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Rearing Codling Moth for the Sterile Insect Technique

V.A. Dyck

Preface

The codling moth ***Cydia pomonella*** (Linnaeus) (Lepidoptera: Tortricidae) is amongst the most severe pests of pome fruit in the temperate regions of the world. Control of this pest has relied mostly on the use of broad-spectrum insecticides with all their negative environmental consequences, but also increasing resistance to a growing list of insecticides. Worldwide, farmers have been demanding alternative control techniques which are not only efficient but also friendly to the environment. These additional control techniques include synthetic growth regulators, mating disruption, attract and kill, microbiological control agents, and the sterile insect technique. The integration of sterile insects with other control practices within the context of area-wide integrated pest management (AW-IPM) offers great potential, as has been demonstrated with great success in the past 15 years in the Okanagan Valley of British Columbia, Canada.

Efficient and effective mass-rearing of the target insect is a fundamental component of the sterile insect technique (Sterile Insect Technique). Mass-rearing knowledge is also needed for other control methods, such as the production of codling moth virus and other microbials, and will also be needed for other genetic control methods that are anticipated in the future. It is a very challenging activity, especially for Lepidopteran pests, and its complexity is very often underestimated. Many years of research and methods development are usually required before all elements of the rearing process have been sufficiently mastered to deliver an end product (the sterile male) that can successfully compete with wild males following sterilization and release.

In the last years, in view of the above described problems, there has been an increasing interest by Member States to develop codling moth SIT for integration with other control tactics. The development of this document is a response to this increased interest, and it compiles and summarizes available information on the rearing of the codling moth, be it in the laboratory or on a larger scale. The information in this document deals with aspects such as colonization, adult and larval diet, sexing, quality control, shipment, disease control, data recording and management. It is not intended to be read from cover to cover, but the information is presented so that individual sections can be consulted by the reader when necessary. Hence, the document does not provide guidelines ***per se***, nor is it a compendium of standard operating procedures, as these will need to be developed for each rearing facility based upon local needs and availability of materials and ingredients. The list of references in this document is exhaustive, and an attempt has been made to be complete. The document is unique as, for the first time, it brings together all existing information on the rearing of the codling moth.

Contents

1. Introduction

1.1. SCOPE

The emphasis in this document is on mass-rearing systems for the codling moth *Cydia pomonella* (L.) (Horak and Brown 1991; Brown 2006) intended for use in the sterile insect technique (SIT) in area-wide integrated pest management (AW-IPM) programmes (Knipling 1966; Butt 1991; K. Bloem et al. 2005; Dyck et al. 2005a; Vreysen and Hendrichs 2005; Hendrichs et al. 2007; Vreysen et al. 2007b). However, it also covers some aspects of laboratory-scale rearing.

This document covers virtually all aspects, both theoretical and practical, of laboratory and mass-rearing of the codling moth, and also includes a major overview on quality control. Since in future international programmes live codling moths are likely to be shipped long distances, current shipping practices are summarized. This document also discusses management issues. Source details on a large number of cited references are provided as is a glossary and list of primary equipment used in a rearing facility.

Standard operating procedures (SOPs) help to standardize and reduce variation in the tasks done in a rearing facility (Schwalbe and Forrester 1984; Bruzzone et al. 1993; IAEA 2008) but they are not provided in this document. As Parker (2005) comments, "Each rearing facility should develop standard operating procedures (SOPs) for rearing operations, quality control operations, and finally responses to adverse quality control findings." The Okanagan-Kootenay Sterile Insect Release Program (OKSIR) facility in Osoyoos, Canada created a series of SOPs for mass-rearing the codling moth (Moore 2003).

1.2. RATIONALE

The information in this document is intended to assist both those who are relatively new at rearing this insect and those who wish to improve existing rearing systems. To date, most experience in rearing the codling moth has been in North America and Europe, and also some in Australia and New Zealand (IAEA 2008). Recently, there has been a considerable increase in interest in the temperate-climate countries of the southern hemisphere where apples and pears are grown commercially (Addison and Henrico 2005; Botto 2006; Kovaleski 2006; Taret et al. 2006).

1.3. BACKGROUND

Most publications deal with laboratory rearing which was initially done using apples, especially immature green (thinning) apples (Dickson et al. 1952; Hamilton and Hathaway 1966; Pristavko and Boreyko 1971; Hathaway et al. 1973). This method is usually used on a relatively small scale, but large numbers of codling moth were reared on thinning apples at the Yakima Agricultural Research Laboratory in Washington State, USA (Hathaway et al. 1971; White and Hutt 1972).

In the 1960s and 1970s, much work was done in North America and Europe on developing artificial larval diets, in some cases using natural food materials but in most cases developing a completely artificial diet from nutritional ingredients (Bathon et al. 1991). However, some ingredients were and still are chemically undefined (House 1961).

