

1. Introduction

1.1. Scope

This document represents the recommendations, reached by consensus of an international group of quality control experts, on the standard procedures for *product* quality control (QC) for mass reared and sterilized tephritid flies that are to be used in Sterile Insect Technique (SIT) programmes. In addition, this international manual describes recommended methods of handling and packaging pupae during irradiation and shipment. A majority of the procedures were initially designed specifically for the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), but they are applicable, with minor modification in some cases, for other tephritid species such as various *Anastrepha* and *Bactrocera* species. The manual is evolving and subject to periodic updates. The future additions will continue to include other fruit flies as the need is identified and data become available.

If followed, procedures described in this manual will help ensure that the quality of mass-produced flies is measured accurately in a standardized fashion, allowing comparisons of quality over time and across rearing facilities and field programmes. Problems in rearing, irradiation and handling procedures, and strain quality can be identified and hopefully corrected before field programmes are affected.

Tests and procedures described in this document are only part of a total quality control programme for tephritid fly production (a list of tephritid fly rearing facilities worldwide is available at www.iaea.org/programmes/nafa/d4/public/sit-may02.pdf). The *product* QC evaluations included in this manual are, unless otherwise noted, required to be conducted during SIT programmes by the field program staff, not the production staff. Additional product QC tests have been developed and their use is optional (see APPENDIX C: ANCILLARY TESTS). *Production* and *process* QC evaluations (e.g., analysis of diet components, monitoring the rearing environment, yield of larvae, development rate, etc.) are not within the scope of this document. Standard Operating Procedures (SOPs) are available at the various mass rearing facilities for the conduct of *production* and *process* QC evaluations.

Quality specifications are included for minimum and mean acceptability of conventional and genetic sexing strains of *C. capitata*, as well as some *Bactrocera* and *Anastrepha* species, for use in SIT programs.

1.2. Background

It became evident in several SIT fruit fly projects in the 1970's that mass reared fruit flies were not performing in the field as expected. In an effort to define and measure the performance of mass-reared flies and wild flies, tests were developed by various workers. During the last 20 years (see APPENDIX A: CHRONOLOGY OF PRODUCT QUALITY CONTROL OF TEPHRITID FLIES FOR USE IN SIT PROGRAMMES), there have been systematic efforts by various groups to develop a series of tests that could be used to compare the quality of sterile tephritid flies produced at different rearing facilities. Those efforts led to the production of several fruit fly quality control manuals (e.g., Orozco *et al.* 1983, Brazzel *et al.* 1986). With the increasing transboundary shipment of sterile insects, and interest from the private sector to invest in the SIT, the need for harmonized standards of quality against which sterile insects can be measured objectively became essential to encourage financial investment in commercial sterile insect mass rearing facilities. To address this need, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture sponsored a Consultant's Meeting to attempt to obtain an international consensus on product quality control procedures for mass-reared tephritid flies.

1.3. Rationale

The Need for Product Quality Control in SIT Programmes

The goal of SIT programmes is to reduce or eliminate wild insect populations. These programmes are effective when high proportions of wild females mate with sterile males and thus fail to reproduce. Successful application of SIT requires that an effective ratio of sterile to wild flies (the "over flooding ratio") be maintained in the field. Mass rearing and releasing a given number of sterile fruit flies into the field is only part of a successful SIT programme.

Managers of SIT programmes also need to ensure that, once in the field, the sterile males compete effectively with wild males and mate with wild females and successfully transfer their sperm. This is especially critical for insects that, like tephritids, have a complex mating system. Effective methods for monitoring and providing feedback on the quality and competitiveness of sterile fruit flies are critical to the success of SIT programmes.

Mating Behaviour and Sterile Male Competitiveness in the Field

Competitiveness, a product of many individual factors, is the overall ability of sterile males to compete for wild females against wild males of the target population. The components of competitiveness can be lumped into broad categories such as ability to survive in the field, mating propensity, mating compatibility, and post-mating factors. The complexity of these various categories, and their relative influence on sterile insect competitiveness, will vary depending on the biology and mating system of the species. A comprehensive QC programme for the mass-reared male fly (end product) should contain a full scope of tests designed to establish fly competitiveness. Tests that are used must be tailored to, and appropriate for, the individual species. To that end, QC tests should ideally be capable of measuring quality parameters from emergence to sperm transfer, and even subsequent effects on the behaviour of wild females flies.

