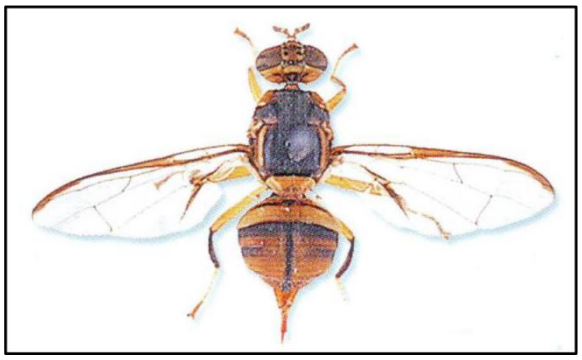
# Tephritidae (Insecta, Diptera) in Palau

Mamoru Terayama



September 2022

(Saitama, Japan)

## Introduction

The family Tephritidae belongs to the suborder Brachycera of the order Diptera, and 4,320 species have been described in about 480 genera in six subfamilies worldwide (Norrbom et al., 1999; Norrbom, 2004). This family includes many pest insects (over 50 species) such as *Bactrocera dorsalis* and *Zeugodacus cucurbitae* that cause very serious damage to crops and are critical in both the protection of domestic crops as well as crop import, export, and quarantine. Diverse research and information in every domain from classification to ecology has been accumulated around the world concerning these important insect species in plant quarantine, and extensive reading of the literature should be sufficient for developing measures in specific areas, but the number of case studies on native Tephritidae species is often limited.

In Palau, recent cooperation with Japan and Taiwan has led to many attempts to cultivate fruits and fruit vegetables. However, several non-native species in the tribe Dacini of Tephritidae that are recognized as particularly serious fruit and fruit vegetable pests have already invaded Palau and inhabited a wide area, and they are causing massive damage (Leblanc et al., 2015). Meanwhile, few surveys and studies have been conducted on native species, resulting in the absence of not only ecological findings, but even a species catalog. Measures to combat Tephritidae are essential for promoting agriculture in Palau and must begin with the collection of extensive information from within and outside of the country. Especially when considering exporting crops as cash crops, eradicating Tephritidae that are pests controlled in import bans in other countries is essential. At the same time, it is necessary to create systems for preventing the invasion of alien Tephritidae species from other countries. In Japan, 18 species in the genus *Bactrocera* alone have been discovered during import quarantine (Yamasako & Sueyoshi, 2020). Looking at the data for Tephritidae fruit flies shows that one is discovered almost daily (Kiritani, 2000; Yokohama Plant Protection Station, 2019). Even when cultivating fruits and fruit vegetables for domestic use, ignoring these fruit flies could result in enormous agricultural damage every year. In any case, correct classification including native species must be carried out before proceeding with pest control research.

This report covers the history and current status of Tephritida*e* fruit fly research in Palau and a review of the species of Tephritidae fruit flies in Palau that are important for conducting future research.

### Research history

In a monograph of Tephritidae in Micronesia, Hardy & Adachi (1956) described 17 species and recorded a total of three species in Palau: *Bactrocera calophylli* (= *Dacus calophylli*) and *B. frauenfeldi* (= *Dacus frauenfeldi*) in the subfamily Dacinae and *Spathulina acroleuca* in the subfamily Tephritinae. *Bactrocera frauenfeldi* has also been reported in Pulo Anna Island in the Southwest Islands. In a 1988–1990 survey of Tephritidae, *B. dorsalis* and *B. umbrosa* were found (Allwood et al., 1999). These two

species were also seen in studies by McGregou (2000) and Allwood et al. (2001). Leblanc (1997) described *Bactrocera dorsalis* causing damage to star fruit *Averrhoa carambola* (discovered in 1996) ahead of a similar report by Allwood et al. (1999). By 1999–2000, *B. dorsalis* was found living across a wide area from Kayangel Island to Babeldaob Island, Koror Island, the Rock Islands, Peleliu Island, and Angaur Island (Leblanc, 2015). SPC (2001) reported finding *Bactrocera philippinensis* (=*B. dorsalis*). GISAC (2015) reported serious damage by *B. philippinensis* to crops such as starfruit, guavas, and bananas, and confirmed infestations in 73% of starfruit and 51% of guavas. Shine (2003)

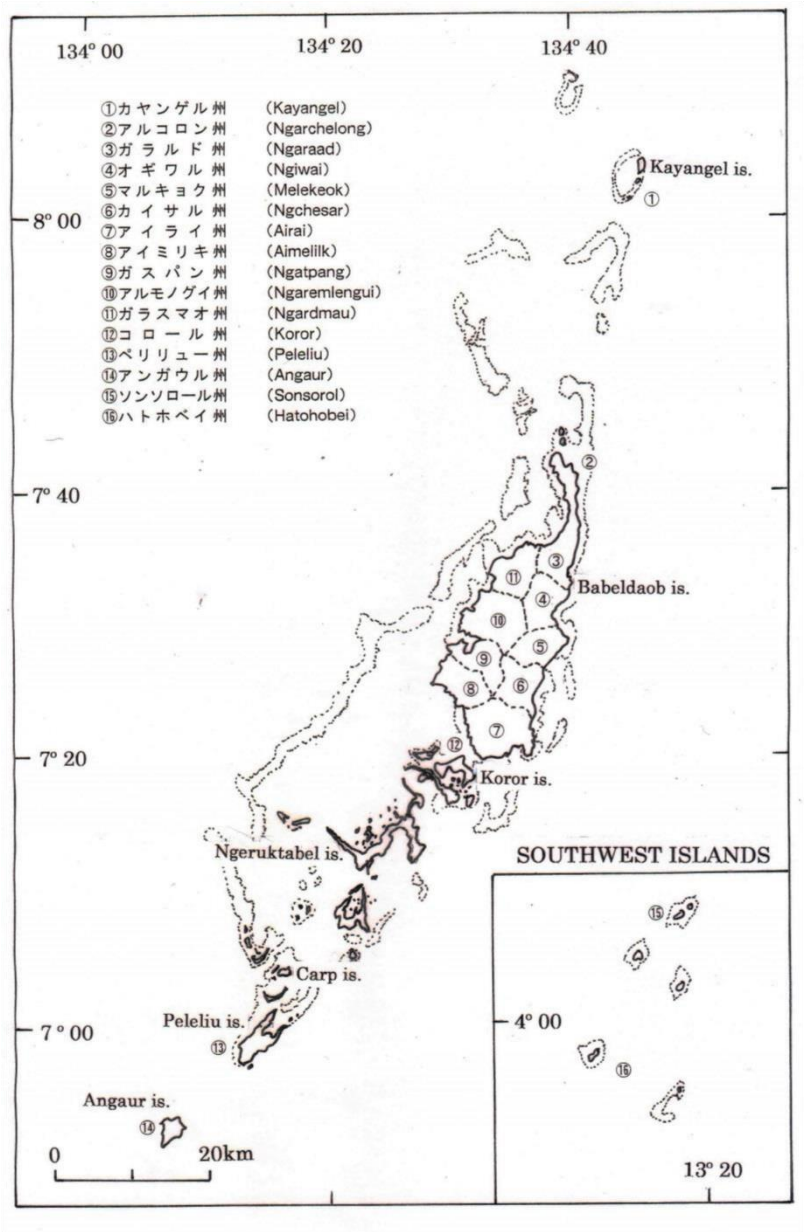



Figure 1. Map of Palau. Surrounded by coral reef.

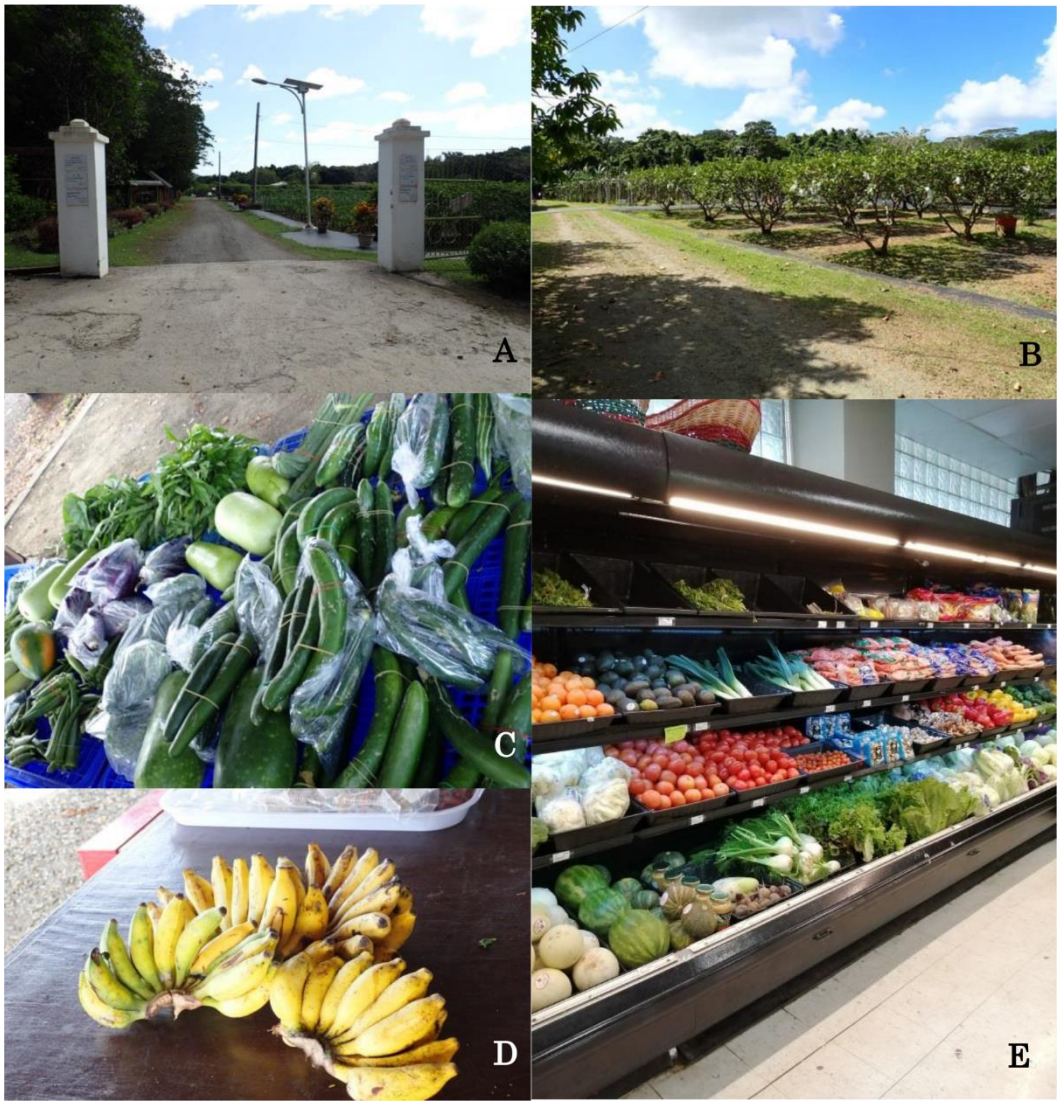



Figure 2. A: Entrance of the experimental farm as part of technical cooperation with Taiwan (Technical mission of the Republic of China (Taiwan) (Babeldaob Island); B: Fruit cultivation at the farm; C: Locally grown vegetables at the market; D: Domestically grown bananas at the same market; E: Fruits and vegetables on the supermarket shelves. Palau has an extremely low self-sufficiency rate for vegetables (10%), with 90% imported from other countries like the U.S. and Taiwan.

reported *B. frauenfeldi* and *B. umbrosa* and Holm & Michaels (2003) reported *B. dorsalis*, *B. frauenfrldi*, *B. umbrosa*, and *B. calophylli* as four species of fruit orchard pests in Palau. Esguerra & Del Rosario (2007) proposed five species of fruit pests in Palau: *Bactrocera frauenfeldi*, *B. umbrosa*, *B. calophylli*, *B. philippinensis*, and *B. occipitalis*. Among these species, *B. philippinensis* is synonymized with *B. dorsalis* and *B. papayae*. In recent classification studies, *B. papayae*, *P.*

*philippinensis*, and *B. invadens*, a species that originated in Africa, were synonymized with *B. dorsalis* (Schtze et al., 2015a; Doorenweerd et al., 2018). In molecular phylogenetic analysis by Leblanc et al. (2015), these types could not be differentiated at the species level and were determined to be intraspecific variation. However, others oppose this view, stating that *B. papayae* is a distinct species from *B. dorsalis* (Drew & Roming, 2022), and the classification is not settled. As mentioned above, the oriental fruit fly migrated from the Philippines to Palau and was thought to be *B. philippinensis*, the species that is found in the Philippines. However, others currently argue that *P. philippinensis* is a synonym of *B. dorsalis* (Doorenweerd et al., 2018) or of *B. papayae* (Drew & Roming, 2013). Findings by Leblance et al. (2015) suggested that *B. occipitalis* does not inhabit Palau. Doorenweerd et al. (2018) states that it is unclear if this species causes any damage to crops and that ambiguous records (especially those in Drew & Hancock, 1994) are being cited carelessly.

