (David Daniels personal communication). These different outbreak thresholds lack a robust scientific basis and create complex administration procedures for trade regulators. An agreed evidence-based Qfly outbreak threshold would harmonize market requirements and thereby facilitate domestic and international trade (Clarke *et al.* 2011). A universal outbreak threshold would have major implications for trade, quarantine and the minimization of pesticides in the environment as part of eradication programs (cover and bait sprays). There is a geometric expansion of areas requiring disinfestation unnecessarily by each kilometre of quarantine radius for outbreaks triggered by a low threshold (Clarke *et al.* 2011).

The purpose of this paper is to review the data from February 2007 to April 2009 for 2-fly and 5-fly thresholds for fruit fly outbreaks in Victoria and New South

Wales, and to examine the published scientific evidence since 1996 regarding incursions, survival, breeding populations and the resultant outbreak thresholds. This review will focus only on male flies as most outbreaks are triggered by the detection of male flies.

Impediments to pest establishment
Founding propagules
It has been shown that the introduction of fruit flies into pest free areas is most often the result of illegal transportation into and the inappropriate disposal of infested host material within the pest free area (Bateman 1991, Dominiak *et al.* 2000, Dominiak and Coombes 2009). This indicates that relatively small parcels of fruit flies are the source of most Qfly detections. Qfly dispersal from these points of introduction is limited by lifespan and the ability to find food to sustain the effort of longer or frequent short flights, survive adverse weather and avoid predation (Meats and Smallridge 2007, Meats and Edgerton 2008, Gilchrist and Meats 2011, Weldon and Taylor 2011). Immature fruit flies disperse for about two weeks in random directions and do not travel in pairs (Fletcher 1974a). Following the introduction of small numbers of Qfly into fruit fly free areas, the chances of a sexually mature male and female occurring in the same tree or group of trees after many days of dispersal is extremely low (Fletcher 1974a, Bateman 1977, Meats 1998, Weldon 2003, 2007, Weldon and Meats 2010). Following the introduction of propagules of infested fruit into fruit fly free areas, Meats (1998) and Meats *et al.* (2003) proposed that flies disperse into a mate-free void and self extinguish, as it becomes increasingly unlikely that they will participate in mating and will therefore not establish a breeding population.

Nutrition
Nutrition is key for Qfly survival, dispersal, reproduction and establishment of new populations. Wild flies must find sugar, minerals, water and protein from products such as bird faeces, honeydew and fruit juice (Bateman 1972, Drew *et al.* 1984, Dalby-Ball and Meats 2000). In dry environments, these products are difficult to find. The average lifespan of Qfly without food and water is approximately 45 hours (Weldon and Taylor 2010, Dominiak unpublished). Qfly require a balanced diet, as diets with too much or too little protein and carbohydrate result in adverse effects on either longevity or reproduction (Prabhu *et al.* 2008, Fanson *et al.* 2009). Protein feeding by post-teneral Qfly has been consistently reported to enhance sexual performance (Perez-Staples *et al.* 2007, 2008, Prabhu *et al.* 2008). Bacteria on the surfaces of leaves and fruit appear to be a key food source for Qfly (Drew

1987, Drew and Lloyd 1987, Fletcher 1987). However in fruit fly free regions of southern Australia, populations of these bacteria may be infrequent and erratic owing to unfavourable climate (Drew *et al.* 1984, Courtice and Drew 1984). In the absence of these bacteria, Qfly must find protein from alternative ephemeral sources (Perez-Staples *et al.* 2007, Weldon and Taylor 2011) and therefore a large proportion of flies may not reach sexual maturity and contribute to population growth.

Climate in the FFEZ is normally dry and crops require irrigation. The combination of low humidity and starvation are considerably more punitive for Qfly survival than starvation alone (Weldon and Taylor 2010). Desiccation resistance is generally lower for females than males and resistance also declines with age. Therefore, the lack of available food resources in the environment diminishes the chance of survival to maturity and the chance to compete for a mating. In summary, the FFEZ usually presents as a hostile environment and affords very limited resources for the establishment and spread of Qfly.

Dispersal before mating
Qfly actually spend very little time flying. Fletcher (1973, 1989) noted that flies spend most of their time making trivial flights or walking within the tree canopy. In response to higher fruit abundance, both male and female Qfly visit more leaves and hence spend more time in trees containing more fruit (Dalby-Ball and Meats 2000). Flies move around the canopy primarily by walking, and when they do fly, it is usually over distances of less than 50 mm in an upward direction. In laboratory observations, wild Qfly spend only about 0.6% of their time in flight with walking (67.5%), inactivity (18.0%) and grooming (14%) taking up the remainder of their time (Weldon *et al.* 2010). In the field, Ero *et al.* (2011) reported that resting was the most commonly observed behaviour for Qfly while feeding was rarely observed.

The flight activity patterns and short-range dispersal patterns of emerged adults are similar for male and female Qfly (Weldon and Meats 2007, Weldon *et al.* 2010). Clarke and Dominiak (2010) found a high correlation between male and female trap catches and suggested that changes in male distribution also reflect the distribution of female Qfly. Fletcher (1973) reported that the weekly declines of released Qfly were similar for males and females. Meats (1998) also assumed that males and females had similar dispersal. Therefore the trapping of male flies is likely to reflect a similar number of female flies in the environment.

Mating after dispersal
Male Qfly use pheromones and acoustic signals to attract sexually receptive

females, and mate only during a brief period of about 30 minutes at dusk (Tychsen and Fletcher 1971). Males gather on the upwind side of trees, where they release pheromone and fan their wings, directing the pheromone stream through the foliage (Tychsen 1977). Male calling is energetically expensive and calling in aggregations maximizes their chances of mating success (Weldon 2007). Males downwind of an aggregation might fly upwind in response to pheromone being released by calling males. There is a period of only about ten minutes during which males could fly to join the flying swarm (Tychsen 1977), and only enough time to mate once at each dusk, although males may mate in many dusk periods over their lifetime (Fay and Meats 1983, Radhakrishnan and Taylor 2008, Radhakrishnan *et al.* 2009). Males do not mate when temperatures at dusk are below 15°C with 50% of males mating at 20°C or higher temperatures (Meats and Fay 2000). Qfly have a relatively poor capacity to locate an odour source and it has been suggested that pheromones operate mainly within a single tree canopy (Meats and Hartland 1999, Weldon 2007). Acoustic cues are only effective over a short distance of about 50 cm (Mankin *et al.* 2004, 2008, Sivinski personal communication). Female Qfly move directly towards the males from up to 50 cm away (Tychsen 1977).

Odour plumes carried by light winds in trees usually become chaotic within a few centimetres of their source and provide few cues as to the direction of the source (Griffiths and Brady 1995). Qfly compensate for the diffused odour by making a series of short flights or walks (Meats and Hartland 1999) or by using large visual cues such as foliage to locate the source of odours (Dalby-Ball and Meats 2000). Female Qfly visit single male Qfly less frequently than aggregations (Weldon 2007). If the Qfly population is sparse, these limitations therefore result in single males being unlikely to attract a female and mate.

Meats (1998) estimated the chance of a successful mating between two Qfly on the same tree of 5 m × 5 m to be about 0.1%. Even in small cages, the chance of mating was only 0.8% (Fay and Meats 1983). A male Qfly has about a one in 400 chance of being in the right place at the right time if the density of males in the area was only one per hectare. Meats (1998) estimated that a single mating was probable when there were six male and six female Qfly present per hectare.

Current outbreak thresholds
Following the detection of small numbers of male Qfly (the number depends on the importing market), trading partners may fear that fruit harvested for trade could contain larvae that might establish populations in areas currently free from this

pest. In Anon. (1996), a breeding population is considered to have three indicators. Two are direct indicators; larvae detected in fruit harvested within the area or a mated female detected in monitoring traps. In fruit fly free areas, larval searches are not routinely undertaken by regulatory authorities at times when no fruit flies are detected, although they are sometimes conducted to meet some importing country requirements. If present, larvae are generally detected and reported by the public but these are rare events in the FFEZ. Because of inefficiency and difficulty of detecting larvae, a monitoring grid or array has been established to provide an early warning of incursions by adult Qfly.