Mating compatibility is defined as the successful interaction of the sterile male and wild female fly, from behaviours that bring them together in a mating arena to effective sperm transfer. In SIT programmes for pest tephritids, mating compatibility is an especially critical issue because of two characteristics of their “lek” mating systems. First, the behaviour required of the male in these mating systems is unusually complex; second, females actively choose a mate from a number of suitors. The following discussion of mating behaviour is specific to *C. capitata* but relates, in principle, to many other pest tephritids that are subject to SIT operations.

To convince a wild female to mate, a sterile male must initially locate a microhabitat suitable for a mating arena and then begin “calling” (releasing sex attractant pheromone). For medfly, the microhabitat is typically on the underside of a well-lit leaf, and locating it probably involves responses to light and other physical stimuli. The multi-component pheromone presumably plays a role in courtship (see below) as well as in luring unmated females. Males also are attracted by the pheromone and, from the sum of physical and chemical factors, usually call in small aggregations that Prokopy and Hendrichs (1979) referred to as leks. Since then, most fruit fly workers have applied the term lek to the mating system of *C. capitata* and other tropical Tephritidae.

When a female approaches a male, he begins his courtship ritual of head-rocking and pulsed wing fanning. Communication to the female may involve chemical (pheromonal), visual, acoustic, and tactile cues. Qualitatively, the ritual is very

stereotyped (the same subset of behaviours almost always occurs), but quantitatively, there is plenty of variation to allow for distinct differences among males, both within and between populations. If females show interest in the male during the courtship or simply remain in the vicinity long enough, the male will usually attempt to mount her. Even if the male mounts the female, she still may choose to shake him off (and does so about half the time) rather than mate. A female typically solicits courtships from a number of males before allowing a male to mate with her.

Laboratory colonization and mass rearing can and do effect phenotypic changes in the behaviour of mass-reared males. Some of these changes have genetic bases; i.e., result from inadvertent selection or genetic drift in the rearing colony. The changes potentially influence such factors as mating age, courtship behaviour, quality or quantity of pheromone produced, and diel periodicity (Cayol 2000). If wild females fail to come in contact with, or reject, sterile males because of these changes, the competitiveness of the sterile flies can be drastically reduced, as shown for a laboratory colony of more than 40 years (McInnis *et al.* 1996).

Changes from the Earlier Manuals

Previous manuals (Orozco *et al.* 1983, Brazzel *et al.* 1986) were intended to provide standardized procedures for routine (daily, weekly) checks of the product of the rearing process. The tests that they outlined were, accordingly, designed to assess emergence, flight ability, mating propensity and indices of the basic viability of the mass-reared flies. In particular, tests outlined by Brazzel *et al.* (1986) were not intended to address mating competitiveness and compatibility or post-mating factors, although the authors noted the need to run regular tests in those areas.

In the years that followed, results of the tests outlined by Brazzel *et al.* (1986) apparently became equated with overall fly quality and competitiveness, at least for Mediterranean fruit fly SIT programmes in the United States. Recent research (e.g. Hibino and Iwahashi 1989, 1991; McInnis *et al.* 1996) suggests that lapses in mating compatibility are a possible cause of poor performance by sterile males in the field and, when severe, can result in the failure of SIT programmes.

The major impetus for Version 4 of this manual was to include *required* tests of mating competitiveness and compatibility. In addition, tests were added to provide better information on survival and dispersal of sterile flies in the field.

Existing laboratory tests were, in some cases, modified to improve standardization. For one test

(mating propensity), the participants in the Consultant's Meeting felt that the results were overvalued and frequently misinterpreted as a) no wild females are involved, b) mating speed is not representative of courtship behaviour, and c) it does not allow for female choice in view that it is carried out at very high densities in small cages. That test was revised, renamed (laboratory mating test), and changed from its "required" status in earlier manuals to an ancillary test. In addition to modifications of tests, specifications and methods were expanded to include genetic sexing strains of *C. capitata* and additional species, including *Anastrepha* and *Bactrocera*.

