

## Documented Pupal Eye Color of Mediterranean Fruit Fly as a Tool for Radiation Sterilization

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**Pupal age is critical when sterilizing pupae of a different strain of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), for a sterile insect technique (SIT) program. Pupal age is usually determined using pupal eye color, which is optimally dark brown for *C. capitata* prior to sterilization. This color is achieved at 9 days of pupal age, 2 days before adult emergence, and upon maturity at 25 °C. However, it is often necessary to use different pupal holding temperatures in order to manipulate pupal development, especially when unforeseen problems occur during the *C. capitata* rearing procedure. Holding pupae at lower temperatures delays pupal development and slows down the progression of eye color changes; at higher temperatures, the opposite occurs. The pupal eye color of *C. capitata* was documented at different ages at different holding temperatures. When maturing pupae at 17, 20, 25 (standard holding temperature), and 28 °C, the developmental duration of pupae was 28, 17, 11, and 9 days, respectively. Using this eye color as the reference guide for timing the irradiation of pupae, the optimum pupal age for irradiation when held at 17, 19, and 28 °C was 23–26, 14–15, and 8 days old, respectively. Documented and close-up photographs of pupal eye color for different pupal holding temperatures are presented here can be used as a reference guide to determine the best time for the irradiation of pupae in an SIT program.**

Keywords: *Ceratitis capitata*, Mediterranean fruit fly, pupal development, pupal eye color, sterile insect technique.

### INTRODUCTION

Mediterranean fruit fly – *Ceratitis capitata* (Wiedemann) – is a major agricultural pest of tropical and subtropical regions worldwide, infesting more than 200 varieties of fruits and vegetables (Christenson and Foote 1960; Vargas *et al.* 1984, 1994, 1996). It causes significant loss to production and limits international trade of fresh commodities (Klassen *et al.* 1994, Hendrichs 1996, Chang *et al.* 2007). It occurs in Europe and the Mediterranean region, South Africa, the Middle East, South and Central

America, Australia, Hawaii, and in continental United States (Saul 1984).

In South Africa, the deciduous fruit industry is of great economic importance with close to 118 million cartons of fruits exported annually, resulting in gross annual export earnings of approximately US\$940 million (Barnes 2016). However, due to the presence of Mediterranean fruit flies, monetary losses in international fruit trade was estimated to exceed US\$3.2 billion per annum in 1997 (Mumford and Tween 1997). To address this situation, the creation of fruit fly-free zones or areas of low pest prevalence was

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therefore an urgent necessity. The sterile insect technique (SIT), integrated with other pest management measures, is widely regarded as the most practical and cost-effective means of establishing such areas. It is an environment-friendly approach to insect control that involves mass-rearing, sterilizing by ionizing radiation, and releasing pest insects in the target area in numbers large enough to outcompete their wild counterparts (Knippling 1955, Dyck *et al.* 2005). In certain cases, this type of insect pest control can lead to eventual eradication of the target pest population (Hendrichs and Robinson 2009).

A pilot project to suppress *C. capitata* in the Hex River Valley, Western Cape, South Africa – a geographically isolated table grape production area – was initiated with an SIT component in 1997, with sterile male releases of the Vienna 7-97 *tsl* genetic sexing strain of *C. capitata* commencing in 1999 (Barnes *et al.* 2004). In 2003, the program was privatized under the name SIT Africa (Pty) Ltd. [now FruitFly Africa (Pty) Ltd.] and, at this time, a new Mediterranean fruit fly Vienna 8 *tsl* genetic sexing strain – with improved production and quality potential – was provided to the SIT Africa Facility by the Entomology Unit of FAO/IAEA Agriculture and Biotechnology Laboratory based in Seibersdorf, Austria. SIT operations using the Vienna 8 *tsl* strain were conducted by aerial and ground releases of sterile males over 5,000 ha of export table grapes. Further details of the pilot project are described by Barnes (2016).