Qfly populations are known to occur naturally in about a 50:50 male:female ratio (Dominiak *et al.* 2008, Clarke and Dominiak 2010). In the FFEZ, female Qfly are poorly attracted to monitoring traps (Dominiak *et al.* 2003a, Dominiak 2006, Dominiak and Nicol 2010). However, these traps and lures may be more successful in tropical regions (Clarke and Dominiak 2010). Due to the lack of reliable female lures, the monitoring array relies primarily on the trapping of male flies and this is a common situation in most countries (International Atomic Energy Agency 2003). In Australia, Willison discovered that male Qfly are attracted to raspberry ketone and subsequently experimented with a related chemical, cuelure (Allman 1958). Cuelure breaks down into raspberry ketone and this process is accelerated in the presence of moisture (Metcalf 1990). Sexually mature male Qfly are attracted to raspberry ketone in nature (Tan and Nishida 1995). While male flies trapped may be sexually mature, there is no current technology which can indicate if a Qfly male has mated and therefore that a breeding population exists. In the absence of this technology, Bateman (1991) proposed that five male flies are an indicator of a breeding population and this is later supported by Meats (1998).

Conditions under the current code
Bateman (1991) and subsequently Anon. (1996) recommended that five male flies trapped within 1 km of each other within a 14 day period was an appropriate outbreak threshold, or in essence indicated unacceptable risk of a breeding population. This standard has been accepted for domestic trade within Australia and by many international trading partners. However, some countries choose lower outbreak thresholds. Presumably, these lower standards are thought to provide a higher level of assurance, but there have been no empirical studies to support this.

As part of the 5-fly standard in Anon. (1996), there is an intermediate step, presumably to further investigate for the presence of a breeding population. When two

male flies are detected within one kilometre of each other within 14 days, 31 supplementary traps must be deployed within 200 metres (the outbreak zone) of the 2-fly detection and fruit must be checked for larvae. Supplementary traps must stay in place for nine weeks and be inspected twice weekly. If fewer than five male Qfly are trapped within 1 km within any 14 day period, an outbreak is not declared. In essence, it is deemed that a breeding population does not exist. If a total of five or more Qfly are detected within any 14 day period, an outbreak is declared for all domestic and international markets. After the outbreak declaration, no produce within the outbreak zone (within 200 m of the detection point) can be traded. All produce between 200 m and 15 km (the suspension area) must be treated with an approved disinfestation protocol before being transported into or sold in fruit fly sensitive markets (Jessup *et al.* 1998, De Lima *et al.* 2007).

The detection date of the last fly trapped is used to determine the reinstatement of area freedom based on generation tables in Anon. (1996). For some countries, these reinstatement periods vary from one generation plus 28 days, 12 weeks, three generations and one year. However apart from noting these differing standards, these reinstatement periods will not be discussed in detail further in this paper. Some countries have adopted the 2-fly threshold (within 400 m) as the outbreak threshold rather than the 5-fly threshold (within 1 km). For Australian exporters and regulators, the different outbreak thresholds result in disrupted trade and an administration burden. Moreover, the disparity in outbreak thresholds and reinstatement periods places regulatory authorities in a difficult position, needing to impose movement controls on host commodities destined for markets with different requirements.

Implications for different outbreak thresholds
Australian states and territories have agreed to the 5-fly threshold as an outbreak threshold. This agreement allows susceptible produce to be traded based on the specified conditions before or after an outbreak is declared. What happens when a trading partner requires a different threshold?

In the Australian response, the detection of two flies requires the deployment of supplementary traps and fruit searches. However since the Australian 5-fly outbreak threshold is not reached, no movement controls are imposed and fruit may move unrestricted from a 2-fly zone to any part of the pest free area or the rest of Australia. Further, no chemical control measures are deployed. This contrasts with countries that are more risk averse

and use a 2-fly threshold. A fruit fly outbreak in any country normally requires an eradication response and movement controls. Since Australia does not deploy these responses for a 2-fly threshold, the interpretation by a 2-fly importing country is that potentially infested produce can move from the area immediately around the 2-fly threshold to any other district.

What is the Australian response to these mixed thresholds? Australia only imposes eradication or movement controls after a 5-fly threshold and therefore countries using the 2-fly threshold may deem the entire or part of the pest free area infested. Trade in fruit fly host commodities under area freedom arrangements into 2-fly sensitive markets is likely to cease for the entire or part of the pest free area. Costly phytosanitary treatments are usually required for these 2-fly markets. The alternative is that Australia aligns its trade standard with the 2-fly threshold, and moves to a lower universal outbreak threshold. This action would decrease fruit fly free trade because the 2-fly threshold is reached more frequently than the 5-fly threshold. Due to the difficulties in servicing markets with different outbreak thresholds, would markets currently accepting the 5-fly threshold then also align with the 2-fly threshold? This possible change in outbreak threshold results in potentially all countries accepting the lowest outbreak threshold. One country is even more risk averse, requiring a 1-fly threshold for Qfly. If this strategy was adopted internationally by all countries for all species, the 1-fly threshold would become an unreasonable burden on all international trade. This strategy would significantly increase pesticide use in field eradication programs and cause most fruit to be unnecessarily treated with undesirable impact on the environment; some chemicals such as methyl bromide are green house gases. There would be significant benefits in harmonizing outbreak thresholds, but empirical evidence is required to support a preferred universal threshold.

New information published since the early 1990s.
Bateman's (1991) report was the basis for the current thresholds for outbreaks and these were adopted as a code of practice (Anon. 1996). More data of Qfly outbreaks have been published since Bateman (1991) and Anon. (1996), and these more recent publications may prove instructive in assessing the relative merits of the 5-fly and 2-fly thresholds. The monitoring grid is either a 400 m array in towns or a 1000 m array in orchards (Anon. 1996, Meats 1998). Fruit flies are reported to rarely disperse as far as one kilometre over their lifetime (Maelzer 1990, Bateman 1991, Meats 1996, Dominiak *et al.* 2003b, Meats *et al.* 2003, 2006, Meats and Edgerton 2008,

Weldon and Meats 2010, Gilchrist and Meats 2011). Given the large size of the FFEZ, we can then surmise that introductions of Qfly usually result from the carriage by humans of infested produce, and this is supported by assessment at roadblocks (Bateman 1972, Dominiak *et al.* 2000, Sved *et al.* 2003, Maelzer *et al.* 2004, Dominiak and Coombes 2009). Clift and Meats (2005) used Bayesian scenario analysis to show that introductions by local inhabitants contributed more to outbreaks than passing travellers. Most humans reside in urban areas and therefore the more intense monitoring array (400 m) in towns is a reflection of the greater risk (Meats 1998, Maelzer *et al.* 2004). Townships also provide better environments for survival and development of fruit flies than the surrounding rural areas (Yonow and Sutherst 1998, Raghu *et al.* 2000, Dominiak *et al.* 2006). Backyard environments are typically well watered and contain both sheltered microclimates and host fruit trees. Larger urban areas have an urban heat island which further minimizes the adverse effects of cold weather (Torok *et al.* 2001, Dominiak *et al.* 2006). The one kilometre grid is used in lower risk rural and orchard areas. These relatively sparsely populated rural areas are unlikely to be the first point of introduction of infested fruit and if they are, rural areas generally provide less favourable environments for fruit fly survival (Dominiak *et al.* 2006).

Meats (1998) suggests that a detection of two male flies within a two week period on the one kilometre grid represents a density between 2.1 and 6.57 flies per hectare within the outbreak zone (200 m radius from the discovery point). The upper estimate of 6.57 flies per hectare represents the most extreme situation in which the source of the incursion is directly in the centre of four adjacent traps in a grid, maximizing its distance from any trap. Meats (1998) proposed that when the density of flies within the outbreak zone exceeded six flies per hectare (of each sex), there was potential (albeit a very low risk) for one pair to mate. Superficially, the upper estimate of 6.57 flies per hectare appears to exceed the minimum density required for a mating to occur by 0.57 flies per hectare. However the theoretical minimum breeding density proposed by Meats (1998) of six male flies is an extremely conservative estimate and is essentially only a 'best guess' based on the information available at that time. Several critical factors used to obtain this theoretical minimum breeding estimate remain poorly understood. Meats (1998) estimated that the probability of a successful mating in the field was less than 0.1 although in calculating the minimum breeding density, the model assumed that it was equal to 0.1. This estimate of 0.1 was based on unpublished observations and has not been substantiated with data or

confirmed experimentally in the field. The model also assumes that there are ten dusk periods available for mating and that mating can occur each and every dusk period.