Distribution surveys conducted in 2001, 2013, and 2014 by Leblanc et al. (2015) using methyl eugenol attractant and cue-lure attractant confirmed the presence of four species: *Bactrocera dorsalis*, *B. frauenfeldi*, *B. umbrosa*, and *B. calophylli*. *Bactrocera dorsalis* and *B. frauenfeldi* are widely distributed in the area from Kayangel Island to Angaur Island, and *B. umbrosa* has been found on Kayangel Island, Babeldaob Island, and Koror Island. *Bactrocera calophylli* that eats *Calophyllum inophyllum* fruit and is therefore not considered an agricultural pest was confirmed on Babeldaob Island (record from Angaur Island in Hardy & Adachi (1956)).

*Chromolaena adorata* that is known as siam weed or devil weed and has a massive impact on crops and native plant communities invaded Palau in the early 1980s and has since spread its distribution. To eradicate this species, *Cecidochares connexa* in the family Tephritidae that forms galls on the plants was introduced into Palau from South America in 1999 (Esguerra, 2002).

Based on this research history, the following is a summary of the Tephritidae fruit flies recorded in Palau to date.

## I. Non-native species

### 1-1. Agricultural pest species

Subfamily Dacinae, tribe Dacini

1) *Bactrocera dorsalis* (Hendel, 1912) (s.l.)

= *Bactrocera papayae* (Drew & Hancock, 1994)

= *Bactrocera philippinensis* (Drew & Hancock, 1994)

= *Bactrocera occipitalis* (Bezzi, 1919): Esguerra & Del Rosario, 2007, misidentification

2) *Bactrocera frauenfeldi* (Schiner, 1868)

3) *Bactrocera umbrosa* (Fabricius, 1805)

### 1-2. Non pest species

Subfamily Dacinae, tribe Dacini

4) *Bactrocera calophylli* (Perkins & May, 1949)

### 1-3. Artificially introduced species

Subfamily Tephritinae, tribe Cecidocharini

5) *Cecidochares connexa* (Macquart, 1848)

## II. Native species

### 2-1. Non pest species

Subfamily Tephritinae, tribe Tephritini

6) *Spathulina acroleuca* (Schiner, 1868)

## Search of Tephritidae

Order Diptera (flies, mosquitoes, horseflies, etc.) comprise a large group, with about 157,000 species recorded around the world, and around one million species are thought to exist. Their hindwings degenerated into small halteres, and so they have two sets of wings. Many have specialized sucking or needle-shaped mouthparts. Tephritidae is one of the families in the suborder Brachycera, , subsection Acalyptratae, superfamily Tephritoidea.

To confirm if a fly discovered belongs to Tephritidae, it should be examined for the following characteristics (Ichinohe & Kaneda, 1992; Yokohama Plant Protection Station, 1988):

1. Has superior fronto-orbital setae and inferior fronto-orbital setae (Figure 2-A).
2. Has paired postocellar setae that grow parallel or divergent when viewed from the front and never cross (Figure 2-A). However, these setae grow poorly in flies in the subfamily Dacinae and are either absent or minute.
3. The wing costal vein (vein C) breaks before and after the humeral crossvein (vein H) and at the end of the subcostal vein (vein Sc) (Figure 2-C).
4. The subcostal vein (vein Sc) and first radial vein (vein $R_1$) are separate, and the subcostal vein (vein Sc) bends abruptly towards the end of the costal vein (vein C) (Figure 2-C).

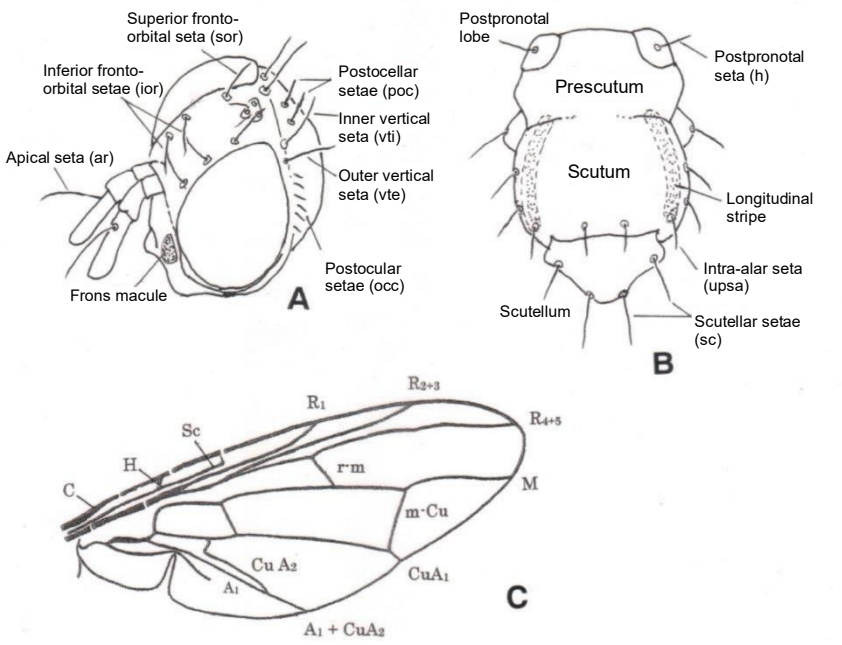



Figure 2. External morphology of Tephritidae. A: Head; B: Thorax, dorsal view; C: Forewing.

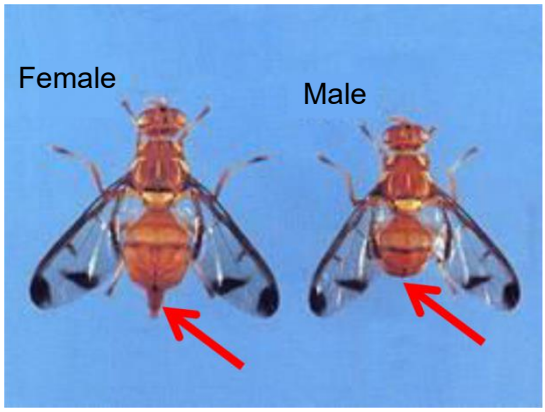



Figure 3. Female and male *Bactocera dorsalis*. The female is easy to distinguish from the male by the long ovipositor extruding from the posterior. (Photo by Okinawa Prefectural Agricultural Research Center: https://www.pref.okinawa.jp/site/norin/byougaichubojo/kawata.html)


Figure 4. A: Exit hole from last instar larvae leaving pumpkin fruit; B: Last instar larva; C: Cocoon.

## Description of each species

Fruit fly species in Palau can generally be differentiated by their wing pattern and body coloring. However, parts of the body may tear off during collection with the traps that are commonly used in monitoring and other surveys. The coloring and markings are often obscured due to the condition of the specimen, and expertise is required for identification.

### Non-native species

**1) *Bactrocera dorsalis* (Hendel, 1912) (s.l.)**

Morphology: Body length of about 7.5 mm. Key pest species known globally as the oriental fruit fly. The wings are hyaline, including the costal cell, with brown transverse bands from the base to the margin. There are yellow spots on the postpronotal lobe and the central setae are absent. The dorsal side of the thorax is black with yellow longitudinal stripes on the sides that extend beyond the intra-alar setae. There is a pair of setae on the scutellum. The abdomen is reddish brown and varies in color but generally has black horizontal stripes on the first to third segments and a median black longitudinal stripe extending from the third segment.

There are many similar species that are difficult to classify morphologically, and the naming *B. dorsalis* species complex or *B. dorsalis* (s.l.) has been used for several species with very similar morphology. In particular, as *B. dorsalis*, *B. carambolae*, *B. papayae*, and *B. philippinensis* are very similar and difficult to identify, these four species have been combined as a single species group. Classification of these species is not settled, with one recent study finding *B. papayae* and *B. philippinensis* to be synonymous with *B. dorsalis* (Schutze et al., 2015a), another suggesting *B. papayae* is a distinct species, and yet another finding *B. philippinensis* to be synonymous with *B. papayae* (Drew & Roming, 2013, 2022). The difficulty of species identification is also evident from the results of genetic analysis. For example, Schutze et al. (2015b) found interspecific hybridization between *B. carambolae* and *B. kandiensis*. This article will therefore use the scientific name *B. dorsalis* in the broad sense (s.l.).

*Bactrocera occipitalis* is a similar species to *B. dorsalis* but can be distinguished from the latter by its wide costal band on the wing that clearly extends below vein $R_{2+3}$ (*B. dorsalis* has a narrow costal band that does not extend beyond vein $R_{2+3}$). *Bactrocera occipitalis* reported by Esguerra & Del Rosario (2007) corresponds to *B. dorsalis* as far as can be determined from photos (p. 83; Fig. 148). *Bactrocera occipitalis* is a species that has been found in parts of Southeast Asia such as the Philippines and Borneo, and the extent of its damage is currently unknown (Doorenweerd et al., 2018). Records of this species from Palau are omitted here.

Damage and ecology: This species damages numerous crops of over 300 species, including not only fruit, but also fruit vegetables like tomatoes. Damage to 40 kinds of crops has been reported in the Pacific Islands alone (Leblanc et al., 2012, 2013a). Females stab the skin of the fruit with their ovipositor and lay eggs in the tissue. The larvae grow by eating away at the fruit from inside and

dropping to the ground after reaching the third instar to form a pupa in the soil. Based on mitochondrial COI analysis and the shape of the male genitalia, the population spreading in Palau is inferred to have invaded from the Philippines (Leblanc et al., 2015).

Distribution: *B. dorsalis* is distributed widely across Southeast Asia, China, Africa, and the Pacific Islands and has been reported in over 65 countries. Although Japan successfully eradicated this species of fruit fly, it has been discovered several times on the Kyushu mainland since 2020, suspected to have flown in from the continent rather than entered the country through the movement of goods. These fruit flies are often detected in imported goods, hand luggage, and mail from overseas, numbering 300 cases a year (Yokohama Plant Protection Station, 2019).

**2) *Bactrocera frauenfeldi* (Schiner, 1868)**

Morphology: Black species with a body length of 6–7 mm. Known as the mango fruit fly. Easily distinguished by a brown transverse band running from vein r-m to vein m-Cu and brown transverse band from the base to the margin. The postpronotal lobe is dark brown and the central setae are absent. The mesothorax is black with a thick dark gray median stripe in the center and short, narrow yellow longitudinal stripes on the sides. There is a pair of setae on the scutellum and a black inverted triangle marking on the base. The abdomen is also black with a dark gray pattern.

Damage and ecology: Extensive eating of fruit. Damage to as many as 73 kinds of crops has been reported in the Pacific Islands (Leblanc et al., 2012, 2013a). Females lay their eggs in fruit, and the larvae eat away at the fruit from the inside.

Distribution: Widely distributed across Southeast Asia, Australia, New Guinea, and the Pacific Islands.

**3) *Bactrocera umbrosa* (Fabricius, 1805)**

Morphology: Body length of 6–7 mm. Distinctive brown marking on the wings. Called the breadfruit fly for its parasitism of fruits like breadfruit. Triangular marking on the base that appears as a pale cutout of the interior, thick median longitudinal stripe with a V-shaped marking at the end. The thorax is black with yellow longitudinal stripes on the sides.