Considerable research on rearing was conducted by J.F. Howell and colleagues at the Yakima Laboratory (Howell 1967, 1970, 1972a, 1972c, 1981; Hathaway et al. 1971; Howell and Clift 1972; Toba and Howell 1991; Howell and Neven 2000) and by others in the USA (Redfern 1964; Rock 1967). Diets were also being developed in Europe (Coutin 1952; Navon 1968; Sender 1969, 1970; Navon and Moore 1971; Huber et al. 1972; Pristavko and Yanishevskaya 1972; Shumakov et al. 1974; Mani et al. 1978; Bathon 1981; Guennelon et al. 1981; Huber 1981). Summaries of codling moth diets can be found in Hamilton and Hathaway (1966), Sender (1969, 1970), Navon and Moore (1971), Huber et al. (1972), Shumakov et al. (1974), Butt (1975), Singh (1977), Ashby et al. (1985) and Reed and Tromley (1985).

Initially, artificial diets were used on a small scale, e.g. in small compartments in plastic trays or in plastic cups, test tubes, etc. (Hamilton and Hathaway 1966; Navon 1968; Howell 1967, 1970; Huber et al. 1972; Bathon 1981; Burton and Perkins 1984; Bathon et al. 1991; Reiser et al. 1993; Keil et al. 2001; Gu et al. 2006) as this avoids possible cannibalism. Later, as individual rearing techniques were changed to mass-rearing procedures, trays (Brinton et al. 1969; Howell 1971; Howell and Clift 1972; Batiste and Olson 1973; Hathaway et al. 1973; Mani et al. 1978; Reiser et al. 1993; Bloem et al. 2000) or boxes (Guennelon et al. 1981) were used. Larger production capability (and at a lower cost) was achieved when Brinton et al. (1969) developed a diet that substituted agar with other ingredients. This diet has been modified by other workers (Wildbolz and Mani 1971). The diet dried out as the larvae grew, matured and formed cocoons, and at adult emergence it was dry and hard. Nevertheless, as only adults were needed, handling larvae and pupae was avoided, reducing rearing costs.

A searchable database of worldwide codling moth literature from 1700–1997 is available at the Codling Moth Index (CMI) from the Codling Moth Information Support System (CMISS 2007). A good source of information on methods of rearing insects, primarily parasitoids and predators for biological control, is the IOBC Working Group on Quality Control of Mass-Reared Arthropods (AMRQC). The proceedings of previous workshops are available (AMRQC 2007).

There are many references on insect rearing, both in general and those dealing with Lepidoptera (Knipling 1966; Martin 1966; Smith 1966; Vanderzant 1966, 1974; Beck and Chippendale 1968; Chippendale and Beck 1968; Gast 1968; Walker 1968; Poitout and Bues 1970, 1972; Dadd 1973; Ivaldi-Sender 1974; Singh 1977; Kakinohana 1982; Leppla et al. 1982; Bartlett 1984, 1985; Collins 1984; Fisher 1984b; Joslyn 1984; King and Leppla 1984; Owens 1984; Schwalbe and Forrester

1984; Sikorowski 1984b; Stewart 1984; Fisher and Leppla 1985; Goodenough and Parnell 1985; Marroquin 1985; Moore 1985; Singh and Ashby 1985; Singh and Moore 1985; Tween 1987; Anderson and Leppla 1992; Mangan 1992; Mastro 1993; Reiser et al. 1993; Ochieng'-Odero 1994; Gooding et al. 1997; Leppla and Eden 1999; Nordlund 1999; Smith 1999; Wood and Wendel 1999; Leopold 2000, 2007; Cohen 2001, 2004; Hagler and Jackson 2001; Fisher 2002; Wyss 2002; Enkerlin and Quinlan 2004; Dowell et al. 2005; Dyck et al. 2005b, c; Parker 2005; Rendón et al. 2005; Taret et al. 2005; IAEA 2008).

2. History of Rearing the Codling Moth

2.1. REARING AND MASS-REARING

Singh (1977) distinguished laboratory and mass-rearing on the basis of scale and economics — in mass-rearing the objective is to produce large numbers of 'acceptable' insects at the lowest possible cost. Chambers (1977) defined mass-rearing as "the production of insects competent to achieve program goals with an acceptable cost/benefit ratio and in numbers per generation exceeding ten thousand to one million times the mean productivity of the native population female." Leppla et al. (1982) defined it as "a systematic enterprise accomplished with machinery in integrated facilities for the purpose of producing a relatively large surplus of insects for distribution".

The key concept is that insects are handled in groups and not as individuals. The various steps in rearing — egg collection, diet infestation and collection of larvae, pupae and adults — are all done by handling insects *en masse*. Mass-rearing usually involves large-scale rearing, but 'large scale' is a relative term and difficult to define. Large-scale rearing is not necessarily mass-rearing. Very large numbers of codling moths could be reared individually in millions of plastic cups using an 'army' of workers; this would certainly be large-scale rearing, but would not be mass-rearing.

