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Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/016/003

Rearing Orgyia leucostigma

Effective Date: 10 March 2015

Canada

TITLE: Rearing Orgyia leucostigma (Ol)

APPROVING OFFICIAL:

DD / MM / YY

Manager, Insect Production Services _____ ___/___/_____

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

NA

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) has been established to ensure that procedures used for the rearing of *Ol* (whitemarked tussock moth) are implemented consistently among Insect Production Unit (IPU) personnel and to minimize the spread of pathogens and microbial contaminants within and between insect colonies.

1.2 Scope

This SOP shall be followed by all IPU personnel for the rearing *Ol*.

1.3 Definitions

Biological Safety Cabinet (BSC) – A class 2 containment cabinet designed for both worker and sample protection; room air is drawn into the front of the unit; the unit is designed in such a way that room air is HEPA filtered before blowing over the work area; air-borne hazardous particles coming off samples in the work area are pulled away from the worker and the air is vented back into the room after HEPA filtration; this type of unit is not suitable for worker protection from chemical fumes.

Chemical Fume Hood – Safety cabinet designed for worker protection but not sample protection; room air is drawn into the front of the unit, chemical fumes or air-borne hazardous particles pulled away from the worker and are vented to the outside of the building.

Controlled Copy – A copy of an SOP distributed to select GLFC personnel having a unique copy number and dated signature of the IPS manager. Controlled copies are intended to ensure that GLFC personnel follow the most recent version of the SOP.

Effective Date – The date from which the procedures given in an SOP are to be implemented.

Environmental Chamber - Reach-in or walk-in environmentally controlled growth chamber for maintaining insects or plants having precise, programmable (manual or electronic) control of temperature, relative humidity and light.

External Clients – Persons not located at the GLFC who receive insects or prepared artificial diets from IPS. These persons may or may not be members of the Canadian Forest Service.

Great Lakes Forestry Centre (GLFC) – One of five Canadian Forest Service (CFS) research facilities in Canada.

Insect Production Services (IPS) – A GLFC work team consisting of the Insect Production Unit (IPU), the Quality Control Unit (QCU) and Insect Quarantine (IQ) personnel who perform insect rearing, quality control and quarantine activities in support of forest pest research activities internal and external to the CFS.

Insect Production Services (IPS) Manager – The individual who has overall responsibility for activities of the IPS team.

Insect Production Supervisor – A member of IPS having supervisory authority over the daily operation of the insectary.

Insect Production Unit (IPU) – A work unit of IPS consisting of personnel who perform insect rearing, diet making and methods development activities at GLFC.

Insectary – A multi-species rearing facility under the control of IPS used exclusively by the IPU for maintaining insect colonies and preparing artificial diets.

Internal Clients – Canadian Forest Service personnel located at the GLFC who receive insects or prepared artificial diets from IPS.

Material Safety Data Sheet (MSDS) – A summary description of a chemical, reagent or substance prepared by the manufacturer or supplier and required by WHMIS legislation to inform workers about procedures required to safely work with the material.

Quality Control (QC) Lab – An analytical laboratory under the control of IPS used by the QCU for monitoring production, process and product control for all IPU insect colonies, and for developing new QC methods and procedures.

Quality Control Unit (QCU) – A work unit of IPS consisting of personnel who conduct routine production, process and product control testing and develop new QC methodology in support of IPU activities.

Standard Operating Procedures (SOPs) – Directives describing routine administrative or technical procedures conducted by IPS personnel or users of the IQ facility.

1.4 Safety

1.4.1 Personal protective safety equipment (i.e., lab coat and disposable chemical protective gloves) shall be worn for the conduct of sections 2.3 through 2.9.
1.4.2 Adults shall be handled within a BSC, or chemical fume hood to provide worker protection.
1.4.3 Safety precautions identified in the referenced SOPs shall be followed.
1.4.4 Personnel shall have access to, and be familiar with, the MSDS for formaldehyde and for sodium hypochlorite (i.e., bleach).

1.5 Materials

1.5.1 Safety equipment:
- (a) class 2 BSC
- (b) lab coat
- (c) disposable chemical protective gloves
- (d) chemical fume hood
- (e) sleeve protectors

1.5.2 Supplies and equipment:
- (a) sanitation supplies/equipment specified in the current version of SOP Number IPS/009, *IPU Personnel Responsibilities*
- (b) consumables including: sweetheart cups, sweetheart lids, Bell diet, sterile paper towel, autoclaved tap water, paper trays, 50 ml screw cap vials, plastic garbage bags, paper bags, 1% bleach solution, 10% formaldehyde solution
- (c) analytical balance
- (d) round plastic dish 6” dia. X 2.5” high with solid lid
- (e) perforated metal trays
- (f) glass pint jar with a screened screw top lid
- (g) magnetic stir plate and stir bars
- (h) wire basket for pupa wash (wire cloth, cylindrical, 20 cm x 15 cm, galvanized) with stainless steel beaker
- (i) crisper box (solid plastic, 26.5 cm long x 19.5 cm wide x 9.5 cm deep)
- (j) crisper box (solid plastic, vented, 26.5 cm long x 19.5 cm wide x 9.5 cm deep)
- (k) sterile scissors