Success of an SIT program depends on the sterilization of the insects at the age at which the mating competitiveness of the released sterile adults with their wild counterparts is best preserved (Seo *et al.* 1987). In all fruit fly mass production facilities, pupae are irradiated 2 days before adult eclosion when held at standard pupal holding temperatures – using pupal eye color to judge the optimal time of irradiation. These pupal sterilization protocols are commonly applied to Mexican fruit fly (*Anastrepha ludens*) and West Indian fruit fly (*A. obliqua*) in Mexico (Hernández *et al.* 2007); Mediterranean fruit fly in Hawaii (Ohinata *et al.* 1971, Williamson *et al.* 1985), South Africa (Barnes *et al.* 2007) and Australia (Fisher 1997); melon fly (*Bactrocera cucurbitae*) in Japan (Teruya & Yukeyama 1979, Teruya & Isobe 1982); South American fruit fly (*A. fraterculus*) in Argentina (Allinhi *et al.* 2007); oriental fruit fly (*Bactrocera dorsalis*) in Thailand (Sutantawong *et al.* 2002); and Philippine fruit fly (*B. philippinensis*) in the Philippines (Resilva *et al.* 2007).

During SIT rearing operations, there are occasional situations that require delaying or speeding up fly emergence – especially when there is inclement weather, mechanical failures with irradiation equipment, large differences in cohort sizes, breakdown in the release operations, or a desirability of smaller or larger releases of flies (FAO/IAEA/USDA 2003). In this situation, eye color

is a reliable indicator of determining the physiological age of pupae (Ruhm and Calkins 1981). This is very useful especially when regulating pupal holding temperature to synchronize pupal development from different ages of pupae for release. In this case, pupal age for irradiation is determined by examination of the pupal eye color (Resilva *et al.* 2007). Pupal eye color of *B. philippinensis* was documented at different holding temperature, and the daily eye color changes were recorded and then matched with Munsel® Soil Color Charts (Resilva and Obra 2016). The Munsel® color system is a means to visually describe and match color using a scientific approach. It enables colors to be accurately portrayed via verbal descriptions and color codes, making it easy to define and express colors within narrow ranges in a very specific way (Gretag Macbeth 2000). The documented pupal eye color can be used as reference guide in the manipulation of pupal holding temperatures during rearing for SIT. In this investigation, similar studies were conducted to document daily pupal eye color changes of *C. capitata* held at different holding temperature regimes. Specifically, the pupal eye color at 25 °C (standard holding temperature) was the calibration point on the day of irradiation. The same pupal eye color serves as an indicator of the irradiation time for the other holding temperatures. The results obtained can be used as a guideline for irradiating *C. capitata* at the optimum time before adult emergence.

## MATERIALS AND METHODS

### Insects

Different strains of Mediterranean fruit fly *Ceratitis capitata* – such as the wild, Argentina, and Vienna 8 *tsl* varieties – have similar pupal development when held at different holding temperature. In this study, the Vienna 8 *tsl* strain was used and obtained from the SIT Africa Mass Rearing Facility; ARC Infruitec-Nietvoorbij; Fruit, Vine, and Wine Research Institute located in Stellenbosch, South Africa. The insects were reared following procedures described by Barnes *et al.* (2007).

### Environmental Conditions for Pupal Development

Mature larval samples of *C. capitata* Vienna 8 *tsl* strain were collected within 1 h after they left the larval diet for pupation to synchronize pupal development and adult emergence. About 500 ml of larvae were mixed with 25% moistened vermiculite, subdivided according to four temperature regimes, and placed in plastic pupation trays covered with cheese cloth to allowed free air circulation. Trays with the larvae were held for pupation in controlled temperature rooms at 17, 20, 25 (pupal holding standard), and 28 °C with 80–85% relative humidity.

### **Pupal Dissections, Eye Color Determination, and Photography**

About 50–100 pupal samples were collected and dissected daily for the different holding temperatures to observe pupal eye color changes from the day of pupation to the day of emergence. During dissection, the shell of the anterior part of the puparium was carefully removed to expose the eyes of the developing imago (Ruhm and Calkins 1981). At the same time, photographs of the eye color of *C. capitata* held at different temperatures were taken using a QX5 computer microscope at 60x magnification (2007 Digital Blue, Inc.; Microsoft Corporation, Atlanta, GA, USA) that was connected to a computer. Each pupa with eyes exposed was positioned under the microscope, focused with proper illumination, and a close-up photograph taken (Resilva and Pereira 2014, Resilva and Obra 2016). The daily eye color at each temperature were recorded and matched with the color scale of the Munsell® Soil Color Charts (Gretag Macbeth 2000). The calibration point at the standard holding temperature (25 °C) was the pupal eye color on the day of irradiation. Then, the same pupal eye color was used as the indicator for the correct physiological age for pupal irradiation at the other holding temperatures.