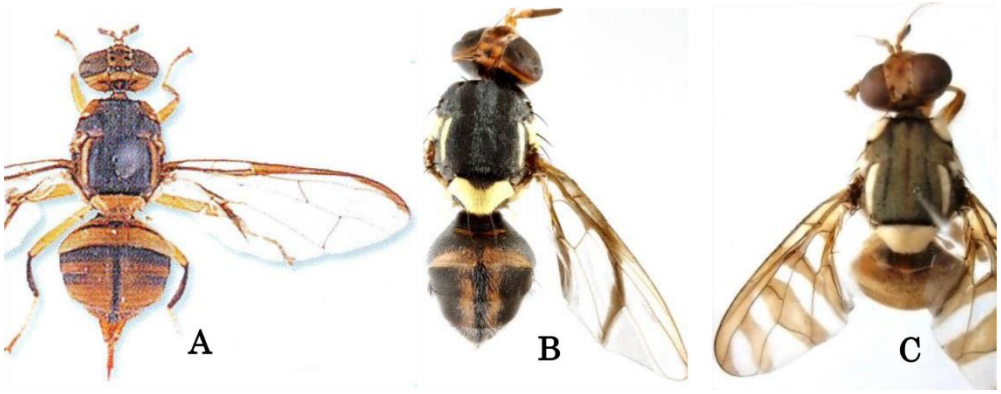



Figure 5. Fruit fly pest species in Palau. A: *Bactrocera dorsalis* (Hendel, 1912); B: *Bactrocera frauenfeldi* (Schiner, 1868); C: *Bactrocera umbrosa* (Fabricius, 1805).

The yellow longitudinal stripes are long, extending to the intra-alar setae. The abdomen is reddish brown with black patterns on the sides and a thin black median stripe extending from the third segment.

Damage and ecology: Generally eats *Artocarpus* plants, with the most damage seen in durians, breadfruit (*A. altillis*), and jackfruit (*A. heterophyllus*).

Distribution: Widely distributed across Southeast Asia, New Guinea, and the Pacific Islands.

**4) *Bactrocera calophylli* (Perkins & May, 1949)**

Classification: Careful examination is required during surveys as it has a similar coloring to *B. dorsalis*. The wings are hyaline, and they can be distinguished from *B. dorsalis* by the absence of brown transverse bands from the base to the margin.

Ecology: Considered a non pest species as it only eats *Calophyllum inophyllum* in the family Calophyllaceae. It should be noted during surveys that *Bactrocera dorsalis* also eats *Calophyllum inophyllum*.

Distribution: Southeast Asia, Japan (Nansei Islands), New Guinea, Australia, and the Pacific Islands.


Figure 6. *Bactrocera calophylli* (Perkins & May, 1949). (Photo by Queensland Museum Network: https://learning.qm.qld.gov.au/resources/1552646/)

### Artificially introduced species

*Chromolaena odorata* is a perennial herb in the family Compositae originating in tropical America. It is a large plant; it is 3 to 7 meters tall and can sometimes grow to over 10 meters. It grows in agitated environments such as farmland, riverbanks, roadsides, and urban areas. This species uses allelopathy to destroy other plants and causes significant damage to crops and native plant species. It has a high reproductive potential and can easily regenerate from the rhizome when mowed. It produces a huge number of seeds that can disperse widely. It is currently distributed across tropical and subtropical zones around the globe and has become a biological threat in the regions it has invaded. The

International Union for Conservation of Nature and Natural Resources (IUCN) has named it one of the "100 of the World's Worst Invasive Alien Species." This species invaded Palau in the early 1980s and is now widely distributed across the country. To eradicate this species, *Cecidochares connexa* in the family Tephritidae that forms galls on the plants was introduced from South America in 1999.

**5) *Cecidochares connexa* (Macquart, 1848)**

Classification: Body length of 5 mm. Easily distinguished from other species by their four thick black bands on their forewings. Orange head, black thorax. Black abdomen with gray horizontal stripes.

Ecology: They create galls in *Chromolaena odorata* in which the larvae grow. For last instar larvae, galls can be as big as 1.8 cm long and wide as 1.1 cm wide.

Distribution: Although originally a neotropical species, they have been artificially introduced into Guam, Australia, Papua New Guinea, India, Thailand, and other countries to eradicate *Chromolaena*

Figure 7. *Cecidochares connexa* (Macquart, 1848). Left: Male; Right: Female. (Photo by Esguerra & Del Rosario, 2007)


Figure 8. A: *Chromolaena odorata*. IUCN: 100 of the World's Worst Invasive Alien Species. B: The arrow shows a larva inside a gall.

*odorata*. *Cecidochares connexa* reared in Indonesia were introduced into Guam, and some of the flies reared in Guam were then introduced to Palau in 1999. This species was still found in Palau as of 2021 and has become established in the country.

### Native species

There is generally no ecological information about native varieties. While some infest wild plants, others infest cultivated plants as well. Although this species does not currently cause agricultural damage, it could potentially become a pest to new crops introduced.

As mentioned earlier, studies are not being conducted in Tephritidae fruit flies that parasitize wild plants in Palau. Collecting general findings on Tephritidae fruit flies in the region is essential for correctly differentiating the key fruit fly species that are subject to invasion alert surveys.

**6) *Spathulina acroleuca* (Schiner, 1868)**

Classification: Small dark brown fruit fly with a body length of 3–3.5 mm. Black wings covered in hyaline spots. Hyaline wing base.

Ecology: The larvae grow in Compositae flowering plants.

Distribution: Wide distribution from tropical Africa to Southeast Asia, China, Japan, and Australia. Also widely distributed across Micronesia.

Figure 9. Forewing of *Spathulina acroleuca* (Schiner, 1868). (Photo by Bhattacharya, K. Kr. et al., 2013)

### Non-native species requiring special attention

The following are key Tephritidae species that should be watched carefully for invasion based on the damage caused around the world and their geographical distribution.

***Bactrocera tryoni* (Froggatt, 1897)**

Body length of 6 mm. English name is the Queensland fruit fly. Similar to *Bactrocera dorsalis* with hyaline wings marked only by a brown transverse band from the base to the margin. Can be distinguished from *B. dorsalis* by the brown costal cell, comparatively short pair of yellow longitudinal

Figure 10. *Bactrocera tryoni* (Froggatt, 1897). (Photo by Fruit Fly ID Australia, Https://www.fruitflyidentification.org.au/species/bactrocera-tryoni/)

stripes on the mesothorax, and absence of intra-alar setae. Infests Citreae plants. A similar species is *B. neohumeralis* (Hardy) that infests guavas.

Found in Southeast Asia and Australia and neighboring islands.

***Bactrocera musae* (Tryon, 1927)**

Body length of 6 mm and slightly similar coloring to *Bactrocera dorsalis*. Called the banana fruit fly as it is a well-known parasite of bananas. Originated in Queensland, Australia. Has invaded Papua New Guinea and been recorded on the Solomon Islands. Although this species is just one of the 17 species that make up *B. musae*, it may actually represent a cryptic species group that includes several species, and more detailed classification research is needed.

***Bactrocera decipiens* (Drew, 1971)**

Called the pumpkin fruit fly owing to its parasitism of pumpkins. The volume of pumpkin import in the South Sea Islands is low, and this species is not treated as important in animal and plant quarantine. Males of this species are not currently attracted to attractant.

***Bactrocera trivialis* (Drew, 1971)**

Important fruit orchard pest that infests more than 10 families of citrus fruits, guavas, and mangoes. Distributed from Indonesia to New Guinea.

***Bactrocera bryoniae* (Tryon, 1927)**

Large fruit fly that lives across Australia from the east coast to the north. Has been found in five families of plants such as Cucurbitaceae, Solanaceae, and Musaceae and is often found in chili peppers. It has also been reported in New Guinea, but is very likely a different species that is similar to the Australian population. It has been found in bananas.

***Bactrocera latifrons* (Hendel, 1915)**

Called the solanaceus fruit fly or Malaysian fruit fly. Body length of 6-7 mm and similar coloring to *Bactrocera dorsalis.* However, it is separated from the latter by the black round spot at near the tip of forewing. Infests Solanaceae plants and Cucurbitaceae plants.

***Zeugodacus cucurbitae* (Coquillett, 1899)**

Body length of 8–9 mm. Major pest known as the melon fly. Called *Bactrocera cucurbitae* for many years, but recently promoted to and positioned in the *Zeugodacus* genus (Doorenweerd et al., 2018). Has a brown marking near the tip of the wing and a brown band starting above vein m-Cu. Costal cell is hyaline. Mesothorax is yellowish brown with three yellow longitudinal stripes on the dorsal side. Abdomen is also yellowish brown. In addition to Cucurbitaceae, eats tropical fruits like papaya and mangoes and also damages crops like green beans and chili peppers.

Originates in Southeast Asia and has spread to Africa, New Guinea, Hawaii, and Guam. A population that invaded the Ryukyu Islands was eradicated in 22-year-long efforts, but they often invade from overseas through imported goods and hand luggage.

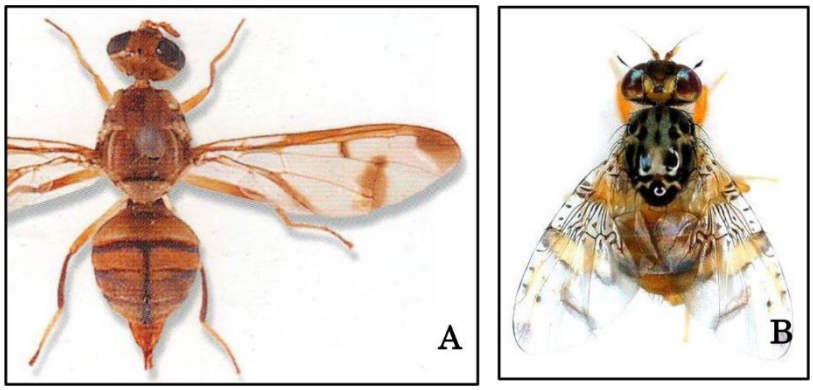



Figure 11. A: *Zeugodacus cucurbitae* (Coquillett, 1899). B: *Ceratitis captata* (Wiedemann, 1824).

***Ceratitis captata* (Wiedemann, 1824)**

Body length of 4.0–5.5 mm. English name is the Mediterranean fruit fly or medfly. Many small brown spots on the base of the wing and brown bands and spots on the other half. Setae on the center of the postpronotal lobe. Mesothorax dorsal side has no yellow longitudinal stripes. There is a pair of setae on the scutellum and black spots on the base and end. They are euryphagous, eating over 200 types of fruits and causing massive destruction. Adult flies lay eggs on the fruit and the larvae eat away at the inside.

Originated in Africa and spread through the area from Spain in Europe to the Middle East in the 19th century, later invading and establishing populations in Australia, Hawaii, and South America. In the contiguous United States, the species invaded California, Florida, and Texas, but has been

successfully eradicated from all three states. Despite this, infestations are often found in North America even today. In the Pacific Islands, the species has invaded Hawaii and efforts are ongoing to eradicate the flies.