Tychsen and Fletcher (1971) concluded that mating only occurs within a 30 minute period each day so that sexually mature flies must be in close proximity at this time for mating to occur. Meats (1998) acknowledges that his estimate of ten dusk periods is also too high as it does not take into account adverse weather, the inhospitable environment, and other factors unfavourable to fruit flies. In reality, mating will only occur under favourable conditions and in the presence of an adequate population. Another factor included in the estimate was dispersal behaviour observed by Fletcher (1973, 1974a, 1974b) in a commercial orchard at Wilton, New South Wales. Fletcher's conclusions are specific to the coastal environment where his study was conducted and cannot be directly applied to inland pest free areas that are much less favourable to fruit flies (Dominiak *et al.* 2006). Meats (1998) also acknowledged in his closing remarks that verification of the models is still required and to date this issue remains unresolved. Meats (1998) recognized that his interpretation of trapping rates on the 1 km grid is conservative, and accordingly did not recommend that the detection of two flies should be the threshold for quarantine precautions, but rather a threshold to intensify the grid.

Data for 2007–2009 period
The period from February 2007 to April 2009 was chosen as a base to compare 2-fly and 5-fly thresholds. Information was provided by the state departments of agriculture in Victoria and New South Wales; there were no outbreaks in the South Australian portion of the FFEZ during this period. Climatically, autumn 2007 experienced near neutral values for the Southern Oscillation Index (SOI) with most parts of New South Wales and Victoria receiving average rainfall (Braganza 2008). The study area received slightly above average rainfall in spring and summer of 2007 followed by dry conditions in autumn, winter and spring 2008 (Duell 2009, Qi 2009). Average to below average rainfall occurred in the FFEZ in summer 2008–2009 and autumn 2008 however several exceptional heatwaves occurred in February 2009 (Mullen 2009). In this period, there were 27 outbreaks and these were allocated to one of two categories.

Category A was a response after detection of two flies, where 31 supplementary traps were deployed and larval searches undertaken according to Anon. (1996). No eradication or product movement controls were imposed. Trade to countries using the 2-fly threshold would have been suspended for that area. Trade was reinstated only after no flies were trapped for a

period of one generation plus 28 days. There was no restriction of trade with any Australian states or any 5-fly markets. There were 19 outbreaks in this category (Victoria: Invergorden 18 March 2008; Cobram 12 March 2008; Barooga 13 March 2008; Shepparton 10 April 2008; Bunbartha 11 April 2008; Katunga 14 April 2008; Numurkah 15 April 2008; Cobram East 2 June 2008; Echuca 18 September 2008; Irymple 24 March 2009. New South Wales: Yenda 11 April 2007; Darlington Point 26 April 2007; Yanco 29 May 2007; Lake Wyangan 12 March 2008; Hillston town 15 April 2008; Yenda 16 April 2008; Yanco 16 September 2008; Leeton town 16 September 2008; Hillston orchard 22 September 2008).

Category B was based on a 5-fly threshold. Subsequent procedures were according to Anon. (1996); supplementary traps and larval searches were conducted, eradication programs and product movement controls were initiated, and a 15 km suspension zone was established. Trade in fruit fly free produce to all domestic and international markets (including countries using the 2-fly threshold) was suspended for all host commodities grown within the suspension zone until there were no flies trapped for one generation plus 28 days. There were eight outbreaks in this category (Victoria: Koonoomoo 2 February 2007; Invergordon 20 March 2008; Bunbartha 22 April 2008; Katunga 13 May 2008; Cobram East 19 June 2008; Shepparton 3 April 2009. New South Wales; Narrandera 23 May 2007; Yanco 28 October 2008.)

Of the 27 outbreaks, 19 Category A outbreaks (70.4% of all outbreaks) did not progress to a Category B outbreak despite supplementary trapping and larval searches. Even with the low level of progression to the 5-fly threshold (29.6%), all susceptible host produce from the pest free area required disinfestation before being exported to markets requiring any threshold other than the 5-fly threshold. Meats *et al.* (2003) found 71% of single Qfly detections did not lead to 5-fly outbreaks and self extinguished without any eradication response. The 2007–2009 data for the 2-fly threshold of 70.4% is consistent with Meats *et al.* (2003).

Riverina trade volume since 1996
There has been considerable trade in host produce from the FFEZ since 1996 using the 5-fly threshold without any reports of larvae found in produce. This confirms that area freedom certification procedures for Australia's pest free area are robust given that consumers are highly likely to report and return damaged fruit to retailers. The volume of produce varies from year to year. Australian Bureau of Statistics (2008) reported that, for the statistic local areas of Carrathool, Griffith, Leeton and Murrumbidgee, 8586, 166 689 and 172 387 tonnes of stone fruit, oranges and

other citrus respectively was produced. These combined industries are valued at $86.492 M (Australian Bureau of Statistics 2008). Given the volume and value of fruit traded annually, if the 2-fly threshold was an accurate indicator of crop infestation, it is likely that Qfly would have been detected in consignments in domestic or international market during the past 15 years.

Closing comments

Qfly is recognized as a poor colonizer in fruit fly free areas such as the FFEZ, owing to hostile conditions for survival and reproduction (Bateman 1972, 1977, Fletcher 1987, Edge *et al.* 2001, Meats *et al.* 2003, Weldon 2007). Even introduction by human activity (jump dispersal) very rarely results in establishment (Maelzer *et al.* 2004, Meats and Edgerton 2008). Given the large volume of produce traded without incident, the 5-fly threshold has a proven track record of success in providing highly effective phytosanitary assurance. Based on the evaluation of outbreak data, there is no indication that the 2-fly threshold provides any additional assurance. On this basis, we recommend that international trading partners adopt the 5-fly threshold as a universal threshold that provides a high level of assurance and also enables increased trading opportunity.

Acknowledgments

References

Allman, S.L. (1958). Queensland fruit fly. New South Wales Department of Agriculture. Conference of Commonwealth and State Entomologists. Brisbane, May 1957. Commonwealth Scientific and Industrial Research Organisation, pp. 68-70.

Allwood, A. (2000). Regional approach to the management of fruit flies in the Pacific Island countries and territories. *In* 'Area-wide control of fruit flies and other insect pests', ed. K.H. Tan, pp. 439-48. (Penerbit Universiti Sains Malaysia, Penang).

Anon. (1996). Code of practice for the management of Queensland fruit fly. Standing Committee on Agriculture and Resource Management, Department of Primary Industries, Canberra.

Australian Bureau of Statistics (2008). Agricultural commodities: small area data, Australia, 2005–2006. Report 71250DO003.

Bateman, M.A. (1972). The ecology of fruit flies. *Annual Review of Entomology* 17, 493-518.

Bateman, M.A. (1977). Dispersal and species interactions as factors in the establishment and success of tropical fruit flies in new areas. *Proceedings of the Ecological Society of Australia* 10, 106-12.

Bateman, M.A. (1991). The impact of fruit flies on Australian horticulture. Horticultural Policy Council Report No. 3. ISBN 0642161100.

Braganza, K. (2008). Seasonal climate summary southern hemisphere (autumn 2007): La Nina emerges as a distinct possibility in 2007. *Australian Meteorological Magazine* 57, 65-75.

Clarke, A.R. and Dominiak, B.C. (2010). Positive correlation of male and female *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) catches in orange-ammonia traps. *General and Applied Entomology* 39, 9-13.

Clarke, A.R., Powell, L.S., Weldon, C.W. and Taylor, P.W. (2011). The ecology of *Bactrocera tryoni* (Diptera: Tephritidae): what do we know to assist pest management? *Annals of Applied Biology* 158, 26-54.

Clift, A. and Meats, A. (2005). Use of a Bayesian Belief Network to identify situations that favour fruit fly incursions in inland SE Australia. International Congress on Modelling and Simulation MODSIM 2005, eds A. Zerger and R.M. Argent, pp. 170-6. (Modelling and Simulation Society of Australia and New Zealand).

Courtice, A.C. and Drew, R.A.I. (1984). Bacterial regulation of abundance in tropical fruit flies (Diptera: Trephritidae). *Australian Zoology* 21, 251-68.

Dalby-Ball, G. and Meats, A. (2000). Influence of the odour of fruit, yeast and cuelure on the flight activity of the Queensland fruit flies, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology* 39, 195-200.

De Lima, C.P.F., Jessup, A.J., Cruickshank, L., Walsh, C.J. and Mansfield, E.R. (2007). Cold disinfestation of citrus (*Citrus* spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science* 35, 39-50.

Dominiak, B.C. (2006). Review of the use of protein food based lures in McPhail traps for monitoring Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *General and Applied Entomology* 35, 7-12.