### **Determination of Adult Emergence and Flight Ability**

Three days before adult emergence, samples of 100 pupae were placed within a ring of paper, which was centered in the bottom of the petri dish. Black plexiglass tubes measuring 10 cm high and 8 cm diameter were placed within the petri dish. The inside of the tube were lightly coated with unscented talcum powder to prevent the flies from walking out. After emerged flies flew from the tube or died, fly emergence and fliers were determined following the standard quality control test procedure (FAO/IAEA/USDA 2003).

## **RESULTS AND DISCUSSIONS**

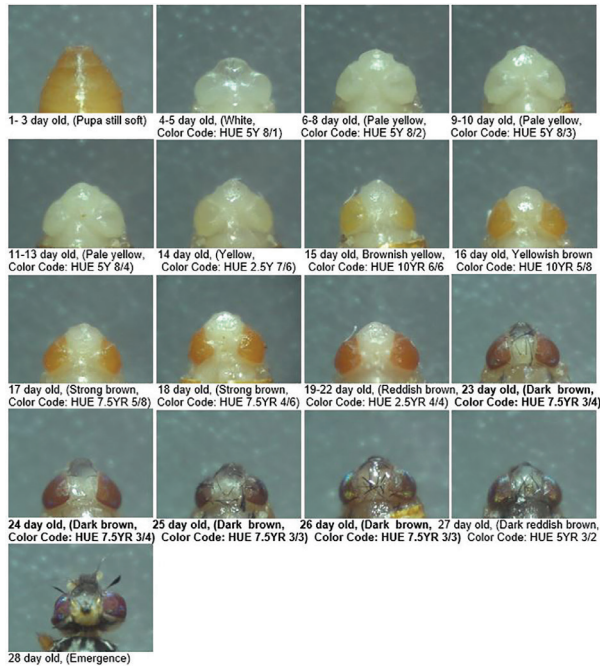
As in the case with *B. philippinensis* (Resilva & Obra 2016), the results of this study showed that the duration of pupal development and eye color in *C. capitata* changes during pupal development when pupae are held at different holding temperatures from the day of pupation to the day of emergence. The fastest pupal development was observed at 28 °C (9 days) followed by 25 °C (11 days). Duration of pupal development was longer when held at 20 °C and 17 °C, (17 and 28 days, respectively). The description of the eye color changes for pupae held at the four different holding temperatures is given below.

### **Holding Temperatures**

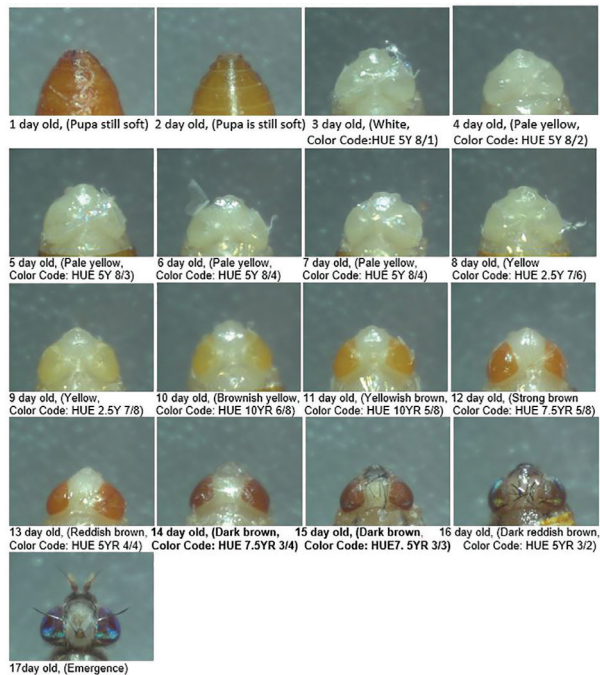
**17 °C.** Pupal duration at 17 °C was 28 days. Dissection of the puparium only became possible 4 days after pupation. Between 4 and 27 days, the sequence of eye color changes were white, pale yellow, yellow, brownish yellow, yellowish brown, strong brown, reddish brown, dark brown, and dark reddish brown (Fig. 1). Holding pupae at this temperature caused retardation in development, which is manifested by slow progression in eye color changes particularly in the early days of pupation. At 4–5 days old, the eye color was white (Color Code: HUE 5Y 8/1). At days 6–8, 9–10, and 11–13, eye color was pale yellow (HUE 5Y 8/2, HUE 5 Y 8/3, and HUE 5 Y 8/4 respectively) – becoming yellow (HUE 2.5Y 7/6) at 14 days. At 15 days, eye color changed to brownish yellow (HUE 10YR 6/6); at 16 days, to yellowish brown (HUE 10YR 5/8); at 17 and 18 days, to strong brown (HUE 7.5YR 5/8 and HUE 7.5YR 4/6); and then from 19 to 22 days, to reddish brown (HUE 2.5YR 4/4). At 23, 24–25, and 26 days, eye color was dark brown (HUE 7.5YR 3/4, HUE 7.5YR 3/3, and HUE 7.5YR 3/2 respectively). On the final day (Day 27), eye color became dark reddish brown (HUE 5YR 3/2). At 17 °C, pupae can be irradiated when they are between 23 and 26 days old. Adults emerged on the 28th day; average emergence was 92.2 % and average flight ability was 88.8%.