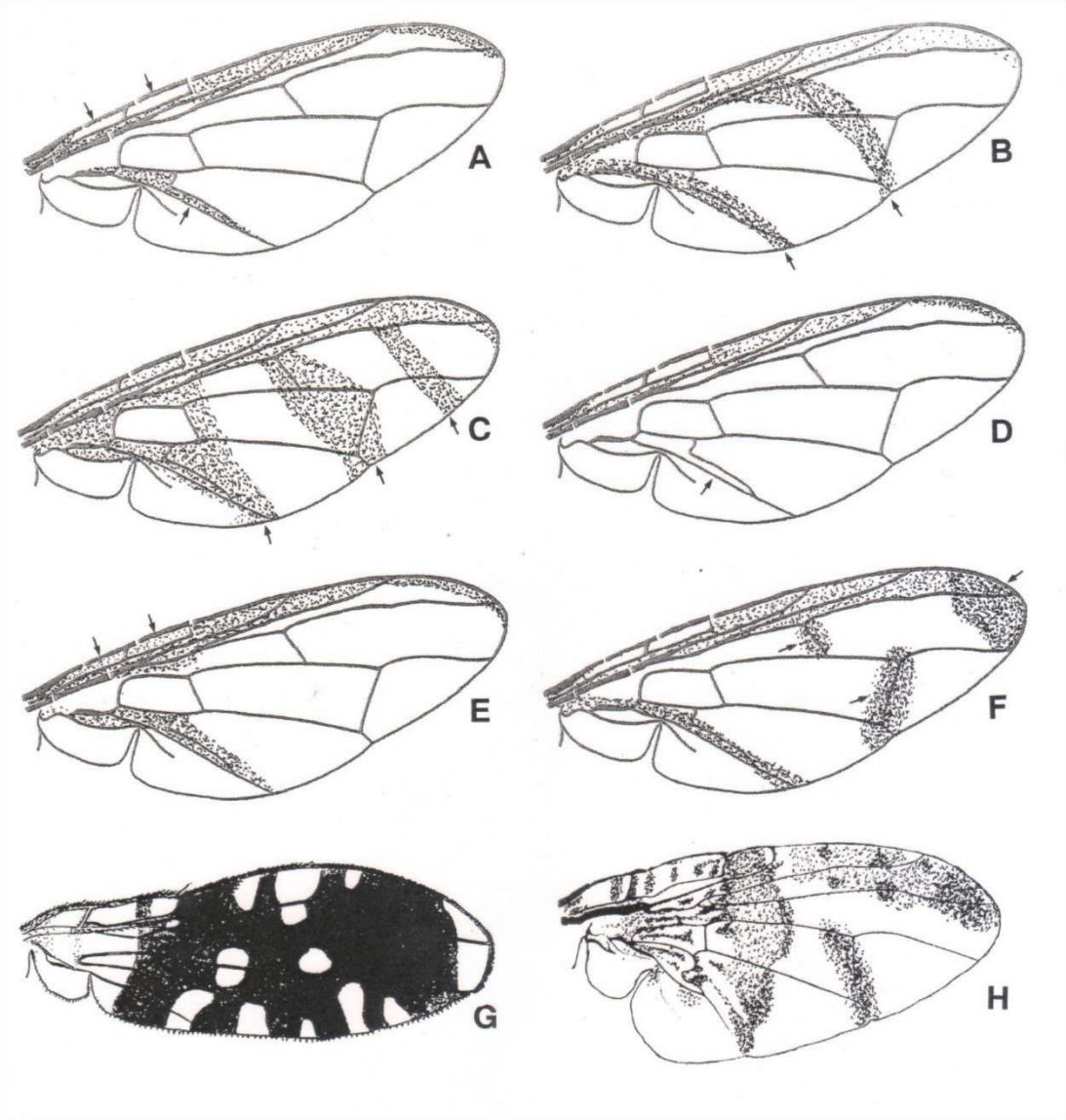



Figure 12. *Tephritidae* fruit fly wings. A: *Bactrocera dorsalis* (Hendel, 1912); B: *Bactrocera frauenfeldi* (Schiner, 1868); C: *Bactrocera umbrosa* (Fabricius, 1805); D: *Bactrocera calophylli* (Perkins & May, 1949); E: *Bactrocera tryoni* (Froggatt, 1897); F: *Zeugodacus cucurbitae* (Coquillett, 1899); G: *Spathulina acroleuca* (Schiner, 1868), (from Hardy & Adachi (1956)); H: *Ceratitis captata* (Wiedemann, 1824).

## Conclusions

This report provides a summary of Tephritidae fruit flies that are key agricultural pests in Palau. Programs to eradicate tephritids are being carried out around the world, and there have been many successes as well as failures. In the Pacific region, Rota in the Mariana Islands successfully eradicated the *Zeugodacus cucurbitae* (1963; first successful case of eradication of a Tephritidae species) and Guam successfully eradicated *Bactrocera dorsalis* in 1964 (Steiner et al., 1965). However, efforts to eradicate *Bactrocera dorsalis* from Rota, Saipan, Tinian, and Aguijan in the Mariana Islands and French Polynesia have all failed. In any scenario, governments must be prepared to expend a massive amount of money, time, and effort. Moreover, if efforts end when the population density decreases, different populations soon invade from other regions and lay eggs or the population density rapidly recovers. For this reason, entire separate islands must generally be treated as single units for a chance for successful eradication (Steiner et al., 1962). That said, *Ceratitis captata* was successfully eradicated from California, Florida, and Texas in the contiguous United States and from New Zealand.

Japan spent 20.4 billion yen and the labor of 440,000 man-days over 22 years to eradicate *Zeugodacus cucurbitae* from the Nansei Islands. It spent about 5 billion yen and a total of 190,000 man-days over 18 years to successfully eradicate *Bactrocera dorsalis* from the Nansei Islands and the Ogasawara Islands (Yoshizawa, 1993). This brings the total amount spent on programs to eradicate *Zeugodacus cucurbitae* and *Bactrocera dorsalis*, excluding labor costs, to 25.4 billion yen. On a visit to Japan in 1996 to receive the 11th Japan Prize, Edward F. Knipling who created the sterile insect technique said that the greatest difficulty arising in his research was raising the funds needed for eradication programs. In 1998, a plan was formed to eradicate *Bactrocera dorsalis* and *Bactrocera umbrosa* from Palau, and the proposed budget for eradication was 1.2 million USD (120 million yen). The plan ended with the failure to raise those funds (Allwood et al., 1999; McGregor, 2000). In the same period (1998–2000), three of the four key pest Tephritidae species were successfully eradicated from Nauru that is located between the Marshall Islands and the Solomon Islands (Allwood et al., 2002).

The main methods used to eradicate tephritids are the sterile insect technique (SIT) and the male annihilation technique (MAT). SIT involves releasing a large number of males that have been sterilized with gamma rays so that the females mate with those males (female tephritids mate only once), thereby reducing the population density. This method requires massive capital expenditure, a large budget, and many staff. MAT (Steiner at al., 1965b) involves placing 4–5 cm square boards (called paperboards and fiberblocks) made of materials such as plant fiber that have been infused with attractants like methyl eugenol, cue-lure, or torido-lure that attracts males and insecticide in fields to eliminate males and annihilate the lineage. In Japan, these types of boards are dropped around Amami Oshima and the Ogasawara Islands in large numbers by helicopter. As the material is plant fiber, it will eventually

return to the soil naturally if left there, eliminating the need for collection. On the Mariana Islands, efforts to eradicate *Bactrocera dorsalis* failed using SIT but succeeded using MAT (Steiner at al., 1970). If a Tephritidae eradication program will be implemented in Palau, controlling populations by dispersing a large number of boards would be a feasible approach.

Other studies are being conducted using a natural enemy of the fruit flies. In an experiment using parasitic wasps, their introduction effectively decreased the population density in Hawaii and Tahiti (Vargas et al., 2007. 2012a, b), but the effectiveness of this type of experiment in Palau could not be determined (Leblanc et al., 2015).

Some approaches for achieving localized control of Tephritidae fruit flies are to disperse insecticide mixed with protein as an attractant (protein bait spray) or to bag fruit before the flies have a chance to lay their eggs. A Vapor Heat Treatment System (VHT) has been developed for exporting fruit. This method kills fruit fly eggs and larvae in the fruit with steam and heat. However, in Japan, this treatment method must be used in advance in the import of fruits by countries that have received conditional lifting of an import ban according to the Plant Protection Act. In Palau, the first step is to decrease the population density of Tephritidae fruit flies, after which discussions can begin about the introduction of the costly VHT. Even before eradication of Tephritidae fruit flies, the most immediate priorities are to improve breeding technologies to achieve a stable production of good quality fruit and increase the domestic self-sufficiency rate and to increase productivity, for example by actively training producers.

## References


Allwood, A. J., J. W. Armstrong, K. Englberger & F. Sengebau, 1999. Feasibility study on eradication of fruit flies attracted to methyl eugenol (*Bactrocera dorsalis* and *Bactrocera umbrosa*) in Palau. FAO/AusAID/UNDP/SPC Project on Regional Management of Fruit Flies in the Pacific. Technical report. 28 pp.

Allwood, A. J., L. Leblanc, E. Tora Vueti & R. Bull, 2001. Fruit fly control methods for Pacific island countries and territories. Plant Protection Service Secretariat of the Pacific Community, Pest Advisory Leaflet, (40): 1-12.

Allwood, A. J., E. Tora Vueti & F. Sengebau, 2001. Implementation plan. Eradication of fruit flies attracted to methyl eugenol-oriental fruit fly (*Bactrocera dorsalis*) and breadfruit fly (*Bactrocera umbrosa*). FAO/AusAID/UNDP/SPC Project on Regional Management of Fruit Flies in the Pacific. Technical report. 29 pp.

Allwood, A. J., E. T. Vueti, L. Leblanc & R. Bull, 2002. Eradication of introduced *Bactrocera* species (Diptera: Tephritidae) in Nauru using male annihilation and protein bait application techniques. *In* Veitch, C. R. & M. N. Clout (eds.), Turning the tide: the eradication of invasive species, 19-25.

Bhattacharya, K. Kr., S. Halder, & D. Banerjee, 2013. New records of fruit flies (Diptera: Thphritidae) from Renuka wetland and wildlife sanctuary, Himachal Pradesh. Rec. Zool. Surv. India: 113 (Part-3): 145-149.

Doorenweerd, C., L. Leblanc, A. L. Norrbom, M. San Jose & D. Rubinoff, 2018. A global checklist of the 932 fruit fly species in the tribe Dacini (Diptera, Tephritidae). ZooKeys, 730: 19–56.

Drew R. A. I. & D. L. Hancock, 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. Bull. Entomol. Res., Supple. Ser., 2: 1-68.

Drew, R. A. I., S. Raghu & P. Halcoop, 2008. Bridging the morphological and biological species concepts: studies on the *Bactrocera dorsalis* (Hendel) complex (Diptera: Tephritidae: Dacinae) in Southeast Asia. Biol. Jour. Linn. Soc., 93: 217-226.

Drew, R. A. I. & M. C. Romig, 2013. Tropical fruit flies of South-East Asia: (Tephritidae: Dacinae). C. A. B. International, Oxfordshire, United Kingdom, 653 pp.

Drew, R. A. I. & M. C. Romig, 2016. Keys to tropical fruit flies (Tephritidae: Dacinae) of South-East Asia: Indomalaya to North-West Australia. CAB International, Wallingford, U. K., 487 pp.

Drew, R. A. I. & M. C. Romig, 2022. The fruit fly fauna (Diptera: Tephritidae: Dacinae) of Papua New Guinea, Indonesia Papua, associated islands and Bougainville. CAB International, UK, 124 pp.

Esguerra, N. M., 2002. Introduction and establishment of the thphrited gall fly *Cecidochares connexa* on siam weed, *Chromolaena odorata*, in the Republic of Palau. ARC-PPRI (2002); 148-151.

Esguerra, N. M. & A. G. Del Rosario, 2007. Economic Entomology in Micronesia. Palau Community College, 224 pp.

Food and Agriculture Organization of the United Nations (FAO), 2019. Fruit fly management in Palau-Advisory leaflet, 4 pp. https://www.fao.org/publications/card/en/c/CA7106EN/

GISAC, 2015. *Bactrocera philippinensis*. http://www.guaminsects.net/gisac2015/index.php?title=Bactrocera_philippinensis

Hardy, D. E. & M. Adachi, 1956. Insects of Micronesia. Vol. 14 No. 1. Diptera: Tephritidae. Bishop Museum. 28 pp.

Holm, T. & P. Michaels, 2003. Republic of Palau. *In* Shine, C., J. K. Reaser & A. T. Gutierrez (eds.), Invasive alien species in the Austral-Pacific Region, Notional Reports & Directory of Resources. GISP, Bishop Museum & US Government), 102-122.

Hirao, J. & K. Umeya, 1974. Report on field survey on fruit flies in mango orchards in the Philippines. Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, 21 pp. [In Japanese.]

Hsu, J.-C., H.-T. Feng & W.-J. Wu, 2004. Resistance and synergistic effects of insecticides in *Bactrocera dorsalis* (Diptera: Tephritidae) in Taiwan. Jour. Econ. Entomol., 97: 1682-1688.

Ichinohe, F. & M. Kaneda, 1992. A practical key to Tephritid species of quarantine importance. Research Bulletin of the Plant Protection Service Japan, 28: 69-74. [In Japanese.]

Ishii, S., K. Kiritani & T. Kocha (Eds.), 1985. Eradication of fruit flies: Theory and practice. Japan

Agricultural Aviation Association, 391 pp. [In Japanese.]