Dominiak, B.C., Campbell, M., Cameron, G. and Nicol, H. (2000). Review of vehicle inspection historical data as a tool to monitor the entry of hosts of Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) into a fruit fly free area. *Australian Journal of Experimental Agriculture* 40, 763-71.

Dominiak, B.C. and Coombes, N.M. (2009). Fruit carrying characteristics of travellers into a quarantine zone in New South Wales in 1999/2000. *Plant Protection Quarterly* 24, 14-19.

Dominiak, B.C., Gilmour, A.R., Kerruish, B. and Whitehead, D. (2003a). Detecting low populations of Queensland fruit fly *Bactrocera tryoni* (Froggatt) with McPhail and Lynfield traps. *General and Applied Entomology* 32, 49-53.

Dominiak, B.C., Mavi, H.S. and Nicol, H.I. (2006). Effect of town microclimate on the Queensland fruit fly *Bactrocera tryoni*. *Australian Journal of Experimental Agriculture* 46, 1239-49.

Dominiak, B.C., McLeod, L.J. and Landon, R. (2003b). Further development of a low-cost release method for sterile Queensland fruit fly, *Bactrocera tryoni* (Froggatt) in rural New South Wales. *Australian Journal of Experimental Agriculture* 43, 407-17.

Dominiak, B.C. and Nicol, H.I. (2010). Field performance of Lynfield and McPhail traps for monitoring male and female sterile (*Bactrocera tryoni* Froggatt) and wild *Dacus newmani* (Perkins). *Pest Management Science* 66, 741-4.

Dominiak, B.C., Sundaralingham, S., Jiang, L., Jessup, A.J. and Barchia, I.M. (2008). Production levels and life history traits of mass reared Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) during 1999/2002 in Australia. *Plant Protection Quarterly* 23, 131-5.

Drew, R.A.I. (1974). The responses of fruit fly species (Diptera: Tephritidae) in the South Pacific area to male attractants. *Journal of the Australian Entomological Society* 13, 267-70.

Drew, R.A.I. (1987). Behavioural strategies of fruit flies of the genus *Dacus* (Diptera: Tephritidae) significant in mating and host-plant relationships. *Bulletin of Entomological Research* 77, 73-81.

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

Drew, R.A.I. and Lloyd, A.C. (1987). Relationship of fruit flies (Diptera: Tephritidae) and their bacteria to host plants. *Annals of the Entomological Society of America* 80, 629-36.

Drew, R.A.I., Zalucki, M.P. and Hooper, H.H.S. (1984). Ecological studies of Eastern Australia fruit flies (Diptera: Tephritidae) in their endemic habitat. I. Temporal variation in abundance. *Oecologia* (Berlin) 64, 267-72.

Duell, R. (2009). Seasonal climate summary southern hemisphere (autumn 2008): a return to neutral ENSO conditions

in the tropical Pacific. *Australian Meteorological and Oceanographic Journal* 58, 63-77.

Edge, V., Gardner, R., Green, A., Cock, K. and Cornish, J. (2001). Technical review of the Tri-State Strategy for Queensland Fruit Fly. Report to Standing Committee on Agriculture and Resource Management. January 2001, 62 pp.

Ero, M.M., Hamacek, E. and Clarke, A.R. (2011). Foraging behaviours of *Diachasmimorpha kraussi* (Fullaway) (Hymenoptera: Braconidae) and its host *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) in a nectarine (*Prunus persica* (L.) Batsch var. *nectarina* (Aiton) Maxim) orchard. *Australian Journal of Entomology* 50, 234-40.

Fanson, B.G., Weldon, C.W., Perez-Staples, D., Simpson, S.J. and Taylor, P.W. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* 8, 1-10.

Fay, H.A.C. and Meats, A. (1983). The effects of age, ambient temperature, thermal history and mating history on mating frequency in males of the Queensland fruit fly, *Dacus tryoni*. *Entomologia Experimentalis et Applicata* 35, 273-6.

Fletcher, B.S. (1973). The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni* IV. The immigration and emigration of adults. *Australian Journal of Zoology* 21, 541-65.

Fletcher, B.S. (1974a). The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni* V. The dispersal of adults. *Australian Journal of Zoology* 22, 189-202.

Fletcher, B.S. (1974b). The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni* VI. Seasonal changes in fruit fly numbers in the areas surrounding the orchard. *Australian Journal of Zoology* 22, 353-63.

Fletcher, B.S. (1987). The biology of dacine fruit flies. *Annual Review of Entomology* 32, 115-44.

Fletcher, B.S. (1989). Movements of tephritid fruit flies. *In* 'Fruit flies, their biology, natural enemies and control' Vol. 3B, eds A.S. Robinson and G. Hooper, pp. 209-19. (Elsevier, Amsterdam).

Gilchrist, A.S. and Meats, A.W. (2011). Factors affecting the dispersal of large-scale releases of the Queensland fruit fly, *Bactrocera tryoni*. *Journal of Applied Entomology* 37, 186-8.

Griffiths, N. and Brady, J. (1995). Wind structure in relation to odour plumes in tsetse fly habitats. *Physiological Entomology* 20, 286-92.

Hancock, D.L., Hamacek, E.L., Lloyd, A.C. and Elson-Harris, M.M. (2000). The distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia. DPI Publications, Brisbane, Australia. ISSN 0727-6273.

International Atomic Energy Agency (2003). Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency, Vienna. pp. 47.

Jessup, A.J., Carswell, I.F. and Dalton, S.P. (1998). Disinfestation of fresh fruits from *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) with combination mild heat and modified atmosphere packaging. *Australian Journal of Entomology* 37, 186-8.

Li, B., Ma, J., Hu, X., Liu, H., Wu, J., Chen, H. and Zhang, R. (2010). Risk of introducing exotic fruit flies, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis rosa* (Diptera: Tephritidae), into southern China. *Journal of Economic Entomology* 103, 1100-11.

Maelzer, D.A. (1990). Fruit fly outbreaks in Adelaide, S.A., from 1948–49 to 1986–87. I. Demarcation, frequency and temporal patterns of outbreak. *Australian Journal of Zoology* 38, 439-52.

Maelzer, D.A., Bailey, P.T. and Perepelicia, N. (2004). Factors supporting the non-persistence of fruit fly populations in South Australia. *Australian Journal of Experimental Agriculture* 44, 109-26.

Mankin, R.W., Anderson, J.B., Mizrach, A., Epsky, N.D., Shuman, D., Heath, R.R., Mazor, M., Hetzroni, A., Grinshpun, J., Taylor, P.W. and Garrett, S.L. (2004). Broadcasts of wing-fanning vibrations recorded from calling male *Ceratitis capitata* (Diptera: Tephritidae) increases captures of females in traps. *Journal of Economic Entomology* 97,1299-309.

Mankin, R.W., Lemon, M., Harmer, A.M.T., Evans, C.S. and Taylor, P.W. (2008). Time-pattern and frequency analyses of sounds produced by irradiated and untreated male *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) during mating behaviour. *Annals of the American Entomological Society* 101, 664-74.

Meats, A. and Fay, F.A.C. (2000). Distribution of mating frequency among males of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), in relation to temperature, acclimation and change. *General and Applied Entomology* 29, 27-30.

Meats, A. and Hartland, C.L. (1999). Upwind anemotaxis in response to cue-lure by the Queensland fruit fly *Bactrocera tryoni*. *Physiological Entomology* 24, 90-7.

Meats, A. and Smallridge, C.J. (2007). Short and long range dispersal of Medfly, *Ceratitis capitata* (Diptera: Tephritidae) and its invasive potential. *Journal of Applied Entomology* 8, 518-23.

Meats, A., Smallridge, C.J. and Dominiak, B.C. (2006). Dispersion theory and the sterile insect technique: application to two species of fruit fly. *Entomologia Experimentalis et Applicata* 119, 247-54.

Meats, A.M. (1996). Demographic analysis of sterile insect trials with Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *General and Applied Entomology* 27, 2-12.

Meats, A.M. (1998). The power of trapping grids for detecting and estimating the size of invading propagules of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) risks of subsequent infestation. *General and Applied Entomology* 28, 47-55.

Meats, A.M., Clift, A.D. and Robson, M.K. (2003). Incipient founder populations of Mediterranean and Queensland fruit flies in Australia: the relation of trap catch to infestation radius and models for quarantine radius. *Australian Journal of Experimental Agriculture* 43, 397-406.

Meats, A.M. and Edgerton, J.E. (2008). Short- and long-distance dispersal of Queensland fruit fly, *Bactrocera tryoni* and its relevance to invasive potential, sterile insect technique and surveillance trapping. *Australian Journal of Experimental Agriculture* 48, 1237-45.