An artificial diet is necessary for mass-rearing because it yields more uniform insects than would be obtained from a natural diet. It also provides predictable quality and reliable production (Leppla and Ashley 1989). Sanitation is also more easily achieved when using an artificial diet.

However, a small 'mother colony', kept separate from the main colony, may be reared differently to produce a virus-free colony or a new strain (Marroquin 1985). It may even be essential in these situations to rear insects individually. Similar to this concept is 'fractional colony propagation' (Hoffman et al. 1984).

2.2. IMMATURE APPLES

Rearing codling moth larvae on mature apples is problematic (Howell 1991) because relatively few newly hatched larvae penetrate and feed in mature apples, and at room temperature they decay rapidly. However, green immature apples (thinning apples) are penetrated much more readily (70% of neonate larvae enter these apples, acoording to Howell (1972a)) and remain as a suitable food for larvae for several weeks; they have been used in many laboratories (Dickson et al. 1952; Proverbs and Newton 1962a, b, c; Hamilton and Hathaway 1966; Hathaway

1966, 1967; Proverbs et al. 1966, 1967, 1969; Rock 1967; Jermy and Nagy 1969, 1971; White et al. 1969, 1970, 1972, 1973; Wildbolz and Riggenbach 1969; Butt et al. 1970, 1973; White and Hutt 1970, 1972; Hathaway et al. 1971, 1972, 1973; Pristavko and Boreyko 1971; Proverbs 1971, 1972; Howell 1972b; Moffitt and Albano 1972b; Moffitt et al. 1972; Butt et al. 1973; Ferro and Harwood 1973; Robinson 1973, 1974, 1975; Robinson and Proverbs 1973; White 1975; White and Mantey 1977; White et al. 1977; Toba and Howell 1991; Howell and Neven 2000).

Before use, apples are washed in water and strong detergent to remove insecticide residues (Hamilton and Hathaway 1966). However, water does not adequately remove residues (Toba and Howell 1991) and a residue analysis is required. Apples are surface-sterilized by dipping in a 0.5% sodium hypochlorite (NaOCl) solution for 5–10 min, washing in water for 5–10 min and then air-drying (Hamilton and Hathaway 1966; Jermy and Nagy 1971).

Thinning apples are collected during manual apple thinning in early summer. These apples, 2.5–4 cm in diameter, keep well in cold storage (0–5°C) for up to one year (Dickson et al. 1952). Many varieties are suitable, e.g. Jonathan, Winesap, Rome Beauty and Golden Delicious (Hamilton and Hathaway 1966; Dickson et al. 1969; Jermy and Nagy 1971).

To infest apples, egg sheets are placed on the apples in trays (Dickson et al. 1952; Hathaway 1967; Jermy and Nagy 1971; Pristavko and Boreyko 1971). Hamilton and Hathaway (1966) used 4.6 eggs for each apple, but later used newly hatched larvae. Moths can also be caged on the thinning apples and eggs laid directly. The tray's inner surfaces must be covered with rough screen because codling moth females lay eggs on virtually any smooth surface. Hatched larvae burrow directly into the apples. During larval development, the infested apples are held in metal or cardboard trays and covered with a tight-fitting screen/mesh/cloth lid to prevent larvae from escaping. The carrying capacity of a 4 cm diameter apple is approximately three larvae (Ferro and Harwood 1973). When larvae are mature (prepupal stage) they emerge from the apples to form cocoons, usually in corrugated cardboard (or fluted fibreboard) strips (Dickson et al. 1952; Hathaway 1967; White and Hutt 1970, 1972; Jermy and Nagy 1971; Pristavko and Boreyko 1971; Toba and Howell 1991; Howell and Neven 2000). Larvae pupate in the cocoons. Provided that a long daylength has been maintained the insects do not enter diapause and adults will emerge. Low light intensities are sufficient for the photoperiod reaction (Wildbolz and Riggenbach 1969). Light intensity should be at least 161 lux at the surface of the apples (Proverbs and Newton 1962a), but Dickson et al. (1952) used only 32 lux and Pristavko and Boreyko (1971) used 500–700 lux. Infested apples can be held at 22–30°C in the laboratory or in a greenhouse under daily fluctuating temperatures. Relative humidity (RH) was maintained at 35% (Dickson et al. 1952; Hamilton and Hathaway 1966), 60% (White and Hutt 1970; Pristavko and Boreyko 1971; Toba and Howell 1991) or 60–70% (White and Hutt 1972). In these conditions the life cycle is completed in about one month. Dickson et al. (1952) obtained a yield of 60 moths from 600