**20 °C.** Pupal duration at 20 °C was 17 days. Dissection of the anterior part of the puparium was possible only on the 3<sup>rd</sup> day of pupation. The color changes in the eyes during the period 3–16 days were white, pale yellow, yellow, yellowish brown, strong brown, reddish brown, dark brown, and dark reddish brown (Fig. 2). At 3 days old, the appearance of the eyes were white (HUE 5Y 8/1), turning pale yellow (HUE 5Y 8/2, HUE 5Y 8/3 and HUE 5Y 8/4) at days 4, 5, 6, and 7 respectively. On the 8<sup>th</sup> and 9<sup>th</sup> day, they became yellow (HUE 2.5Y 7/6 and HUE 2.5Y 7/8 respectively). At 10 days, eye color was brownish yellow (HUE 10YR 6/8); at 11 days, yellowish brown (HUE 10YR 5/8); at 12 days, strong brown (HUE 7.5YR 5/8); at 13 days, reddish brown (HUE 5YR/4/4); at 14 and 15 days (ready for irradiation), dark brown (HUE 7.5YR 3/4 and HUE 7.5YR 3/3 respectively); and finally at 16 days, dark reddish brown (HUE 5YR 3/2). Adult emergence was on the 17<sup>th</sup> day with an average emergence of 97.6% and average flight ability of 97.0%.

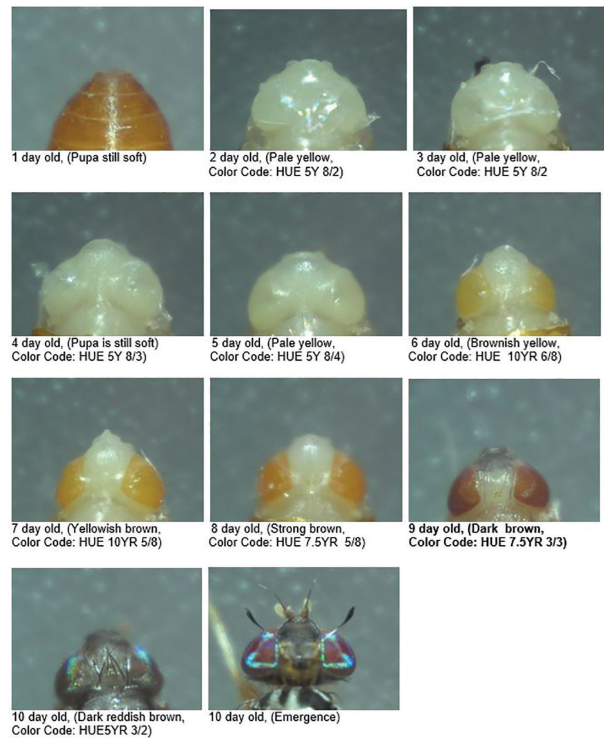
**25 °C (Pupal holding standard).** The developmental duration of pupa to adult at 25 °C was 11 days. Dissection of the anterior part of the puparium only became possible on the 2<sup>nd</sup> day. Based on the Munsell® Soil Color Chart the progressive color changes in the eyes during the period of 2 to 10 days were pale yellow, brownish yellow, yellowish brown, strong brown, dark brown, and dark reddish brown (Fig. 3). At 2, 3, 4, and 5 days old, the pupal eye colors



**Figure 1.** Photographs of the daily eye color changes of Mediterranean fruit fly *Ceratitidis capitata* (*Vienna 8 tsl* strain) taken during pupal development at 17 °C. Pupal age (*i.e.*, time for irradiation) and related color for optimum irradiation is given in bold). Color Tables from the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition) were used.