Metcalf, R.L. (1990). Chemical ecology of Dacine fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America* 83, 1017-30.

Mullen, C. (2009). Seasonal climate summary southern hemisphere (summer 2008-09): a weak, brief La Nina returns. Bumper wet season in tropical Australia: exceptional heatwaves in southeastern Australia. *Australian Meteorological and Oceanographic Journal* 58, 275-84.

Oliver, J. (2007). Summary of national fruit fly-related activities released. Plant Protection News. February 2007. Department of Agriculture, Fisheries and Forestry.

Perez-Staples, D., Harmer, A.M.T., Collins, S.R. and Taylor, P.W. (2008). Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. *Agricultural and Forest Entomology* 10, 255-62.

Perez-Staples, D., Prabhu, V. and Taylor, P.W. (2007). Post-teneral protein feeding enhances sexual performance of Queensland fruit flies. *Physiological Entomology* 32, 127-35.

Plant Health Australia (2010). National Plant Biosecurity Strategy. Plant Health Australia, Deakin, Australian Capital Territory.

Prabhu, V., Pérez-Staples, D. and Taylor, P.W. (2008). Protein: carbohydrate ratios promoting sexual activity and longevity of male Queensland fruit flies. *Journal of Applied Entomology* 132, 575-82.

Qi, L. (2009). Seasonal climate summary southern hemisphere (spring 2008): La Nina pattern returning across equatorial Pacific. *Australian Meteorological and Oceanographic Journal* 58, 199-208.

Radhakrishnan, P., Perez-Staples, D., Weldon, C.W. and Taylor, P.W. (2009). Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. *Animal Behaviour* 78, 839-46.

Radhakrishnan, P. and Taylor, P.W. (2008). Ability of male Queensland fruit flies to inhibit receptivity in multiple mates, and the associated recovery of accessory glands. *Journal of Insect Physiology* 54, 421-8.

Raghu, S., Clarke, A.R., Drew, R.A.I. and Hulsman, K. (2000). Impact of habitat modification on the distribution and abundance of fruit flies (Diptera: Tephritidae) in southeast Queensland. *Population Ecology* 42, 153-60.

Stanaway, M.A., Zalucki, M., Gillespie, P.S., Rodriguez, C.M. and Maynard, G.V. (2001). Pest risk assessment of insects in sea cargo containers. *Australian Journal of Entomology* 40, 180-92.

Sved, J.A., Yu, H., Dominiak, B. and Gilchrist, A.S. (2003). Inferring modes of colonization for pest species using heterozygosity comparisons and shared-allele test. *Genetics* 163, 823-31.

Stephenson, B.P., Gill, G.S.C., Randall, J.L. and Wilson, J.A. (2003). Biosecurity approaches to surveillance and response for new plant pest species. *New Zealand Plant Protection* 56, 5-9.

Tan, K.H. and Nishida, R. (1995). Incorporation of raspberry ketone in the male rectal glands of the Queensland fruit fly *Bactrocera tryon*i Froggat (Diptera: Tephritidae). *Applied Entomology and Zoology* 30, 494-7.

Torok, S.J., Morris, J.G., Skinner, C. and Plummer, N. (2001). Urban heat island features of southeast Australian towns. *Australian Meteorological Magazine* 50, 1-13.

Tychsen, P.H. (1977). Mating behaviour of the Queensland fruit fly, *Dacus tryoni* (Diptera: Tephritidae), in field cages. *Journal of the Australian Entomological Society* 16, 459-65.

Tychsen, P.H. and Fletcher, B.S. (1971). Studies on the rhythm of mating in the Queensland fruit fly, *Dacus tryoni*. *Journal of Insect Physiology* 17, 2139-56.

Weldon, C. (2003). Effectiveness of coloured unbaited sticky traps for monitoring dispersal of gamma-irradiated Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *General and Applied Entomology* 32, 55-60.

Weldon, C. (2007). Influence of male aggregation size on female visitation in *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology* 46, 29-34.

Weldon, C. and Meats, A. (2007). Short-range dispersal of recently emerged males and females of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) monitored by sticky sphere traps baited with protein and Lynfield traps baited with cue-lure. *Australian Journal of Entomology* 46, 160-6.

Weldon, C. and Meats, A. (2010). Dispersal of mass-reared sterile, laboratory-domesticated and wild male Queensland fruit flies. *Journal of Applied Entomology* 134, 16-25.

Weldon, C.W., Prenter, J. and Taylor, P.W. (2010). Activity patterns of Queensland fruit flies (*Bactrocera tryoni*) are affected by both mass-rearing and sterilization. *Physiological Entomology* 35, 148-53.

Weldon, C.W. and Taylor, P.W. (2010). Desiccation resistance of adult Queensland fruit flies *Bactrocera tryoni* decreases with age. *Physiological Entomology* 35, 385-90.

Weldon, C.W. and Taylor, P.W. (2011). Sexual development of wild and mass-reared male Queensland fruit flies in response to natural food sources. *Entomologia Experimentalis et Applicata* 139, 17-24.

White, I.M. and Elson-Harris, M.M. (1992). 'Fruit flies of economic significance: their identification and bionomics'. (CAB International, Oxon, UK).

Yonow, T. and Sutherst, R.W. (1998). The geographical distribution of the Queensland fruit fly, *Bactrocera (Dacus) tryoni*, in relation to climate. *Australian Journal of Agricultural Research* 49, 935-53.

APPENDIX 2

The influence of mixtures of parapheromone lures on trapping of fruit fly in New South Wales, Australia

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Abstract

Tephritid fruit flies of economic importance are monitored using traps containing either cuelure (CL) or methyl eugenol (ME) as an attractant. There would be potential economic advantages if both lures could be combined in a single trap without compromising trapping efficiency. This study presents results from two trials testing combinations of cuelure (4.4 mL) and methyl eugenol (0.5 mL and 2.2 mL) in Lynfield traps near Griffith, NSW and in Sydney.

For the Griffith trial, the addition of 2.2 mL of methyl eugenol to the standard cuelure wick quadrupled the overall capture of sterile Queensland fruit fly (Qfly) although significant differences were detected in only one of four trials. Traps were placed between 5 and 55 m from the release point, and distance had no significant effect on the number of flies trapped. Time after trap deployment and all time interactions were significant. The proportion of sterile Qfly trapped within three weeks in the first three releases was >91% of total flies trapped in the CL–ME combinations while the CL only treatment recaptured <83% in the same period. Newman fruit fly were trapped with all treatments but not analysed.

In Sydney, the combined lure trapped fewer Qfly, although overall the treatments were not significantly different. There was a seasonal effect with cuelure alone attracting more flies than the combination lure in February and August and less in March and April. The combined lure lowered the capture of fruit fly attracted to methyl eugenol by 88%. Reasons for the discrepancies between the trials are discussed, as well as the potential advantages for surveillance for Qfly and exotic fruit flies. Two additional species attracted to the lure combination are noted.

Keywords: Queensland fruit fly, cuelure, methyl eugenol, *Bactrocera*.

Introduction

There are about 4500 species of fruit flies world-wide. In the Pacific area alone, there are 350 species of which at least 25 species are regarded as being of major economic importance (Allwood 2000). The genus *Bactrocera* contains over 400 species, distributed primarily though the Asia-Pacific area including Australia (Drew 1974). Tephritidae fruit flies cause direct losses to many fresh fruit and some vegetable industries, resulting in adverse impacts on trade and potentially to the economies of many countries (Stephenson *et al.* 2003). With the increasing globalization of trade, fruit flies are a major quarantine concern triggering the implementation of regional surveillance programs (Stanaway *et al.* 2001, IAEA 2003, Reid and Malumphy 2009). Males of these species are generally regarded as being attracted to the parapheromones cuelure or methyl eugenol (CL and ME respectively) but not both lures.

The National Exotic Fruit Fly monitoring program is deployed in most Australian ports as an early warning program for the entry of exotic fruit flies (Gillespie 2003). At each monitoring site for exotic *Bactrocera* species, separate monitoring traps are baited with either CL or ME (Drew 1974, Cunningham 1989, Gillespie 2003, IAEA 2003). The possible advantages of CL–ME mixtures in wicks has been reported in sub-tropical Australia (Hooper 1978) and in other countries with different species (Umeya and Hirao 1975, Ito *et al.* 1976, Liu 1989, Vargas *et al.* 2000, Shelley *et al.* 2004). The economic benefit of combining lures in one trap would be considerable. In surveillance programs, each additional trap that requires inspection is a cost in staff and materials to service and administer. In a national stocktake, Oliver (2007) reported that $128.7 million would be spent on fruit fly related activities in Australia from July 2003 to June 2008. Within this figure, $34.3 million would be spent on surveillance. In neighbouring New Zealand that has no endemic fruit flies, approximately NZ$1 million is spent annually on an early detection surveillance program for fruit flies (Stephenson *et al.* 2003). Any improvement in surveillance efficiency is likely to have significant financial benefits for Australia and New Zealand.

