IJIE Int. J. Indust. Entomol. 33(1) 15-23 (2016)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2016.33.1.15

Artificial rearing of the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) for use in the Sterile Insect Technique: improvements of the egg collection system

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Abstract

One major constraint in the development and implementation of a successful and cost-effective area-wide integrated pest management (AW-IPM) programme with a SIT component for *Bactrocera oleae* (Diptera: Tephritidae) is the ability to produce a large number of high quality mass-reared individuals. The aim of this study was to develop a more efficient and practical egg collection system in an attempt to improve the mass-rearing of this species. The following basic parameters were examined: egg production per female, egg hatch, pupal recovery, pupal weight, adult emergence and percentage of fliers. Three different strains (Israel wild-type, France wild-type, and Greece laboratory) were tested and each strain was evaluated for six generations. Female flies of the Israel strain produced significantly more eggs per female than the other two strains, but egg hatch was significantly lower. Egg hatch of the France wild type and the Greece laboratory strain was similar. For all other parameters, there was no significant difference between strains; however, there was a significant generational effect for all parameters observed. As a result of this study, a protocol was developed for the mass-rearing of this species that included the use of large adult holding cages that could house up to 96,000 flies per cage. The newly developed method of egg collection using a flat wax panel as one of the sides of an adult holding cage proved to be cost-effective, efficient, making colony growth easier for industrial mass-rearing.

Int. J. Indust. Entomol. 33(1), 15-23 (2016)

Received : 25 Aug 2016
Accepted : 19 Sep 2016

Keywords:
artificial rearing,
flat egging panel,
hybridization,
olive fruit fly

Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) is a serious pest of economic importance for the production of olive fruits in the Mediterranean basin and the Middle East. It is highly invasive and has become established in North America after its first

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detection in 1998 in California (California, Arizona and northern Mexico) (Rice *et al.*, 2003). *B. oleae* larvae infest olive fruits and causes premature fruit drop (Collier and Van Steenwyk, 2003), mediates bacterial and fungal contamination of remaining fruit (Zalom *et al.*, 2009), causes undesirable staining of olive oil (Neuenschwander and Michelakis, 1978) and renders table olives unmarketable (Kapatos, 1989).

Traditionally, *B. oleae* infestations have been controlled using indiscriminative applications of synthetic insecticides. The insecticides have however serious limitations due to their toxic effects, residues in the olive fruit and olive oil, causing outbreaks of secondary insect pests and other adverse effects on the environment (Ferreira and Taínha, 1983). Therefore, there is a great demand to develop control methods which are environmentally friendly, efficient and cost effective. The sterile insect technique (SIT) is an eco-friendly control tactic for the management of selected insect pests. It relies on the mass-rearing of the target insect, sterilizing the male sex using ionizing radiation and releasing the sterile males in the target area where they will mate with virgin wild females and transfer their sterile sperm which results in unviable eggs (Knipling, 1955). Successive, regular and sustained releases of sterile insects will gradually reduce the density of the target population to a very low, economically acceptable level and in some cases eradication might be achievable (Knipling ,1955). The SIT has been proven to be very effective for the suppression, containment, prevention or eradication of several fruit flies species such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) in Mexico (Hendrichs *et al.*, 1983), the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) in Thailand (Orankanok *et al.*, 2007), the Mexican fruit fly, *Anastrepha ludens* (Loew) in north-western Mexico (Reyes Flores *et al.*, 2000), the melon fly, *Bactrocera cucurbitae* Coquillett in the Okinawa Islands, Japan (Koyama *et al.*, 2004) and the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) in western Australia (Fisher, 1996;Jessup *et al.*, 2007).

Despite its many successes and increased applications world-wide, there is still a need to improve several aspects of the SIT package for certain pest insect species. The SIT requires not only the ability to produce large quantities of insects at a reasonable cost but the mass-produced must display a behavioural repertoire that allows the released sterile males to compete with wild males for wild females, transfer their sterile sperm and induce sterility in the target population.

The development and implementation of the SIT as part of area-wide integrated pest management (AW-IPM) strategies (Vreysen *et al.*, 2007) against *B. oleae* has great potential in view of the extensive economic losses it causes. In addition, due to the monophagous nature of this pest that limits greatly its distribution, the probability of successful application of the SIT against this pest is considered high. However, the main limitation for its successful and efficient application remains the lack of efficient mass-rearing methods for this species.

The main bottleneck for the use of the SIT against B. oleae is the lack of consistency in producing large numbers of flies. The challenges does not only include the adaptation of wild flies to an artificial laboratory environment (Ahmad *et al.*, 2014), but also the development of efficient egg collection systems using artificial devices, proficient larval cultivation in artificial diets and adequate pupal recovery. With respect to oviposition substrates, the common practice has been to use paraffin domes that were exchanged daily or sometimes at longer intervals (Moore, 1959; Cantarino and Rey, 1970; Hagen *et al.*, 1963), parafilm (Silva, 1973), smooth surface or paraffin coated nylon gauze (Tzanakakis, 1971) and nylon gauze (Cavalloro and Girolami, 1968). All of these methods however are labour intensive and the number of egg obtained and their percentage hatch was not satisfactory. The production of large numbers of olive fruit flies required for the application of the SIT therefore necessitated the development of a better adult holding and egg collection system. The objective of this study was to assess improvements in egg collection system for olive fruit fly.

Materials and Methods

Origin of flies

The *B. oleae* populations were maintained at the FAO/IAEA Insect Pest Control Laboratory (IPCL), Seibersdorf, Austria under the conditions described below. Insects originated from: a) a colony maintained at the Demokritos laboratory of the National Centre of Scientific Research, Athens, Greece that was initiated from wild material collected in 2003 and reared in the laboratory for > 500 generations before being shipped to the IPCL, where a colony was established and reared for a further > 90 generations. This colony is referred to as 'lab strain' b) a colony that was established from wild pupae collected in Israel and cultured at the