Chapter 9

Sampling, Conserving and Identifying Fruit Flies

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INTRODUCTION

Among the fruit pests of tropical America, the fruit flies are considered of greatest economic importance, as they are key-pests for most of the fruit crops. They are multivoltine insects with relatively high biotic potential, and great capacity to infest different native and exotic fruit species. They belong to the order Diptera, family Tephritidae. Five genera are important as pests: *Anastrepha*, *Bactrocera*, *Ceratitis*, *Rhagoletis* and *Dacus*, which are spread globally throughout the continents, except Antarctica (White and Elson-Harris, 1992). A few studies have highlighted the ecology and aetiological aspects of fruit flies, mainly focusing on the pupal and larval phases (Silva et al, 1996; Zucchi et al, 1996). Fruit flies have complex behaviour and taxonomy (Bateman, 1972; Steck and Wharton, 1988). Classification is exclusively based on adult morphological characteristics. The sexes are easily distinguished, as females have an ovipositor quite prominent at the end of the abdomen, with a long and fine tip. Taxonomic characteristics differentiating gender in larvae and pupae are not yet established (Salles, 2000).

Damage is caused during the immature phase, a period in which the larvae destroy the fruit pulp, making them unsuitable for harvesting and consumption. Before they reach the adult stage, the larvae migrate from the fruit to pupate in the soil (Plate 8a). Thus, fruit flies are temporary soil inhabitants, because they live only part of their life cycle in soil. After infested fruits have dropped, larvae move on the soil surface to find suitable soil conditions, penetrate to approximate 10cm depth and pupate. This depth can vary according to physical soil conditions, mainly temperature, humidity and texture. The entire pupal stage occurs in soil. Therefore, for a part of their life cycle, fruit flies can be considered soil organisms. The pupal phase lasts from eight to ten days depending on temperature and humidity. The pupal phase is the one most vulnerable to abiotic stress and to many natural enemies, mainly predators and entomopathogens such as bacteria, fungi and nematodes which are also part of the soil biota (see Chapter 10). These antagonists play an important

role in the biological control of fruit flies. Even if larvae are parasitized (infected) inside the fruits, the adult parasites can emerge in the soil. Therefore, it is of fundamental importance to consider soil biodiversity as an important factor influencing larval and pupal mortality when designing integrated pest management strategies.

Because the life cycle involves the aerial parts of the plant host as well the soil, sampling procedures vary according to the stage and purpose. For the adult fly, which is diurnal and responds to visual and olfactory stimuli, the use of traps containing food baits is most suitable. However, this technique does not unambiguously establish the relationship between the fly and its host. This can only be achieved by collecting infested fruits, branches or seeds, where the fruit-fly larvae are lodged.

SAMPLING FRUIT FLIES

Sampling of adult fruit flies is carried out by using plastic McPhail traps (Plate 8b) containing a food bait, usually 200ml of hydrolyzed corn protein (5 per cent in water preserved with sodium tetraborate, at pH between 8.5 and 9.0). Alternatively, it is possible to use fruit juice at 10 per cent, sugar at 10 per cent or sugar cane syrup at 10 per cent. The food baits should be replaced weekly and trapped specimens removed. The traps are installed in the middle of the tree canopy and the location is georeferenced (GPS). The number of traps per unit area can vary according to project objectives. If the objective is pest monitoring, one trap per 4ha is sufficient. When the objective is integrated control, the density should be three to five traps per ha or according to the extant land-use systems, that is, all land uses represented and monitored with the same trap density. Up to five traps per ha can be installed in areas with shrubs or trees (home gardens), while up to three per ha are suitable in herbaceous (arable) plantings. In the case of herbaceous plants the trap can be supported by three sticks positioned to form a tripod-like structure, or placed in an adjacent tree if one is available. Traps must be positioned equidistant from each other.

COLLECTING THE TRAPPED ADULTS

As several insect species of diverse taxonomic categories are likely to be trapped, it is first necessary to sort out fruit flies from other specimens. This can be done in the field or in the lab. When food baits are replaced, the contents of the trap should be passed through a fine nylon mesh of 1.5mm to remove the flies. Using curved forceps, the fruit flies are sorted out from other taxonomic groups. Their sex is recorded and they are stored in labelled glass jars (around 50ml), containing 70 per cent alcohol for further identification. The label must include basic sampling information and the trap number. For example:

- Manaus-AM
- Brazil
- 04° 05`S; 60° 04´W
- 23/3/2006
- Silva, N. M.
- Trap No 5

Other taxonomic groups collected in the traps should also be noted.

FRUIT SAMPLING

To establish the relationships of the *Anastrepha* species with their/its host(s), fruits are collected randomly in different land-use systems and at different stages of maturation. The fruits must be collected directly from the trees or immediately after they fall to the soil. Fruits sampled are separated by species, protected in cloth bags and transported in insulated cool boxes to the lab. After that, they are weighed, counted and separated by sampling point into plastic trays containing a layer of vermiculite or fine sand, which serves as substratum for pupation. Finally, the trays are then covered by a voile cloth, secured tightly with rubber bands to prevent the escape of any adult flies that emerge.

OBTAINING THE PUPAE

The substratum of vermiculite or fine sand is passed through a galvanized iron mesh of 1.5mm to separate the pupae, which are placed in cages with the respective identification labels, to allow the emergence of the adult fruit flies and/or parasitoids. The cages should be examined daily. Emerged adults are retained in the cages for 48 hours, to allow cuticular hardening and the full development of the wing spots (Plate 8c), which are of great importance for taxonomic identification.