The objective of this study was to evaluate the efficacy of the addition of ME to standard CL wicks in attracting Qfly in the dry inland environment and the coastal environment in Sydney. Other species were trapped and are reported here but not analysed.

Materials and methods

Traps, lures and identification

Lynfield traps were used to monitor fruit flies in both locations. These traps consisted of a 1 L cylindrical clear plastic pot with a screw lid. The pots were 120 mm in diameter and 120 mm deep. There were four 2 mm drain holes in the bottom to prevent the accumulation of rainwater. Four equally-spaced 25 mm diameter holes were cut into the side of the trap. These holes allow the egress of pheromone and ingress of insects. Wicks are made using four dental cotton rolls (1 × 4 cm long) held together by metal clamp and suspended from the middle of the trap lid.

Four lure mixtures were evaluated. Treatment A consisted wicks baited with 5 mL of solution containing eight parts CL and one part Maldison (1150 gL⁻¹ active ingredient); this is the standard CL lure in New South Wales. Treatments B and C consisted of wicks baited with Treatment A to which was added either 0.5 or 2.2 mL of ME respectively. In Sydney, Treatment D was available with wicks baited with 2 mL of solution containing eight parts ME and one part Maldison (1150 gL⁻¹ active ingredient). Trapped flies were sent for identification to the Agricultural Scientific Collections Unit at the Orange Agricultural Institute, Orange, New South Wales (NSW). All detections and identifications were recorded on the state database and data retrieved later for analysis (Dominiak *et al.* 2007).

Griffith trials

A trial comparing Treatments A, B and C was established in an orchard near Griffith

in inland NSW. The three treatments were hung on adjacent trees with an average of 5.8 m between traps (range 3.1 to 9.3 m). The treatments were replicated at 10 different sites within the orchard. All traps were inspected 30 times from 19 March 2003 to 30 December 2003. Traps were not inspected in June or July (winter). Treatment D (ME only) was not used in the Griffith trial site since there are no naturally occurring ME-responsive species in that area.

Since wild Qfly are quickly eradicated at Griffith due to trade requirements, a test population of sterile Qfly from a mass rearing strain was released. Flies were mass reared, dyed, irradiated and transported to Griffith under standard protocols established for sterile releases (Dominiak *et al.* 2008). Sterile flies were released four times (5 March, 22 August, 1 November and 5 December 2003) in the orchard. A single release site was used and fruit flies were released using a pupal release technique similar to Dominiak *et al.* (2003a). No additional protein, sugar or water was provided for adults. The GPS coordinates of the release site and trap sites were taken using hand held equipment and the distance from the release point to each trap was calculated. The proportion of flies recaptured in the three weeks following release was calculated. *Dacus newmani* (Perkins) (Newman fly), an Australian native fruit fly came from the local environment. While the trappings are reported here, the results were not analysed as the species is of no economic importance.

Sydney trial
There is an extensive fruit fly trapping array in Sydney maintained as part of the National Exotic Fruit Fly Monitoring program to detect both CL- and ME-responsive species (Gillespie 2003). All flies came from the local environment. We used nine of these trapping sites in the present study. Each experimental site already had two Lynfield traps in separate trees (treatment A and D).

At the nine experimental sites, an additional Lynfield trap was deployed containing a mixture of CL and ME, corresponding to Treatment C above. Traps were inspected 22 times (fortnightly) from 12 January 2007 to 22 October 2007. New CL lures were deployed in January and September 2007 as part of the normal replacement procedure for the program.

Data analysis
In the Griffith experiment, the number of male sterile Qfly (Y) for each trap was fitted with a linear mixed model as follows: $\log_{10} (Y+1)$ = fixed terms (treatment, release, time after release, distance and all interactions) + random terms (replicate and its interaction with cuelure and release). All parameters were estimated using Residual Maximum Likelihood (REML) estimation. All analyses were run on Genstat Windows Version 9 (VSN International Ltd 2006).

For the Sydney data, the number of male wild Qfly (Y) for each trap was fitted using a linear model: $\log_{10} (Y+1)$ = fixed terms (treatment, fortnight and interactions). Non-significant terms were dropped from the final model. All analyses were carried out in Genstat Versions 13 (VSN international Ltd 2010). Other species were not analysed due to the low numbers trapped.

Results
Griffith trials
The total number of flies recaptured in the Griffith trials is shown in Table 1. While trappings varied greatly between trials, there was no overall significant difference between treatments, but there were significant differences within releases (P <0.001). For example, there were significant differences in capture rates between treatment A and C for the release on 11 November, but not for releases on 5 March, 22 August and 5 December. The distance parameter (P = 0.60) and all distance interactions were not significant. Trap catches decreased as trapping time after release increased (P <0.001). Regarding the proportion of sterile Qfly trapped within three weeks, the first three releases resulted in >91% of total flies trapped in the CL–ME combinations while the CL treatment recaptured <83% in the same period. The fourth release was monitored for only 20 days and was not included in these calculations. *D. newmani* was trapped in the August, November and December release periods in all three treatment lures (Table 1).

Sydney trial
The total number of each species trapped with each lure in the Sydney trail is shown in Table 1. In contrast to the Griffith trial, fewer CL-responsive flies were trapped in mixed lure traps (treatment C) than in CL traps (treatment A) (P <0.001). The treatment by fortnight interaction was significant (P <0.001), indicating different relative trapping rates through the year (see Figure 1). Treatment C trapped more Qfly than treatment A in the March-May period. During winter, both treatments trapped very small numbers of Qfly. In July-September, treatment A attracted more Qfly than treatment C, after which

Table 1. Numbers of each species trapped by each lure for the Griffith and Sydney trials.

|	Types of lures			
	Treatment A	Treatment B	Treatment C	Treatment D
Species	CL (4.4 mL)	CL (4.4 mL) + ME (0.5 mL)	CL (4.4 mL) + ME (2.2 mL)	ME (2.0 mL)
Griffith – CL responsive species				
5 March sterile Qfly release				
B. tryoni (sterile)	350	2042	261	*
D. newmani	0	0	0	*
22 August sterile Qfly release				
B. tryoni (sterile)	1023	2048	5283	*
D. newmani	528	118	260	*
1 November sterile Qfly release				
B. tryoni (sterile)	2223	326	9086	*
D. newmani	128	150	140	*
5 December sterile Qfly release				
B. tryoni (sterile)	1425	2300	2293	*
D. newmani	41	25	54	*
Sydney – CL responsive species				
B. tryoni	4848	*	2648	32
D. aequalis (Coquillett)	104	*	83	0
D. absonifacies (May)	74	*	45	2
Sydney – ME responsive species				
B. cacuminata (Hering)	0	*	277	2267
B. endiandrae (Perkins and May)	0	*	0	1

* = no data
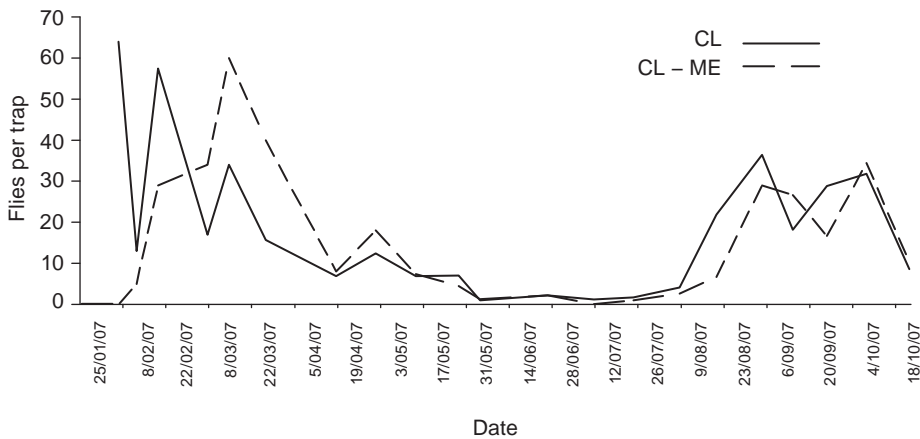
Figure 1. Trappings in Sydney of Qfly into cuelure and the cuelure–methyl eugenol combination traps.

no treatment was consistently more effective.