Ishikawa, K., F. Ichinohe & S. Arai, 1981. Comparative studies of the properties in the Ogasawara and the Amami populations of the oriental fruit fly, *Dacus dorsalis*. Research Bulletin of the Plant Protection Service Japan, 17: 19-24. [In Japanese.]

Ito, Y., 1980. Release of insects to annihilate insects: Private records of efforts to eradicate the melon fly from Okinawa. Chuokoron-sha, 183 pp. [In Japanese.]

Jin, T., L. Zeng, Y.-y. Lin, Y.-y. Lu & G.-w. Liang, 2011. Insecticide resistance of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), in mainland China. Pest Manag. Sci., 57: 370-376.

Kaneda, M. & M. Sasaki, 2019. Trap improvement for *Baxtrocera correcta* (Bezzi) (Diptera: Tephritidae) by mixing methyl eugenol and β-caryophyllene attractants. Research Bulletin of the Plant Protection Service Japan, 55: 21-23. [In Japanese.]

Kamiji, T., 2021. Fruit flies (Diptera: Tephritidae) intercepted by Japanese Import Plant Quarantine at Narita International Airport. Res. Bull. Prot. Japan, 48: 33-42.

Kamiji, T. & M. Kadoi, 2021. Effectiveness of adenosine triphosphate (ATP) assays for rapid judgment of life or death of the melon fly, Bactrocera cucurbitae (Diptera: Tephiritidae), eggs in quarantine. Jpn. Jour. Environ. Entomol. Zool., 32: 69-73.

Kamiji, T., M. Kaneda & M. Sasaki, 2018. Suppressive effects of trap capture on fertilized egg production in *Bactrocera correcta* (Bezzi) (Diptera: Tephritidae): comparison of two attractants, β-caryophyllene and methyl eugenol, in laboratory cages. Jpn. J. Environ. Entomol. Zool., 29: 127-134.

Kiritani, K., 2000. Dunnage that accompanies fruits flies arrives in Japan every day. Japanese J. Conserv. Ecol., 5: 187-189. [In Japanese.]

Koyama, J., 1994. A battle with 53 billion flies: History of melon fly eradication. Tsukiji Shokan Publishing, 220 pp. [In Japanese.]

Krosch, M. N., M. K. Schutze, K. F. Armstrong, Y. Boontop, L. M. Boykin, T. A. Chapman, A. Englezou, S. L. Cameron & A. C. Clarke, 2013. Piecing together an integrative taxonomic puzzle: microsatellite, wing shape and aedeagus length analyses of *Bactrocera dorsalis* s.l. (Diptera: Tephritidae) find no evidence of multiple lineages in a proposed contact zone along the Thai/Malay Peninsula. Syst. Entomol., 38: 2-13.

Leblanc, L., 1997. Fruit fly fauna in Federated States of Micronesia, Guam, Palau, Kiribati, Northern Marianas and Marshall Islands. *In* A. J. Allwood & R. A. I. Drew (eds.), Management of Fruit Flies in the Pacific. ACIAR Proceedings No. 76, 64-67.

Leblanc, L., E. Tora Vueti, R. A. I. Drew & A. J. Allwood, 2012. Host plant records for fruit flies (Diptera: Tephritidae: Dacini) in the Pacific Islands. Proc. Hawaiian Entomol. Soc., 44: 11-53.

Leblanc, L., E. Tora Vueti & A. J. Allwood. 2013a. Host plant records for fruit flies (Diptera:

Tephritidae: Dacini) in the Pacific Islands: 2. Infestation statistics on economic hosts. Proc. Hawaiian Entomol. Soc., 45: 83-117.

Leblanc, L., R. I. Vargas & R. Putoa, 2013b. From eradication to containment: invasion of French Polynesia by *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and releases of two natural enemies: a 17-year case study. Proc. Hawaiian Entomol. Soc., 45: 31-43.

Leblanc, L., F. Harry, F. Sengebau, M. San Jose1, D. Rubinoff & R. Pereira, 2015a. A Survey of fruit flies (Diptera: Tephritidae: Dacinae) and their Opiine parasitoids (Hymenoptera: Braconidae) in Palau. Proc. Hawaiian Entomol. Soc., 47: 55-66.

Leblanc, L., M. San Jose, N. Barr & D. Rubinoff, 2015b. A phylogenetic assessment of the polyphyletic nature and intraspecific color polymorphism in the *Bactrocera dorsalis* complex (Diptera, Tephritidae). Zookeys, 540: 339-367.

Liu, H., D.-j. Zhang, Y.-j. Xu, L. Wang, D.-f. Cheng, Y.-x. Qi, L. Zeng & Y.-y. Lu, 2019. Invasion, expansion, and control of *Bactrocera dorsalis* (Hendel) in China. Jour. Integrative Agri., 18: 771-787.

Maung, K., Y. Mon, M. Khine, K. Chan, A. Phyoe, A. So, T. Han, W. Myo, S. San & A. Khai, 2021. Current Knowledge of Mango and Fruit Fly (Diptera: Tephritidae) Control in Myanmar: A Review. Advances in Entomol., 9: 49-58. doi:10.4236/ae.2021.91005.

McGregor, A., 2000. A feasibility study of eradication of Oriental fruit fly (*B. dorsalis*) and breadfruit fly (*B. umbrosa*) from the Republic of Palau. FAO/AusAID/UNDP/SPC Project on Regional Management of Fruit Flies in the Pacific. Technical report. 45 pp.

Ministry of Agriculture, Forestry and Fisheries, 2003. Commentary on main pest insects that have not invaded. Plant Protection Station Pest Information, (70): 6-7.

Norrbom A. L., L. E. Carroll, F. C. Thompson, I. M. White & A. Friedberg, 1999. Systematic database of names. *In* Thompson, F. C. (ed.), Fruit fly expert identification system and systematic information database. Myia 9, 65-251.

Norrbom A. L., 2004. Updates to Biosystematic Database of World Diptera for Tephritidae through 1999. Diptera Data Dissemination Disk (CD-ROM) 2.

Plant Health Australia, 2018. The Australian handbook for the identification of fruit flies. Ver. 3.1, 157 pp. Plant Health Australia, Canberra, ACT.

Raga, A. & M. E. Sato, 2006. Time-mortality for fruit flies (Diptera: Tephritidae) exposed to insecticides in laboratory. Arq. Inst. Biol., Sao Paulo., 73: 73-77.

San Jose, M., L. Leblanc, S. M. Geib & D. Rubinoff, 2013. An evaluation of the species status of *Bactrocera* invadens and the systematics of the *Bactrocera dorsalis* (Diptera: Tephritidae) complex. Ann. Entomol. Soc. Amer., 106: 684-694.

Schutze, M. K., N. Aketarawong, W. Amornsak. K. F. Armstrong, A. A. Augustinos, N. Barr, W. Bo, K. Bourtzis, L. M. Boykin, C. Cáceres, S. L. Cameron, T. A. Chapman, S. Chinvinijkul, A.

Chomič, M. De Meyer, E. Drosopoulou, A. Englezou, S. Ekesi, A. Gariou-Papalexiou, S. M. Geib, D. Hailstones, M. Hasanuzzaman, D. Haymer, A. K. W. Hee, J. Hendrichs, A. Jessup, Q. Ji, F. M. Khamis, M. N. Krosch, L. Leblanc, K. Mahmood, A. R. Malacrida, P. Mavragani-Tsipidou, M. Mwatawala, R. Nishida, H. Ono, J. Reyes, D. Rubinoff, M. San Jose, T. E. Shelly, S. Srikachar, K. H. Tan, S. Thanaphum, I. Haq, S. Vijaysegaran, S. L. Wee, F. Yesmin, A. Zacharopoulou & A. R. Clarke, 2015a. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on 20 years of integrative morphological, molecular, cytogenetic, behavioural, and chemoecological data. Syst. Entomol., 40: 456-471.

Schutze, M. K., K. Mahmood, A. Pavasovic, W. Bo, J. Newman, A. R. Clarke, M. N. Krosch & S. L. Cameron, 2015b. One and the same: Integrative taxonomic evidence that *Bactrocera invadens* (Diptera: Tephritidae) is the same species as the Oriental fruit fly *Bactrocera dorsalis*. Syst. Entomol., 40: 472-486.

Sengebau, F., N. Waqa & E. Tora Vueti, 2005. Fruit flies in Palau. Plant Protection Service Secretariat of the Pacific Community, Pest Advisory Leaflet, (44): 1-4.

Sonda, M., K. Kitajima, T. Suetsugu & M. Uechi, 1985. Battle to eradicate *Tephritidae* fruit flies, 39(5): 195-200. [In Japanese.]

SPC (Secretariat of the Pacific Community), 2001. *Bactrocera philippinensis* and *Bactrocera occipitalis* in Palau. Pest Alert No. 23, 1. https://www.spc.int/Pacifly/Pest_Adv_leaflets/ Pest-Alert-23-NewFlies-Palau.pdf

Steiner, L. F., E. J. Harris, W. C. Mitchell, M. S. Fujimoto & L. D. Christenson, 1965. Melon fly eradication by overflooding with sterile flies. Jour. Econ. Entomol., 58: 519-522.

Steiner, L. F., W. G. Hart, E. J. Harris, R. T. Cunningham, K. Ohinata & D. C. Kamakahi, 1970. Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. Jour. Econ. Entomol., 63: 131-135.

Steiner, L. F., W. C. Mitchell & A. H. Baumhover, 1962. Progress of fruit-fly control by irradiation sterilization in Hawaii and the Mariana Islands. Inrer. Jour. Appl. Radiation & Isotopes, 13: 427-434.

Steiner, L. F., W. C. Mitchell, E. J. Harris, T. T. Kozuma & M. S. Fujimoto, 1965b. Oriental fruit fly eradication by male annihilation. Jour. Econ. Entomol., 58: 961-964

Sueyoshi, M., 2014. Family Tephritidae. Entomological Society of Japan. Edited by the Editorial Committee of Catalogue of the Insects of Japan, Catalogue of the Insects of Japan, Volume 8: Diptera (Part 2: Brachycera Schizophora), Touka Shobo, 555-588. [In Japanese.]

Tahir, H. M & R. Yaqood, 2015. Insecticide resistance in Bactocera dorsalis H. (Diptera: Tephritidae). Lap Lambert Academic Publ., KG, 73 pp.

Tanaka, A., 2021. *Bactrocera dorsalis*, *Zeugodacus cucurbitae*: Records from invasion to eradication

in the Amami Islands. Nanpou Shinsha, 184 pp. [In Japanese.]

Vargas, R. I., L. Leblanc, R. Putoa & A. Eitam, 2007. Impact of introduction of *Bactrocera dorsalis* (Diptera: Tephritidae) and classical biological control releases of *Fopius arisanus* (Hymenoptera: Braconidae) on economically important fruit flies in French Polynesia. J. Econ. Entomol., 100: 670-679.

Vargas, R. I., L. Leblanc, E. J. Harris & N. C. Manoukis, 2012a. Regional suppression of *Bactrocera* fruit flies (Diptera: Tephritidae) in the Pacific through biological control and prospects for future introductions into other areas of the world. Insects, 3: 727-742.

Vargas, R. I., L. Leblanc, R. Putoa & J. C. Piñero, 2012b. Population dynamics of three *Bactrocera* spp. fruit flies (Diptera: Tephritidae) and two introduced natural enemies, *Fopius arisanus* (Sonan) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), after an invasion by *Bactrocera dorsalis* (Hendel) in Tahiti. Biol. Contr., 60: 199-206.

Vargas, R. I., J. C. Pinero & L. Leblanc, 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific region. Insects, 6: 297-318

Yamasako, J. & M. Sueyoshi, 2020. Foreign Tephritidae collection at the Institute for Agro-Environmental Sciences, NARO (Insecta: Order Diptera). J. NARO R&D, 5: 21-30. [In Japanese.]

Yokohama Plant Protection Station, 1988. Fruit flies discovered in invasion alert surveys (I). Plant Protection Station Pest Information, 27: 3. [In Japanese.]

Yokohama Plant Protection Station, 1989a. Fruit flies discovered in invasion alert surveys (II). Plant Protection Station Pest Information, 28: 3. [In Japanese.]