After emergence adult flies are fed with a 10 per cent aqueous solution of honey, changed daily. Dates of emergence and the number and sex of adult fruit flies or parasitoids must be recorded. Finally, specimens are fixed in 70 per cent alcohol. The determination of sexual ratio (SR) (i.e. females to males ratio) for both adult fruit flies and parasitoids, is made according to Silveira-Neto et al (1976) using the equation below:

$$\mathbf{SR} = \frac{\text{No of females}}{\text{No of females + No of males}}$$

SPECIES IDENTIFICATION

The taxonomic identification of tephritid species is based on the ventral examination of the female apical aculeus, under a stereoscopic microscope (40×) or in mounted slides for examination in the transmission microscope (100×). It is first necessary to secure the extroversion of the aculeus with the help of needles or forceps (Plate 8d), as described by Zucchi (1988). Species identification within *Anastrepha* is based on the adult females, by reference to the wing pattern, body colour, mesonotum, mediotergite, abdomen and especially the morphological characteristics of the apical aculeus (Plate 8e), which are compared with museum specimens and submitted to taxonomic keys according to Lima (1934), Stone (1942), Foote (1967), Steyskal (1977), Silva (1993), Ronchi-Teles (2002), Zucchi (1978 and 2000) and Norrbom (1985). It is recommended that voucher species are deposited at a museum or added to the collection of the institutional laboratory.

REFERENCES

Bateman, M. A. (1972) 'The ecology of fruit flies', *Annual Review Entomology*, vol 17, pp493–518

Foote, R. H. (1967) 'Family Tephritidae', in M. Vanzolini (ed) *A Catalogue of the Diptera of the Americas South of the United States*, Secretaria da Agricultura, Departamento de Zoologia, São Paulo

Lima, A. C. (1934) 'Moscas-de-frutas do gênero *Anastrepha* Schiner, 1868 (Diptera: Trypetidae)', *Memórias do Instituto Oswaldo Cruz (Rio de Janeiro)*, vol 28, issue 4, pp487–575

Norrbom, A. L. (1985) 'Phylogenetic analysis and taxonomy of the *cryptostrepha*, *daciformis*, *robusta* and *schausi* species groups of *Anastrepha* Schiner (Diptera: Tephritidae)', PhD thesis, The Pennsylvania State University, Pennsylvania

Ronchi-Teles, B. (2002) 'Ocorrência e flutuação populacional de espécies de moscas-das-frutas e parasitóides com ênfase para o Gênero *Anastrepha* (Diptera: Tephritidae) na Amazônia Brasileira', PhD Thesis, Federal University of the Amazon (UFAM) and National Institute for Agricultural Research (INPA), Manaus, Brazil

Salles, L. A. (2000) 'Biologia e ciclo de vida de *Anastrepha fraterculus* (Wied.)', in A. Malavasi and R. A. Zucchi (eds) *Moscas-das-frutas de importância econômica no Brasil: Conhecimento básico e aplicado*, Ribeirão Preto, Holos, Brazil

Silva, N. M. (1993) 'Levantamento e análise faunística de moscas-das-frutas (Diptera: Tephritidae) em quatro locais do Estado do Amazonas', PhD Thesis, Escola Superior de Agricultura 'Luiz de Queiroz', University of São Paulo, São Paulo

Silva, N. M., Zucchi, R. A., Silveira Neto, S. (1996) 'The natural hosts plants of *Anastrepha* (Diptera: Tephritidae) in the State of Amazonas, Brazil', in G. J. Steck and B. A. MacPherson (eds) *Fruit Fly Pests: A World Assessment of their Biology and Management*, CRC Press, Boca Raton

Silveira Neto, S., Nakano, O., Barbin, D. and Nova, N. A. V. (eds) (1976) *Manual de Ecologia dos Insetos*, Ceres, São Paulo

Steck, J. and Wharton, R. A. (1988) 'Description of immature stages of *Anastrepha interrupta*, *A. limae*, and *A. grandis* (Diptera: Tephritidae)', *Annals of the Entomological Society of America*, vol 81, issue 6, pp994–1003

Steyskal, G. C. (1977) *Pictorial Key to Species of the Genus* Anastrepha *(Diptera: Tephritidae)*, Entomological Society of Washington, Washington DC

Stone, A. (1942) 'The fruit flies of the genus *Anastrepha*', *United States Department of Agriculture Miscellaneous Publications*, vol 439, pp1–112

White, I. M. and Elson-Harris, M. M. (1992) *Fruit Flies of Economic Significance: Their Identifications and Bionomics*, CAB International, Wallingford

Zucchi, R. A. (1978) 'Taxonomia das espécies de *Anastrepha* Schiner 1868 (Diptera: Tephritidae) assinaladas no Brasil', PhD Thesis, Escola Superior de Agricultura 'Luiz de Queiroz', University of São Paulo, São Paulo

Zucchi, R. A. (1988) 'Moscas-das-frutas (Dip., Tephritidae) no Brasil: taxonomia, distribuição geográfica e hospedeiros', in H. L. M. de Souza (ed) *Moscas-das-frutas no Brasil*, Campinas, Fundação Cargill

Zucchi, R. A. (2000) 'Taxonomia', in A. Malavasi and R. A. Zucchi (eds) *Moscas-das-frutas de importância econômica no Brasil: Conhecimento básico e aplicado,* Ribeirão Preto, Holos

Zucchi, R. A., Silva, N. M. and Silveira Neto, S. (1996) '*Anastrepha* species (Diptera: Tephritidae) from the Brazilian Amazon: Distribution, hosts and lectotype designations', in G. J. Steck and B. A. MacPherson (eds) *Fruit Fly Pests: A World Assessment of their Biology and Management*, CRC Press, Boca Raton