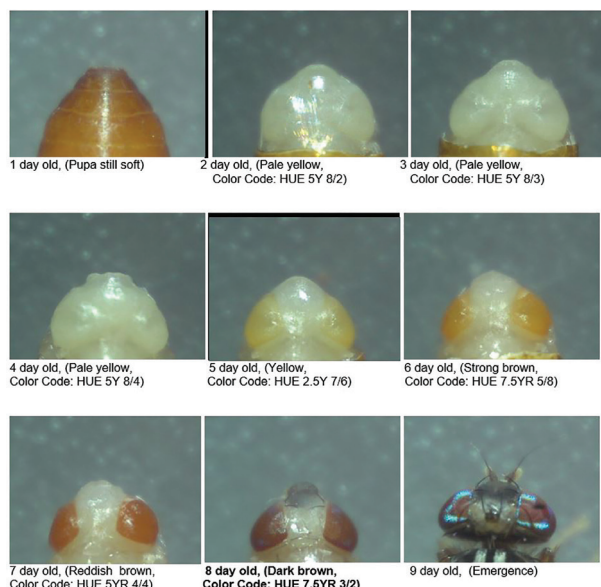
**Figure 2.** Photographs of the daily eye color changes of Mediterranean fruit fly *Ceratitidis capitata* (*Vienna 8 tsl* strain) taken during pupal development at 20 °C. Pupal age (*i.e.*, time for irradiation) and related color for optimum irradiation is given in bold). Color Tables from the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition) were used.



**Figure 3.** Photographs of the daily eye color changes of Mediterranean fruit fly, *Ceratitidis capitata* (*Vienna 8 tsl* strain), taken during pupal development at 25 °C (pupal holding standard). Pupal age (*i.e.*, time for irradiation) and related color for optimum irradiation is given in bold). Color Tables from the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition) were used.

were all pale yellow (Color Codes: HUE 5Y 8/2, HUE 5Y 8/2, HUE 5Y 8/3, and HUE 5Y 8/4, respectively). At Day 6, eye color changed to brownish yellow (HUE 10YR 86/8), turning yellowish brown (HUE 10YR 5/8) on the 7<sup>th</sup> day. Rapid pupal development was observed on Day 8 and eye color changed abruptly to strong brown (HUE 7.5YR 5/8). The pupae were ready for radiation sterilization at 9 days old when the eye color became dark brown (HUE 7.5YR 3/3). When pupae approached 10 days old, the appearance of the compound eyes became dark reddish brown (HUE 5YR 3/2). Adult emergence on the 11<sup>th</sup> day was an average of 97.6% and flight ability was 94.2%.

**28 °C.** Pupal duration at 28 °C was 9 days. Pupae could not be dissected on the 1<sup>st</sup> day of pupation because they were still soft. Dissection of the anterior part of the puparium was possible on the 2<sup>nd</sup> day. The sequence of color changes of the eyes from the 2<sup>nd</sup> to 8<sup>th</sup> days were pale yellow, yellow, strong brown, reddish brown, and dark brown (Fig. 4). At 2, 3, and 4 days old, the eye colors were pale yellow (HUE 5Y 8/2, HUE 5Y 8/3, and HUE 5Y 8/4), respectively. After 5 days, pupal eye color was yellow (HUE 2.5Y 6/6), turning strong brown (HUE 7.5YR 5/8) on the 6<sup>th</sup> day after pupation. Pupae continued to develop



**Figure 4.** Photographs of the daily eye color changes of Mediterranean fruit fly *Ceratitis capitata* (*Vienna 8 ts/* strain) taken during pupal development at 28 °C. Pupal age (*i.e.*, time for irradiation) and related color for optimum irradiation is given in bold. Color Tables from the Munsell® Soil Color Charts (Year 2000 Revised Washable Edition) were used.

until the eye color became reddish brown (HUE 5YR 4/4) after 7 days. At this holding temperature, pupae are ready for irradiation on the 8<sup>th</sup> day of pupation when the eye color is dark brown (HUE 7.5YR 3/2). Average adult emergence on the 9<sup>th</sup> day was 94.6% and flight ability was an average of 94.0%.