Yokohama Plant Protection Station, 1989b. Fruit flies discovered in invasion alert surveys (III). Plant Protection Station Pest Information, 29: 3. [In Japanese.]

Yokohama Plant Protection Station, 1989c. Fruit flies discovered in invasion alert surveys (IV). Plant Protection Station Pest Information, 30: 3. [In Japanese.]

Yokohama Plant Protection Station, 2019. Key pests discovered in import quarantine in 2017. Plant Protection Station Pest Information, 117: 8. [In Japanese]

# Survey of Tephritidae in Palau

## 1. Manual for inhabitation and fruit fly faunal survey

### Survey with bait traps

Survey method: This is the most important survey for determining current distribution and density of harmful Tephritidae fruit flies in Palau. Place the traps for surveying across as wide an area as possible, collect the flies attracted to the traps every two weeks (six times in total), and examine the species composition and distribution.

Survey tools:

- A: Steiner traps (clear plastic cylinder, 10 cm diameter × 15 cm length, 2.7 cm openings on both ends) that are often used in fruit fly monitoring surveys in Japan, 350 traps (Plan A) or 300 traps (Plan B). B: McPhail traps (22 cm height x 17 cm diameter), 350 traps (Plan A) or 300 traps (Plan B). The trap should choose in either A or B which it is easy to use a Palauan side.
- Mixture (e.g. Euge-lure D8) containing Euge-lure attractant components (methyl eugenol, cue-lure) and attractant insecticide (insecticidal component: dibrome). 4,620 g (4.6 L)
- Cotton swabs for trap surveys (Roll Cotton; 1.0 cm diameter × 3 cm) 350 swabs × 6 times = 2,100 swabs (Steiner traps)
- Wire (used for setting traps): Can be prepared in Palau
- Tweezers: 30
- Sample vials for fruit fly collection (glass can break and should be avoided; it is best to use sumiron or PP) in three sizes: small, medium, and large. 330 × 6 surveys for a total of 1,980 vials. (500 large, 500 medium, and 1,500 small)
- Preservative solution for sample: Isopropanol (IPA) (Comment: If a sample needs to be sent to Japan, ethyl alcohol cannot be used as incendiary substances are prohibited. In that case, it is safe to use non-flammable IPA. Ethyl alcohol can be obtained in Palau, but obtaining IPA in Palau is probably difficult.)
- Counters: 10

#### Survey guidelines

About the setting of the bait traps, collection of the samples, and fruit fly identification and counting, I show two plans here.

**Plan A:** Members of the Bureau of Agriculture conduct setting of the bait traps, collection of the samples (lured flies), and fruit fly identification and counting.

Four teams of two people each (4 cars, 8 people) each visit their designated locations over a one- to three-day period (one team visits Peleliu Island and Angaur Island). The teams travel by car, setting bait traps as they go.

Number of traps to set: 200 on Babeldaob Island, 30 on Koror Island, 20 on Arakabesang Island, 20 on Malakal Island, 30 on Peleliu Island, and 30 on Angaur Island for a total of 330 traps.

**Plan B:** Each state workers conduct setting bait traps and collection of the samples (lured flies) (20 traps in a state; 20 x 14 states excluding Sonsorol and Hatohobei = 280 traps). Members of the Bureau of Agriculture conduct fruit fly identification and counting.

### A. Setting bait traps

0. Before setting the traps, attach a sticker that says, "Fruit fly Under Examination."
1. Fix the trap to a tree trunk or other structure with wire. Set it 1.5 meters from the ground (at eye-height) in a shady place out of direct sunlight.
2. Place a cotton swab for trap surveys infused with attractant and insecticidal components inside the trap and close the lid.
3. Write the trap number on the trap in permanent marker (e.g. B-001 for trap No. 001 on Babeldaob Island; the others would be C-001, N-001, M-001, P-001, and A-001) and write the location where it was set on the map.
4. Move to the next spot to set a trap. Generally set traps along the road (on Babeldaob Island in particular, traps can probably only be set on the side of the road, on farms, or in villages on the perimeter).


Figure 1. A: Steiner trap; B: Setting a steiner trap; C: McPhail trap.

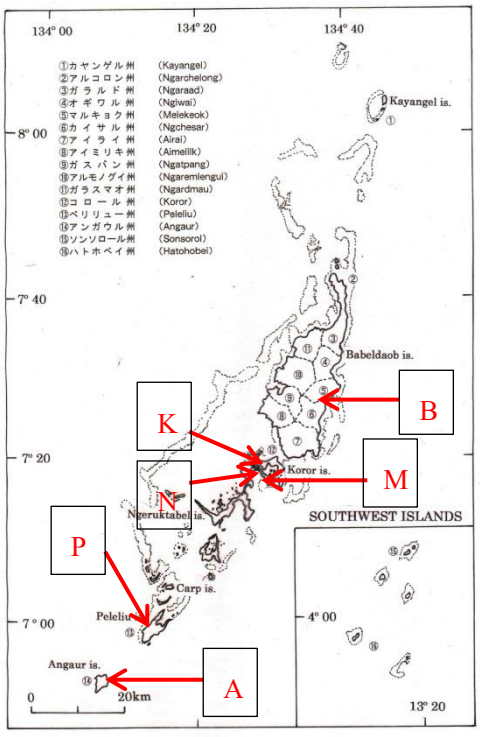



Figure 2. Trap setting area. B: Babeldaob, 200; K: Koror, 30; N: Ngarekebesang, 20; M: Malakal, 20; P: Peleliu, 30; A: Angaur, 30. Total, 330 traps. (Plan A)

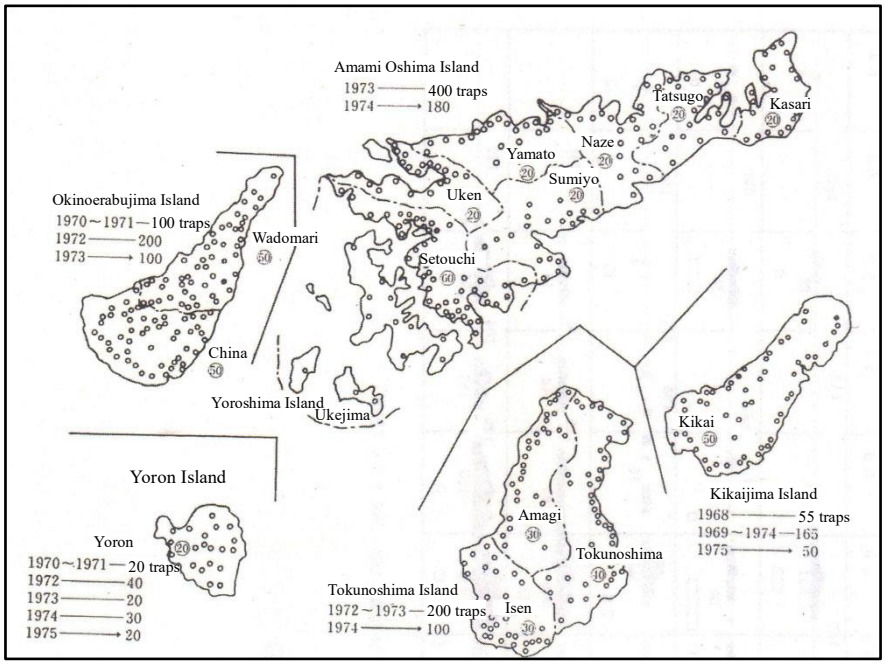



Figure 3. Example of trap setting (Japan: Amami Islands). Amami Oshima 712 km$^2$, 180–400 (number of traps set); Tokunoshima 105 km$^2$, 100–200; Okinoerabujima 93 km$^2$, 100–200; Kikaijima 57 km$^2$, 50–165; Yoron 21 km$^2$, 20–40. (cf. Babeldaob 331 km$^2$) (Modified from Tanaka, 2021)

## B. Collected of lured flies

Collect the fruit flies killed by the traps every two weeks. When collecting the flies, replace the cotton swab for trap surveys.

1. Open the lid on the trap and transfer any flies that are inside the trap to a sample vial filled with isopropanol.
2. In pencil (not pen as the ink will disappear), write the trap number and date (e.g. 25. Oct. 2022) on a piece of paper, put the paper in the vial, and close the lid.
3. In the case of steiner traps: Replace the cotton swab for trap surveys infused with attractant and insecticidal components with a new one (they should actually last a month).

Figure 4. Sample vials.

### C. Fruit fly identification and counting

Tabulate the data from the sample vials brought back for each island. Data should be taken within a week from the day the sample was collected.

1. Remove the fruit flies from the sample vial and place them on a petri dish.
2. Use the identification manual to separate the samples by species. If necessary, look at the samples under a stereoscopic microscope, and determine the species name.
3. Write the trap number and date on the tabulation sheet and write the names of the flies and number collected. If the name of a fly cannot be determined, write "Unknown, xx individuals." → Unidentified samples will be identified by the Japanese team.
4. Return all of the flies to the sample vial and store the vial.
5. Lastly, make a copy of the tabulation sheet and file it away along with the original. One copy will be taken to Japan for data analysis.

When collecting flies with bait traps, parts of their body are often damaged and the coloring and markings are often obscured due to the condition of the specimen, and so expertise is required for identification. When local staff carrying out identification and measurement in Palau come across a specimen that seems difficult to identify or is suspected to be a new species (new to Palau, not new to science), that fact should be written on the tabulation sheet. In addition, such specimens should be transferred to a separate sample vial. They will be taken back to Japan for further examination.

### D. Data analysis

A Japanese side performs the analysis of data provided in this investigation.

Major contents of the analysis

1. Show distribution in each species in Palau
2. Show the relative density at each area in each species

### E. Survey schedule

Late April: Set bait traps
Mid-May: First fruit fly collection
Late May: Second fruit fly collection
Mid-June: Third fruit fly collection
Late June: Fourth fruit fly collection
Mid-July: Fifth fruit fly collection
Late July: Sixth fruit fly collection: Collect traps
August: Data analysis by a Japanese side.

### Other (reference):

#### 1. Need for collaboration with the Belau National Museum

The insect collection at the Belau National Museum should be examined for the purpose of investigating the species of Tephritidae fruit flies inhabiting Palau.

#### 2. Confirmation of *Ceratitis captata* (Mediterranean fruit fly) existence

Mediterranean fruit flies are not attracted to methyl eugenol or cue-lure. In the Pacific Islands, this species has invaded Hawaii and caused damage. If Palau wants to determine whether Mediterranean fruit flies have invaded the country, add 20 more bait traps for Mediterranean fruit fly surveying. Attractant component (Trimedlure) will also need to be procured.

#### 3. Compliance with the Convention on Biological Diversity (CBD)

Based on access to genetic resources according to the Convention on Biological Diversity (CBD) and Access and Benefit-Sharing (ABS; referred to as the Nagoya Protocol), international laws pertaining to the use of genetic resources and national laws for the country collecting samples must be followed appropriately. Nowadays, it is generally required when using genetic resources from overseas to write appropriate evidence showing correct compliance with ABS (e.g. official document number, name of the local organization in charge) on any scientific papers or reports.

To carry out collaborative research with Palau and bring genetic resources (in this case fruit flies) back to Japan for research purposes, a collaborative research agreement must be signed with a research institute in Palau according to the ABS (Nagoya Protocol). For the present agricultural cooperation agreement, an MOA (or MOU) for Tephritidae research must be signed by the Japanese Ministry of Agriculture, Forestry and Fisheries and Palau's Ministry of Natural Resources,

Environment & Tourism. The contact person in Palau concerning ABS is Mr. King M. Sam from the Ministry of Natural Resources, Environment & Tourism who can probably prepare the necessary document easily if asked by the Ministry of Agriculture, Forestry and Fisheries. Also, in Palau, the Bureau of Agriculture in the Ministry of Natural Resources, Environment & Tourism issues approval to bring specimens or samples outside of the country.

## Reference cited

Tanaka, A., 2021. *Bactrocera dorsalis*, *Zeugodacus cucurbitae*: Records from invasion to eradication in the Amami Islands. Nanpou Shinsha, 184 pp. [In Japanese.]

# Survey of Tephritidae in Palau

## 2. Manual for fresh fruit survey

### Survey of fruit fly infestation of fruit from cultivated plants and wild plants

Survey method: Pick a lot of fruit of as many varieties as possible from places around Palau, store them indoors, and collect and survey the fruit flies that emerge from the fruit. The survey team will be centered around a fruit expert.