Management of Product Quality Control

Conflicts may arise in the production facility with the need to mass rear predetermined quantities and at the same time to produce high quality flies. Examples include: (1) attempts to increase production levels may result in reducing the size and quality of the sterile flies; (2) production managers may not find it advantageous to report or admit to lapses in the quality, and (3) production managers might be hesitant to replace an older strain with a newer less laboratory-adapted strain that is initially more difficult to rear but more competitive in the field. Because of these conflicts, it is strongly recommended that *product* QC for SIT programmes be conducted by a unit that is not directly responsible for the production of the sterile flies. The *product* QC unit should not report to the Rearing Manager but to the Programme Manager. However, the *product* QC unit must work closely with rearing personnel involved in *production* and *process* QC evaluations and provide continuous feedback necessary to maintain an effective rearing process. Post-irradiation QC evaluations that are conducted at the production facility should be corroborated at emergence facilities and at field release sites utilizing the same methodology.

The most important part of the QC programme is to ensure that the mass reared sterile males interact successfully with the wild females of the target population. To ensure that the sterile males are competitive and compatible with the wild females, field evaluations must be conducted routinely. These tests should include wild flies collected from the area where releases are to occur or conducted in the location that is a likely source of pest introductions. Because this activity is critical to programme success, sufficient funding and other resources must be allotted for this purpose. The full-time staff dedicated to conducting field evaluations should include personnel trained in behavioural and ecological aspects of fruit fly biology. Both the Programme Manager and the end user (increasingly not the same) should be responsible for ensuring that these tests are

conducted, reports are submitted to all concerned parties, and appropriate actions are taken.

Future Plans to Update this Manual

As recommended by the consultant's, updates to this manual are anticipated as improvements are made. In this regard, ongoing Coordinated Research Projects sponsored by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, have among their specific research objectives the standardization and improvement of quality control (QC) tests, data management and information exchange on a global level.

This international manual and future revisions will become available through the Joint FAO/IAEA Division Home Page on the Internet (www.iaea.org/programmes/nafa/d4/index.htm).

1.4. Data Analysis, Presentation and Communication

Establishing specifications for the quality of insects to be used in an SIT programme is only the first step. Specifications have little value if the user cannot expect that they will be routinely met. That expectation requires an adequate QC programme at the production facility; a second requirement is analysis, verification, and communication between producer and user. Simple, routine procedures are available for collecting and preparing data on product quality that allow producer and user to evaluate the continuing processes of production and to predict trends leading to reduced quality. Production facilities *should*:

Prepare a capability analysis of each of the variables identified in the list of required routine quality tests. A sample of ≈ 50 measurements of each parameter will be accumulated during a period when the rearing process is thought to be "under control" (i.e., when fly quality, is, subjectively "good"). Mean values or overall proportions from each of those 50 tests results are then used to compute a measure of central tendency (for example, overall mean) and control limits. Typical lower control limits might include a "warning limit" (set at a level where, with normal variation, measurements would be expected to exceed the warning limit 97.5% of the time) and an "action limit" (99.5% level). For continuous variables (e.g., pupal weight), the "warning" and "action" limits would, for example, be 2 and 3 standard deviations below the mean. For data in which each individual falls into one of two categories (for example, emerged or unemerged, male or female), statistical procedures based on the binomial

distribution (for example, *P*-charts) may be more appropriate.

Routinely (e.g., weekly) produce graphs that track the values of each QC parameter over time.

Graphs should also show lower control limits and perhaps the minimum levels specified in the respective section of this manual.

Provide copies of the charts to users and rearing personnel.

Values below the action limit, or consecutive values below the warning limit, would indicate a problem with the production process that requires the attention of rearing personnel. Extended periods when measurements consistently fall below the central value also indicate potential problems. Charts are provided to the user to enhance communication regarding variations and trends in fly quality. Analysis and charting of each measured parameter at the receiving station will be similarly conducted to allow comparison.

With advances in microcomputer technology, entry, storage, manipulation, and graphing of QC data can be easily automated. Data can be stored conveniently in electronic databases (use secure back-up systems), and database or spreadsheet forms can be designed that mimic the QC Forms such as those presented in this manual. Statistics such as flight ability, percent emergence, and mating propensity can be computed automatically, reducing the chance of human error. Data can be exported to spreadsheets or other applications for routine analysis or production of graphs. A number of statistical software packages are available that include specific routines for computing control limits and producing quality control graphs such as Shewhart *P*- and *X*-charts (e.g., SPSS 1996).

Control limits are effective for identifying deviations from normal levels in production processes because they are based on statistical evaluations of production data from a given facility, process, and strain of fly.

1.5. Relevant Literature

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