All results on pupal duration, recommended pupal age for irradiation, and percentage adult emergence and flight ability from the four holding temperatures are given in Table 1. Irradiation of *C. capitata* pupae at the SIT Africa Fruit Fly Facility for the SIT program was done 2 days before emergence at the standard holding temperature (25 °C), when the pupae were 9 days old – at which stage the eye color was dark brown (HUE 7.5YR 3/3). Using this eye color as the reference guide for optimum radiation sterilization of *C. capitata*, the optimum pupal

age for irradiation when held at 17, 20, and 28 °C was 23–26, 14–15, and 8 days old, respectively. Data on the percentage adult emergence and percentage flight ability observed at all pupal holding temperatures tested exceeded the minimum specification set for *C. capitata* in the FAO/IAEA/USDA Quality Control Manual (2003), which ranged from 92.2 – 97.6 and 88.8 – 97.0 %. Table 1). The high percentage of adult emergence and flight ability observed at all holding temperatures tested suggests that development of *C. capitata* can adequately be manipulated by delaying or speeding up pupal growth by holding pupae from as low as 17 °C to as high as 28 °C. In addition, pupae can be irradiated using eye color as a reference guide to achieve irradiation sterilization without affecting adult emergence and flight ability. This is very useful when there are failures in the rearing operations in the facility or with release operations in the field, and pupal development needs to be manipulated with temperature.

## SUMMARY AND CONCLUSIONS

A study on eye color changes during pupal development in Mediterranean fruit fly, *Ceratitis capitata*, at different pupal holding temperatures of 17, 20, 25, and 28 °C for optimal timing of irradiation sterilization was conducted at the SIT Africa Fruit Fly Mass Rearing Facility at Stellenbosch, South Africa. The recommended age for irradiating *C. capitata* for optimum sterilization and minimal damage to the pupae and adults is 2 days before adult emergence. At the standard pupal holding temperature of 25 °C, pupae should be irradiated when 9 days old (2 days before emergence) – at which stage the pupal eye color should be dark brown (HUE 7.5YR 3/3) as classified by the Munsell® Soil Color Charts. However, problems in the rearing operations in the facility or with release operations in the field can require pupal development to be hastened or retarded in order to meet sterile fly release schedules – requiring that pupal maturation temperatures be increased or decreased. Using an eye color of dark brown (HUE 7.5YR 3/3) as a reference guide for optimum radiation sterilization, we

**Table 1.** Pupal development, recommended pupal age for irradiation, adult emergence and flight ability of *C. capitata* at different holding temperature\*.

Holding Temperature (°C)	Pupal Duration (days)	Recommended Pupal Age for Irradiation (days)	Adult Emergence (%)	Flight Ability (%)
17	28	23 – 26	92.2 ± 0.66	88.8 ± 0.58
20	17	14 – 15	97.6 ± 0.40	97.0 ± 0.71
25	11	9	97.6 ± 0.68	94.2 ± 1.24
28	9	8	94.6 ± 0.93	94.6 ± 0.71

Note:

\*Mean of 5 replicates.

determined that the optimum age for pupal irradiation when pupae are held at 17, 20, and 28 °C was 23–26, 14–15, and 9 days old, respectively.

Quality control data on adult emergence and flight ability using the four holding temperatures exceeded the minimum specifications set in the FAO/IAEA/USDA Quality Control Manual (2003), which ranged from 88.8 to 97.6 % (Table 1). The results of this study indicate that for the Vienna 8 *ts/* strain of Mediterranean fruit fly used for SIT programs anywhere in the world, pupae destined for radiation sterilization can be maintained at holding temperatures between 17 and 28 °C without affecting fly quality. The dark brown pupal eye colors (HUE 7.5YR 3/2 to 3/4) identified in the Munsell® Soil Color Charts for each holding temperature can be used as baseline information in the mass rearing facility to determine the optimum time for radiation sterilization of pupae kept at the required holding temperature to speed up or delay pupal development. This is a very useful tool to avoid or solve potential problems that may be encountered in mass rearing operations in an SIT release program.

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