Survey tools:

- About 200–300 large plastic storage containers (about 30 × 40 cm) (can be bought in Palau)
- 2,000 sample vials (glass can break and should be avoided; it is best to use sumiron or PP) in three sizes: small, medium, and large (500 large, 500 medium, and 1,000 small)
- Preservative solution for sample: Isopropanol (IPA) (Comment: If a sample needs to be sent to Japan, ethyl alcohol cannot be used as incendiary substances are prohibited. In that case, it is safe to use non-flammable IPA. Ethyl alcohol can be obtained in Palau, but obtaining IPA in Palau is probably difficult.)
- 20 sets of tweezers
- 10 sample aspirators

Survey guidelines:

1. Pick a lot of fruit of as many varieties as possible from places around Palau (Must prepare funds for purchasing fruit from cultivated plants). As much as possible, pick fruit from wild plants in addition to fruit from cultivated plants. Do not forget to record the collection place and date.

For cultivated plants, collect fruit from several locations (farms or plantations) even for the same variety, pick as much fruit as possible from each location (ideally at least 100 for statistical purposes), and bring the fruit back to the laboratory.

2. Lay sand on the bottom of a large plastic storage container and place a piece of wood on top of the sand (to keep the fruit from touching the sand directly). Place the fruit that was picked inside the container (different varieties in different containers) and store the containers for at least 10 days. Cut an opening in the center of the lid and place a net over the opening to allow ventilation.

3. Fruit flies burrow in the sand and pupate (pupa stage is 10 or more days). Capture the flies that emerge from the sand with a sample aspirator and store them in sample vials filled with isopropanol.

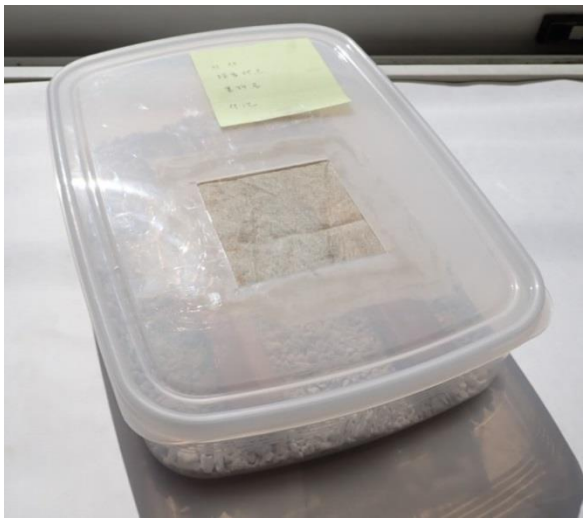


Figure 1. Large plastic storage container for fruit survey (about 30 × 40 cm). Lay sand on the bottom, place a piece of wood on top, and then place the fruit picked on top of that. Cut an opening in the center of the lid and place a net over the opening to allow ventilation. Attach a data label (e.g. date picked, location where picked, name of fruit).

Figure 2. Survey of fresh fruit infestation rate. Fresh fruit is placed in the plastic storage containers and they are monitored for fruit fly emergence.


Figure 3. Holes in the skin of a pumpkin from which *Zeugodacus cucurbitae* fruit flies emerged.
Figure 4. Last instar *Bactrocera dorsalis* larvae.
Figure 5. *Bactrocera dorsalis* pupa.

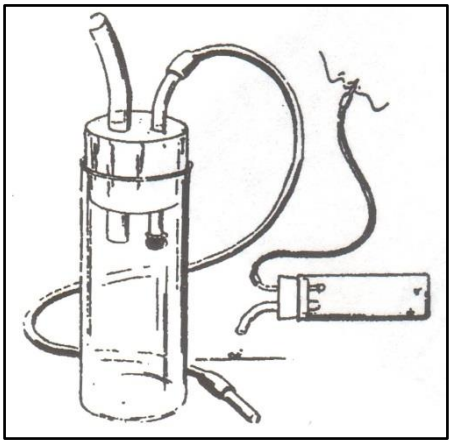


Figure 6. Sample aspirator. Tool for collecting adult fruit flies that have emerged by aspiration.

Data tabulation:

1. Look at the fruit exterior to determine if it is infested with fruit flies (can tell by holes in the skin of the fruit; Figure 3).

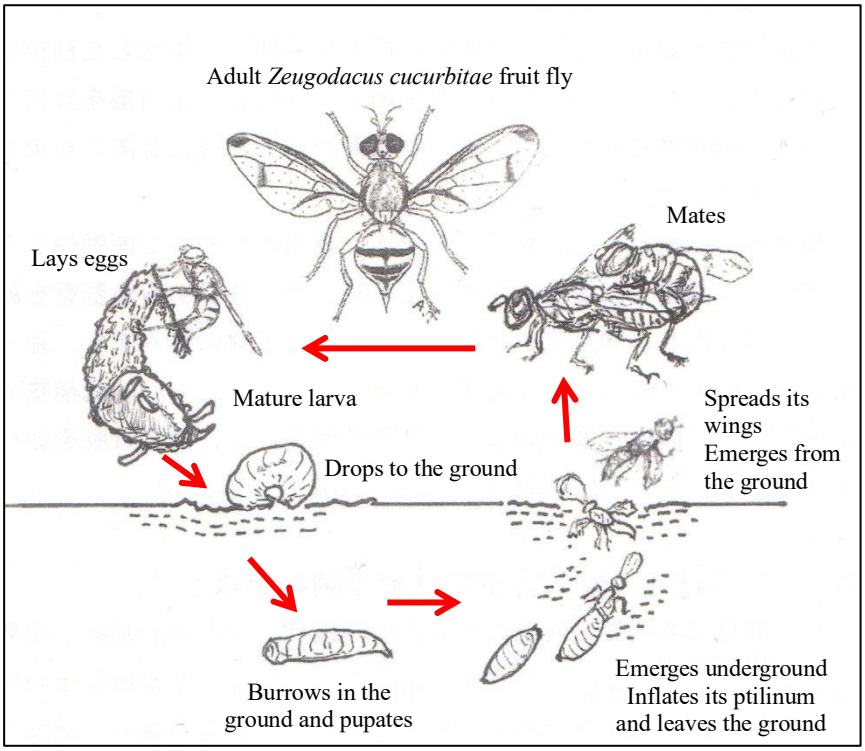
2. Identify the flies that emerge.

3 Write the name of the fresh fruit, place where it was picked, date picked, name of the fruit fly, number of fruits that were infested with fruit flies, and number of fruits that were not infested with fruit flies on the data tabulation sheet (no need to calculate the number of fruit flies).

Figure 7. Sample vials. For storing adult fruit flies that have emerged. Fill with isopropanol.

## Reference: Life cycle of a *Tephritidae* fruit fly

*Bactrocera dorsalis*: 2–3 days in eggs, 1–3 weeks as larvae, 10 days or more in pupae
*Zeugodacus cucurbitae* (under 26°C conditions): 1–2 days in eggs, 5–7 days as larvae, 10 days in pupae




Life cycle of a fruit fly (Modified from Tanaka, 2021).

## Reference cited

Tanaka, A., 2021. *Bactrocera dorsalis*, *Zeugodacus cucurbitae*: Records from invasion to eradication in the Amami Islands. Nanpou Shinsha, 184 pp. [In Japanese.]

# Survey of Tephritidae in Palau

## 3. Manual for testing chemical effectiveness

### Experiment 1: Test to examine the baiting effectiveness of attractant components
### Experiment 2: Test to examine the effectiveness of insecticidal components

When performing drug resistance tests, it is important to have as many test specimens as possible, which requires a relatively high amount of time and labor. A room must also be secured for used as a rearing room. This manual provides an experimental design based on the premise of confirming the minimum necessary chemical effectiveness. We must carry out this experiment in at least *Bactrocera dorsalis* and *B. frauenfeldi*.

### Preparation of test specimens (from a large reared population):

As the level of fruit fly attraction differs by age of the adults, it is necessary to rear specimens to the right age for the experiments. For example, *Bactrocera dorsalis* has a very weak attraction response to methyl eugenol (Me) immediately after emergence when they are not sexually mature. Males respond very strongly 12 days after emergence. Accordingly, same-age specimens must be obtained by rearing a large number of fruit flies on artificial feed. Adults that have emerged should be reared on plenty of water (agar soaked in water is good) and adult feed (1:4 ratio of hydrolyzed yeast (AY-65) to granulated sugar).

Tools for rearing:
- Plastic cups (9 cm diameter): 300
- Trays (for rearing larvae; about 20 × 30 cm): 50
- Rearing containers (for rearing adults): 30
- Artificial feed for larvae
- Adult feed
- Agar

The following table shows the ingredients in the artificial feed (for *Zeugodacus cucurbitae*: 1.5 L):

| | | |
|---|---|---|
| Water | 1,556 | mL |
| Sodium benzoate (preservative) | 1 | g |
| 3.5% hydrochloric acid | 88 | mL |
| Tissue | 40 | g |
| Brewer's yeast | 60 | g |
| Sugar | 112 | g |
| Soybean cake | 60 | g |
| Wheat bran (No. 1 Canadian White) | 300 | g |
| Strained lees of pumpkin | 60 | g |
| Total | 2 | L |

Inoculate this volume of culture medium with about 20,000 eggs to obtain 16,000 to 18,000 mature larvae.

## Rearing method:

1. Put female fruit flies (*B. dorsalis* or *B. frauenfeldi*) in a small plastic container and rear them until they lay eggs (Figures 1 and 2).
2. Transfer the agar holding the eggs to a tray (about 20 × 30 cm) with artificial feed and rear them indoors (Figure 3).
3. When larvae reach the last instar stage, they reach beyond the container limits and drop downward. Place a tray with sand on the ground for the larvae to pupate.
4. When the adults emerge from the pupa, collect all the same-age fruit flies and rear them in rearing containers. Add agar for water replenishment and adult feed to the container as needed.
5. Use fruit flies that have reached a certain age in the experiments.

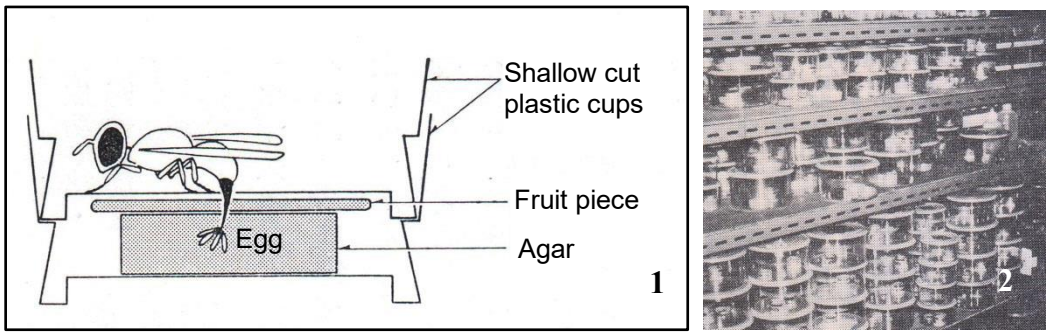



Figure 1. Method for inducing female fruit flies to lay eggs. Stack two plastic or glass cups. (Modified from Iwahashi et al., 1976)

Figure 2. Fruit flies being reared to lay eggs.

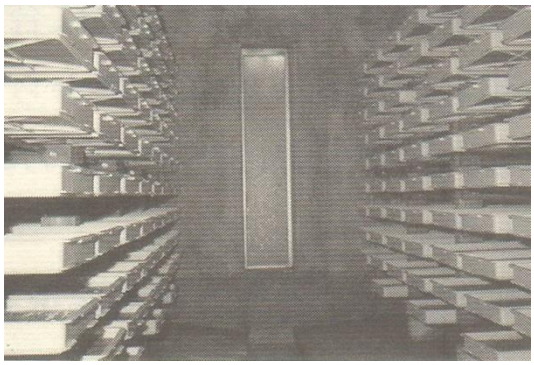


Figure 3. Fruit fly larvae rearing room. Larvae are reared on artificial feed in shallow trays.