For the ME attracted species, the combined lure (treatment C) greatly reduced the numbers of flies trapped. Most notably, the number of *B. cacuminata* (Hering) attracted by the combined lure traps was only 12% of treatment D (ME-only).

Discussion
General
There is a general taxonomic concept that fruit flies are attracted to either CL or ME but not a combination of both lures (Drew 1974), even though both of these lures or their derivatives are plant extracts. More recent publications suggest that different mixture combinations or spatial proximity of different lures may affect trap catches. *B. cucurbitae* (Coquillett) is normally attracted to CL only. Shelley *et al.* (2004) found that the addition of ME placed in the same wick or within 3 m of CL resulted in an increase in the capture of *B. cucurbitae*. Vargas *et al.* (2000) found the response of *B. cucurbitae* to low levels of cross mixtures resulted in significant differences and that the season had a significant effect.

Liu (1989) found a mixture with 10% and 20% ME added to CL was more effective than CL alone at attracting *Dacus cucurbitae*. Hooper (1978) noted a Taiwanese report that reported *D. cucurbitae* trapping almost doubled as a result of adding ME to CL. Of the species normally attracted to CL, Hooper (1978) found that the addition of ME to the CL wick did not significantly decrease the capture of *Dacus tryoni*, *Dacus neohumereralis* (Hardy) or *Callandra aequalis* (Coquillett). However the capture rate was significantly improved when CL and ME lures were hung side by side.

Griffith trials
Our results have some similarities to those of Vargas *et al.* (2000) and Hooper (1978). Like Vargas *et al.*, we found that the

relative numbers of sterile Qfly trapped by the different lure mixtures varied greatly between the seasons. But like Hooper, we found no overall significant effect of the different treatment lures on numbers trapped. Our treatment B was similar to the 10% ME addition to CL tested by Liu (1989) who found a 10% ME mixture was more effective than CL alone. There are a number of possible confounding effects that could be affecting relative trapping rates. Firstly, there could be differences in fly physiology in different seasons affecting the reaction of the flies to lures. Secondly, environmental variation though the year (temperature and/or humidity) could affect the quantity or quality of the volatiles produced by the different mixtures. Differences in the availability of natural food sources could also vary seasonally, affecting fly responses. Thirdly, the responses of the mass reared strain may also be different to that of wild flies due to the genetic effects of adaptation to the mass rearing environment. Overall, the variability between the different trials at Griffith suggests that more trials will be required to identify factors affecting Qfly trapping rates.

Nevertheless for Qfly, treatment C did not result in a significant decrease in sterile Qfly numbers in three of the four evaluations. We infer that using this CL–ME mixture for Qfly is unlikely to have any detrimental impact on catches. However, for treatment B, there was a notable decrease in Qfly trapped in the third release, lending caution to the conclusion that ME has no detrimental impact on catches.

In our evaluations, a small number of traps caught most of the flies. This clumping effect was independent of distance (at distances up to 55 m) and was similar to the findings of Horwood and Keenan (1994) and Meats (2007). Meats (2007) reported that wild and sterile Qfly had clumped distributions, particularly at low densities.

We found that trappings did not vary significantly over short distances from the release point, i.e. within 55 m of the release point. Our results are consistent with Weldon and Meats (2007) who found no significant trend in the recapture rate with distance from release point up to 88 m. Fletcher (1974) however, proposed a rule that the number of the flies captured was proportional to the inverse distance from release point. Weldon and Meats (2007) suggested that Fletcher's rule probably became operational at some point after 100 m from the release point. Meats and Edgerton (2008) reconciled both short and longer distance trapping results by showing that a long-tailed (Cauchy) distribution provides an adequate dispersal model for all distances up to 1000 m.

Dacus newmani were trapped in the August, November and December periods but not in March. Our results are consistent with Gillespie (2003) who reported that this species has a major flight in spring and was captured in small numbers at other times of the year. Our report appears to be the first peer reviewed report of *D. newmani* being attracted to the CL–ME combination. The addition of ME to CL attracted very few non-target species. This would be a positive outcome if the lure combination was adopted as an enhanced male attractant. The trapping of large numbers of non-target species is an undesirable attribute of wet protein traps (Dominiak *et al.* 2003b, Dominiak 2006).

Longevity of sterile flies in the field is a significant issue impacting on the frequency of release. Some species survive less than a week and require weekly releases (Hernandez *et al.* 2007). The March and November releases for CL attracted 82.5% and 71.3% respectively (within three weeks) of the total treatment catch. This is consistent with Dominiak and Webster (1998) who reported 85.7% recaptured after three weeks. The CL–ME combinations seem to attract more flies within the 21 day period compared with CL alone in the March and November releases. Given the perception that the ME plume travels a longer distance than the CL plume, we suggest that the addition of ME might attract more flies from longer distances more quickly compared with CL alone. This could be an advantage for the trapping out technique to quickly deplete a population, prior to a sterile release deployment. This chemical combination could also be useful in the male annihilation technique. Vargas *et al.* (2000) found the combination lure lasted well in fibreboard discs in the field.

Sydney trial
The Sydney trial contrasted with the Griffith trial. In Sydney, the mixed lure traps caught only half of the number of Qfly which were trapped in CL traps. We can

only speculate the reasons for these differences. The environmental conditions in the drier inland may create a different result compared with the moister environment of the Sydney basin (our results) or the Queensland coast (Hooper 1978, Dominiak *et al.* 2006). Alternatively the difference between the trials may be due to strain differences: sterile flies were used in the Griffith trial and the wild flies were trapped in the Sydney trial. Weldon and Meats (2010) reported no significant differences in the capture of sterile and wild flies in Sydney, but that result may be relevant to the harsher inland environment. Our Sydney trial and that of Hooper (1978) were conducted in humid coastal environment. Hooper used lower amounts of lure (1.5 mL of CL and ME) than the present trials.

The range of species trapped in this trial was consistent with those reported for Sydney by Osborne *et al.* (1997) and Gillespie (2003). This trial showed that treatment C attracted CL responsive species (Qfly, *D. aequalis* and *D. absonifacies*) but only attracted 10% of the ME responsive *B. cacuminata* compared with ME alone. Hooper (1978) found that captures of *B. cacuminata* were reduced by the CL–ME mixture in comparison to ME alone. Shelly *et al.* (2004) also found the same asymmetry between CL and ME responsive species. They speculated that this may indicate that ME response evolved later in Dacinae than CL response. Since *B. cacuminata* is not of economic importance this reduction should not influence the use of combined traps for surveillance. However, since some economically important exotic *Bactrocera* species are ME-responsive, this aspect requires further investigation. As in the Griffith trial, the CL–ME mixture attracted very few non-target species.

Variation between trials

Overall, our results show that relative effectiveness of different lures was dependent on season and location. Fitt (1983) found the response of male *Dacus opiliae* (Drew and Hardy) to methyl eugenol traps varied with seasonal patterns of humidity associated with 'wet' and 'dry' seasons. Recent research has shown that the attractiveness of CL can be improved by the addition of other compounds. Apart from ME as discussed earlier, Khoo and Tan (2000) reported that zingerzone added to CL had potential to improve the monitoring of *B. cucurbitae*. More research is required before the CL–ME mixture can be recommended as a replacement for the standard CL monitoring lure for Qfly or Newman fly. In the Australian context, our results are consistent with Hooper (1978) indicating that *B. tryoni* and *D. aequalis* were attracted to the CL–ME combination. This paper appears to be the first to report that *D. newmani* and *D. absonifacies* are

attracted to the CL–ME combination. Any improvement in surveillance efficiency is likely to have significant financial benefits for all countries monitoring fruit flies.

Additionally, the CL–ME lure combination could also be useful in the male annihilation technique in drier inland areas (Dominiak *et al.* 2009) and is worthy of additional research. Vargas *et al.* (2000) found the combination lure lasted well in fibreboard discs in the field. Our results indicate that, on occasion, large numbers of CL-responsive flies are attracted to mixed lure traps. However, that response was highly variable and we know little about the factors leading to the highly clumped distribution of Qfly in that region. The CL–ME combination in monitoring and male annihilation is worthy of further research.
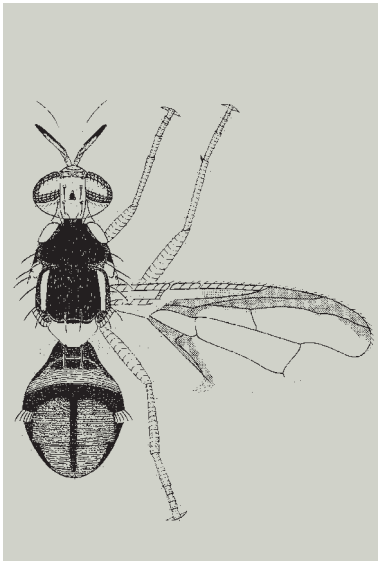
Acknowledgments

References

Allwood, A. (2000). Regional approach to the management of fruit flies in the Pacific Island countries and territories. *In* 'Area-wide control of fruit flies and other insect pests', ed. K.H. Tan, pp. 439-48. (Penerbit Universiti Sains Malaysia, Penang).

Cunningham, R.T. (1989). Parapheromones. *In* 'Fruit flies, their biology, natural enemies and control', eds A.S. Robinson and G. Hooper, World Crop Pests Volume 3A, pp. 221-230. (ElsevierScience Publishers, Amsterdam).

Dominiak, B.C. and Webster, A. (1998). Sand bed release of sterile Queensland fruit fly *Bactrocera tryoni* (Froggatt) at Young, NSW. *General and Applied Entomology* 28, 9-11.

Dominiak, B.C., McLeod, L.J. and Landon, R. (2003a). Further development of a low-cost release method for sterile Queensland fruit fly, *Bactrocera tryoni* (Froggatt) in rural New South Wales. *Australian Journal of Experimental Agriculture* 43, 407-17.

Dominiak, B.C., Gilmour, A.R., Kerruish, B. and Whitehead, D. (2003b). Detecting low populations of Queensland fruit fly *Bactrocera tryoni* (Froggatt) with McPhail and Lynfield traps. *General and Applied Entomology* 32, 49-53.

Dominiak, B.C., Mavi, H.S. and Nicol, H.I. (2006). Effect of town microclimate

on the Queensland fruit fly *Bactrocera tryoni*. *Australian Journal of Experimental Agriculture* 46, 1239-49.

Dominiak, B.C. (2006). Review of the use of protein food based lures in McPhail traps for monitoring Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *General and Applied Entomology* 35, 7-12.

Dominiak, B.C., Gillespie, P.S. and Tom, G. (2007). Changes in data management and the impediments to change – a case study for fruit fly surveillance. *Plant Protection Quarterly* 22, 95-8.

Dominiak, B.C., Sundaralingham, S., Jiang, L., Jessup, A.J. and Barchia, I.M. (2008). Production levels and life history traits of mass reared Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) during 1999/2002 in Australia. *Plant Protection Quarterly* 23, 131-5.

Dominiak, B.C., Clifford, C.S. and Nielsen, S.G. (2009). Queensland fruit fly (*Bactrocera tryoni* Froggatt) attraction to and chemical analysis of male annihilation blocks using three concentrations of cuelure at Dubbo, New South Wales, Australia. *Plant Protection Quarterly* 24, 157-60.

Drew, R.A.I. (1974). The responses of fruit fly species (Diptera: Tephritidae) in the South Pacific area to male attractants. *Journal of the Australian Entomological Society* 13, 267-70.

Fitt, G.P. (1983). The influence of seasonal climatic factors on the development of the methyl eugenol response in male *Dacus opiliae*. *Entomologia Experimentalis et Applicata* 33, 171-8.

Fletcher, B.S. (1974). The ecology of a natural population of the Queensland fruit fly, *Dacus tryoni* V. The dispersal of adults. *Australian Journal of Zoology* 22, 189-202.

Gillespie, P. (2003). Observations on fruit flies (Diptera: Tephritidae) in New South Wales. *General and Applied Entomology* 32, 41-7.

Hernandez, E., Orozco, D., Breceda, S.F. and Dominguez, J. (2007). Dispersal and longevity of wild and mass reared *Anastrepha ludens* and *Anestrepha obliqua* (Diptera: Tephritidae). *Florida Entomologist* 90, 123-35.

Hooper, G.H.S. (1978). Effect of combining methyl eugenol and cuelure on the capture of male tephritid fruit flies. *Journal of the Australian Entomological Society* 17, 189-90.

Horwood, M.A. and Keenan, P.J. (1994). Eradication of Queensland fruit fly. Final report CT336. Horticultural Research and development corporation, Canberra.

IAEA (2003). Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency, Vienna. 47 pp.

Ito, T., Teruya, T. and Sakiyama, M. (1976). Attractiveness of fibre-blocks containing mixture of methyl eugenol and cue-lure on *Dacus dorsalis* and *Dacus cucurbitae*. *Bulletin of Okinawa Agricultural Experimental Station* 2, 39-43.

Khoo, C.C. and Tan, K.H. (2000). Attraction of both sexes of Melon fly, *Bactrocera cucurbitae* to conspecific males – a comparison after pharmacophagy of cue-lure and a new attractant – zinger-zone. *Entomologia Experimentalis et Applicata* 97, 317-20.

Liu, Y.C. (1989). Development of attractants for controlling the melon fly, *Dacus cucurbitae* Coquillett, in Taiwan. *Chinese Journal of Entomology*. Special Publication 4, 128-9.

Meats, A. (2007). Dispersal of fruit flies (Diptera: Tephritidae) at high and low densities and consequences of mismatching dispersions of wild and sterile flies. *Florida Entomologist* 90 (1), 136-46.

Meats, A.M. and Edgerton, J.E. (2008). Short- and long-distance dispersal of Queensland fruit fly, *Bactrocera tryoni* and its relevance to invasive potential, sterile insect technique and surveillance trapping. *Australian Journal of Experimental Agriculture* 48, 1237-45.

Oliver, J. (2007). Summary of national fruit fly-related activities released. Plant Protection News. February 2007. Department of Agriculture, Fisheries and Forestry.

Osborne, R., Meats, A., Frommer, M., Sved, J.A., Drew, R.A.I. and Robson, M.K. (1997). Australian distribution of 17 species of fruit flies (Diptera: Tephritidae) caught in cuelure traps in February 1994. *Australian Journal of Entomology* 36, 45-50.

Reid, S. and Malumphy, C. (2009). Fruit flies (Diptera: Tephritidae) intercepted on plant produce imported into England and Wales. *The Entomologist's Monthly Magazine* 145, 213-26.

Shelly, T.E., Pahio, E. and Edu, J. (2004). Synergistic and inhibitory interactions between methyl eugenol and cue lure influence trap catch of male fruit flies, *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Diptera: Tephritidae) *Florida Entomologist* 87, 481-6.

Stanaway, M.A., Zalucki, M., Gillespie, P.S., Rodriguez, C.M. and Maynard, G.V. (2001). Pest risk assessment of insects in sea cargo containers. *Australian Journal of Entomology* 40, 180-92.

Stephenson, B.P., Gill, G.S.C., Randall, J.L. and Wilson, J.A. (2003). Biosecurity approaches to surveillance and response for new plant pest species. *New Zealand Plant Protection* 56, 5-9.

Umeya, K. and Hirao, J. (1975). Attraction of Jackfruit fly, *Dacus umbrosus* F. (Diptera: Tephritidae) and lace wing, *Chrysopa* sp. (Neuropteran: Chrysopidae) by lure traps baited with methyl eugenol and cue-lure in the Philippines. *Japanese Society of Applied Entomology and Zoology* 10, 60-62.

Vargas, R.I., Stark, J.D., Kido, M.H., Ketter, H.M. and Whitehand, L.C. (2000). Methyl eugenol and cue-lure traps for suppression of male oriental fruit flies and melon flies (Diptera: Tephritidae) in Hawaii: effects of lure mixtures and weathering. *Journal of Economic Entomology* 93, 81-7.

VSN International Ltd (2006). Genstat for Windows Release 9.2. Lawes Agricultural Trust. United Kingdom.

VSN international Ltd (2010). Genstat for Windows Release 13.1. Lawes Agricultural Trust. United Kingdom.

Weldon, C. and Meats, A. (2007). Short-range dispersal of recently emerged males and females of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) monitored by sticky sphere traps baited with protein and Lynfield traps baited with cue-lure. *Australian Journal of Entomology* 46, 160-6.

Weldon, C. and Meats, A. (2010). Dispersal of mass-reared sterile, laboratory-domesticated and wild male Queensland fruit flies. *Journal of Applied Entomology* 134, 16-25.

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