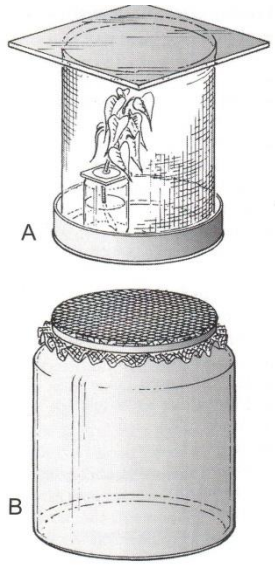



Figure 4. Rearing container for rearing adult fruit flies. Use containers with ventilation. Place feed for rearing adult fruit flies and water-soaked agar in the container. (From Gibb & Oseto, 2006)

Reference:

Life expectancy of a *Bactrocera dorsalis* fruit fly (female): Three months or longer
Number of eggs hatched by one female: Average of 630 eggs (average of 18 eggs per day)

Life expectancy of a *Zeugodacus cucurbitae* fruit fly (female): Three months or longer
Number of eggs hatched by one female: Average of 540 eggs (average of 11 eggs per day under 25ºC conditions)

Starts laying eggs about 20 days after emergence.

## Experiment 1. Examine the baiting effectiveness of attractant components

Some designs involve creating a large netted enclosure outside (about 5 × 3 × 2.5 m) in which to perform baiting experiments, but the experimental design used here can be used indoors.

### Tools for experiment:

- Rearing cage (Figure 6: About 30 × 30 cm with a height of 45 cm): Store-bought ones are expensive. Making a cage is easy. It is best to make two or three cages to perform multiple experiments at the same time.
- Simple traps (Figure 5): 90 mm diameter clear plastic cup with two plastic tubes with an inner diameter of 10 mm attached and a 90 mm diameter filter paper on the bottom (Figure 4). Prepare six traps.
- Micropipette: 1
- Filter paper (90 mm diameter): 20 sheets
- Sample aspirators: 3
- Plastic centrifuge tubes (for 45 mL): 10
- Micro Tubes (for 1.5 mL): 100
- Measuring cylinders (with 1 mL increments): 5
- Stop watches or timers: 3

Experimental reagents:

- Methyl eugenol (Me) (Not containing insecticidal components. Same concentration as the chemicals used outdoors.)

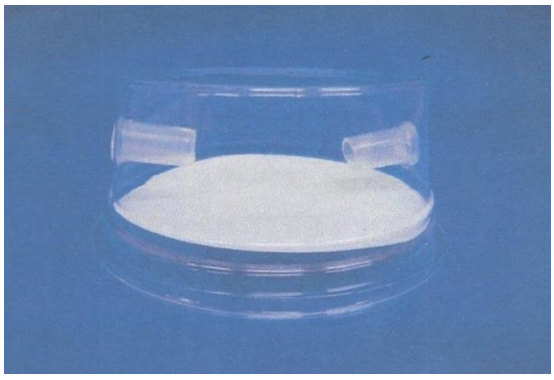


Figure 5. Simple traps (attract traps) used in this experiment. (From Kaneda & Sasaki, 2019)

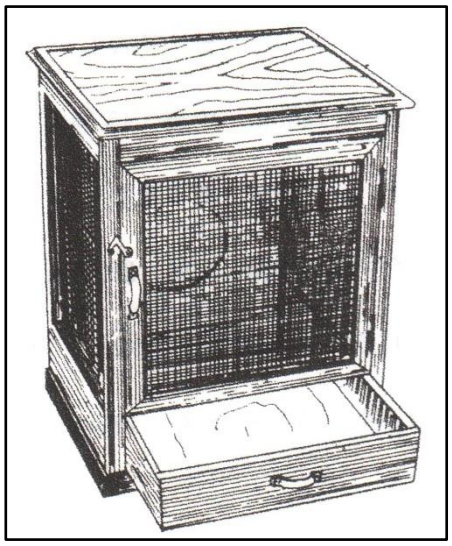


Figure 6. Rearing cage. Prepare a cage that is about 30 × 30 cm with a height of 45 cm.

Experimental guidelines:

1. Release 20 adult *Bactrocera dorsalis* (or *B. frauenfeldi*) fruit flies 12 days after emergence into the rearing cage (use the sample aspirator).
2. Leave them for one hour to allow them to calm down.
3. Point the micropipette to the center of the filter paper in the simple trap and drop 5 μL of methyl eugenol (Me) onto the filter paper. Place the trap inside the rearing cage and start measurement.
4. Count the number of fruit flies attracted to the traps (inside the traps) every 10 minutes for a total of six times.

Conduct this experiment six times (10 times). Use the same start time and temperature conditions (as much as possible) for the experiment (n = 6 is the minimum number of repetitions).

If the attraction rate obtained is 80% or higher, it can be concluded that there is no resistance to the attractant.

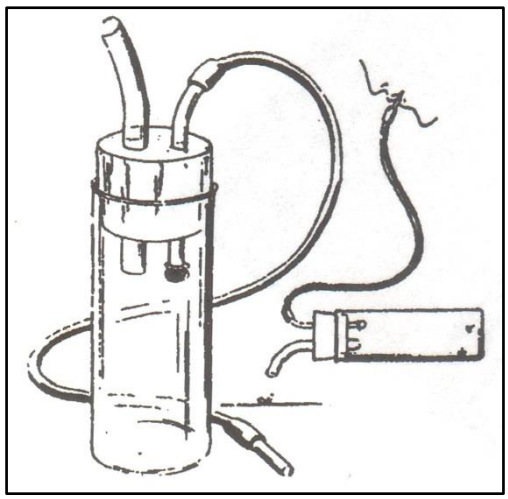


Figure 7. Sample aspirator.

## Experiment 2: Examine the effectiveness of insecticidal components

Usually the topical application method or the filter paper method is used. In this experiment, a simplified filter paper method will be used.

Tools for experiment:

- 90 mm diameter clear plastic cups. Place a 90 mm diameter filter paper on the bottom (Figure 8). Stretch a net across the lid for good ventilation and stick damp cotton to the top at the start of the experiment. As it is discarded for each trial, prepare a large amount (50 in one species).
- Pipettes (for mL measurement): 5
- Filter paper (90 mm diameter): 50 sheets

The following are the same as experiment 1:

- Sample aspirators: 3
- Plastic centrifuge tubes (for 45 mL): 10
- Micro Tubes (for 1.5 mL): 100
- Measuring cylinders (with 1 mL increments): 5
- Stop watches or timers: 3

Experimental reagents:

- Insecticidal components: Dibrome (BRP) (If it is possible that it may be used in Palau: Diazinon, malathion, sumithion, etc.)
- Acetone: 1 bottle

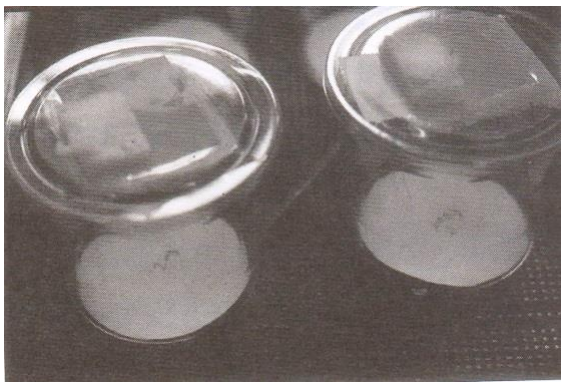


Figure 8. Clear plastic cup used in the experiment. Cut an opening in the center of the lid and attach a net. Stick damp cotton to the top and put filter paper on the bottom.

Experimental guidelines:

1. Drop 1 mL of solution diluted to the prescribed concentration of acetone (four steps from 5 to 0.01%) onto the center of the filter paper, let it drop at room temperature, and then lay it on the bottom of a clear plastic cup.
2. Put 10 male (or female) fruit flies in the clear plastic cup and then count the number that have died every 10 minutes (for 60 minutes in total).

   At the same time, record the time at which half (five fruit flies) have died (the $KT_{50}$ value).
3. Leave the cup as is and again count the number that have died after 24 hours.

Repeat this experiment six times (n = 6 is the minimum number of repetitions). Discard the clear plastic cup after one use.

When testing the effectiveness of dibrome:

Prepare 5% solution → 0.05 mL per 1 mL = ca. 50 mg (rough content in a bait trap)
Prepare 1% solution → 0.01 mL per 1 mL = ca. 10 mg
Prepare 0.1% solution → 0.001 mL per 1 mL = ca. 1 mg
Prepare 0.01% solution → 0.0001 mL per 1 mL = ca. 0.1 mg

Conduct the experiment with the above four concentrations.
You will need 10 fruit flies × 6 trials (repeated experiment) × 4 concentrations (5 to 0.01%) × 2 (for males and for females) = 480 fruit flies (240 males and 240 females). If doing 10 repetitions, you will need at least 400 males.

Strictly speaking, you cannot determine dibrome resistance without experimental data showing that there are susceptible species with no resistance to (tolerance of) dibrome. However, you can also measure the insecticidal effects of the content used in bait traps in this experiment. While it is best to perform the same experiment using other insecticides such as diazinon as well, it is also acceptable to decide whether or not to conduct those experiments after determining the effectiveness of dibrome.

Reference: Differentiation of males and females

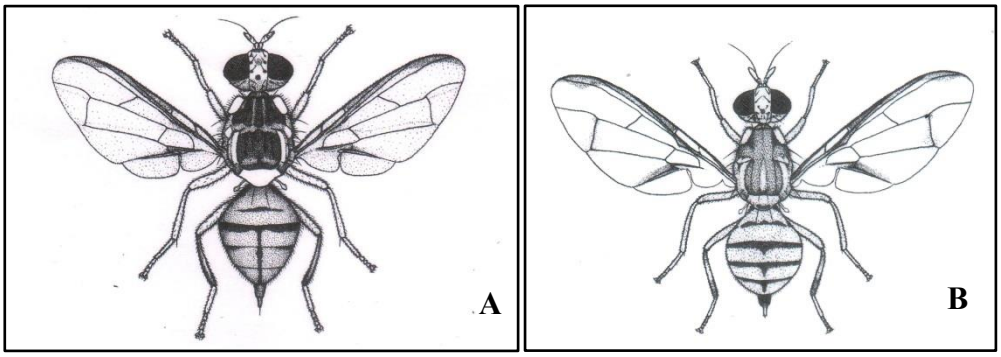



Figure 9. *Bactrocera dorsalis* female (A) and *Zeugodacus cucurbitae* female (B) (Modified from Hirao & Umeya, 1974)

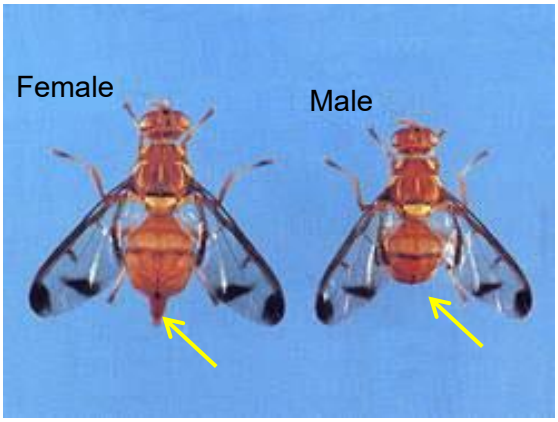



Figure 10. *Zeugodacus cucurbitae* female and male. The end of the abdomen is thin and pointed in females and rounded in males. (Photo by Okinawa Prefectural Agricultural Research Center: https://www.pref.okinawa.jp/site/norin/byougaichubojo/kawata.html)

## References cited

Gibb, T. J. & C. Y. Oseto, 2006. Arthropod collection and identification. Laboratory and field techniques. Academic Press, 311 pp.

Hirao, J. & K. Umeya, 1974. Report on field survey on fruit flies in mango orchards in the Philippines. Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, 21 pp. [In Japanese.]

Iwahashi, O., Y. Ito, H. Zukeyama & Y. Yogi, 1976. A progress report on the sterile insect releases of

the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) on Kime Is., Okinawa. Appl. Ent. Zool., 11: 194-201.

Kaneda, M. & M. Sasaki, 2019. Trap improvement for *Baxtrocera correcta* (Bezzi) (Diptera: Tephritidae) by mixing methyl eugenol and β-caryophyllene attractants. Research Bulletin of the Plant Protection Service Japan, 55: 21-23. [In Japanese.]