1.5.3 Forms:
- (a) *Ol Distribution* (IPS Form Number 0013/005, Appendix 1)
- (b) *Ol Rearing Schedule* (IPS Form Number 0025/002, Appendix 2)
- (c) *Ol Tracking* (IPS Form Number 0014/007, Appendix 3)
- (d) *Ol Discards* (IPS Form Number 0140/004, Appendix 4)

2.0 PROCEDURES

2.1 Facility Sanitation Procedures

Procedures identified in the current version of SOP Number IPS/009, *IPU Personnel Responsibilities*, shall be followed.

2.2 Records for Distribution of Insects

2.2.1 No eggs shall be distributed until results of QC analysis have been received by the IPU and identified as suitable for use (refer to section 2.11).

2.2.2 Orders for *Ol* eggs shall only be accepted from internal and external clients through the IPS web-based storefront (i.e., requests submitted through standard email or telephone are not to be accepted).

2.2.3 The IPU email account shall be reviewed daily (excluding weekends and holidays) for the receipt of orders from the storefront. Orders shall be printed, stamped as "Received" and shall be signed/dated by the technician receiving the order. The electronic copy shall be filed in the electronic archive for either internal or external clients, as applicable. The printed copy shall be placed into the in-box (labelled as "outstanding orders") for either internal or external clients, as applicable. In-boxes shall be reviewed by each IPU technician on a daily basis (excluding weekends and holidays) and shipping performed on the requested shipping date (subject to availability of eggs). Upon completion of the shipment, the printed order shall be stamped as "Completed", signed and dated by the technician, then placed into the out-box labelled as "completed orders" for either internal or external clients, as applicable. At the end of each month, IPU personnel shall photocopy completed external orders (i.e., those not already paid by credit card) and deliver them to the GLFC finance department for billing according to the current fee schedule. All printed copies of completed orders (internal and external) shall be maintained with IPU records. All distribution of *Ol* to clients internal and external to GLFC shall be documented on the *Ol Distribution* form (IPS Form Number 0013/005, Appendix 1). A separate form shall be used for each cohort of every generation and maintained with IPU records.

2.2.4 Upon the initiation of each *Ol Distribution* form, the following records shall be documented:

(a) ID code for the cohort
(b) total grams egg masses in diapause
(c) # grams kept for colony maintenance
(d) box ID of eggs kept for colony maintenance
(e) # grams discarded for QCU

(f) # grams available for distribution

2.2.5 *Ol* shall only be distributed to clients as eggs (i.e., the IPU will not conduct larval rearing for clients; eggs for internal clients may be set up on diet but shall be distributed prior to thinning). All distributions shall be documented on the form, including:
(a) date distributed
(b) # eggs requested
(c) # grams egg masses distributed (distribute 1 gram egg mass for every 1000 eggs requested; this will provide 20% more eggs to account for mortality during diapause, etc.)
(d) recipient name and affiliation
(e) diet ID, where applicable
(f) initials of technician distributing the eggs

2.2.6 Eggs shall be distributed after 20 to 32 weeks (optimum 24 weeks) of diapause, unless requested otherwise by the client. Unused eggs shall be autoclaved and discarded after 32 weeks in diapause. The number of grams of eggs discarded from each cohort of every generation shall be documented (including date of discard) on the *Ol Distribution* form.

2.2.7 Upon completion of 2.2.1 through 2.2.7, the following information shall be determined and recorded on the electronic multi-generation summary (i.e., *Ol Discards*, IPS Form Number 0140/004, Appendix 4) maintained on the IPU network drive:
a) 32 week discard date
b) # grams egg masses used for colony maintenance
c) # grams egg masses distributed to QC
d) # egg masses distributed during diapause to clients (internal, external and CFS)
e) # grams egg masses discarded after 32 weeks in diapause
f) % discarded

2.2.9 Upon distribution or discard of all insects from each cohort, IPU personnel shall review the *Ol Distribution* form to ensure that the sum of distributions and discards is equal to the number that were available.

2.3 Surface Sterilization of Eggs

2.3.1 Egg masses from each of the mating boxes (maximum 5) from a cohort of *Ol* will have been identified by the QCU for use in either colony maintenance, distribution to clients, or to be discarded due to potential quality issues.

2.3.2 Egg masses destined for colony maintenance shall be removed from diapause on the date identified on the *Ol* rearing schedule (refer to 2.13). An *Ol Tracking* form (IPS Form Number 0014/007, Appendix 3) shall be initiated at this time and shall follow the insects throughout the entire rearing and mating process to record: date out of diapause, # of days in diapause and # grams egg masses taken out.

2.3.3 Egg masses that are to be set up on diet for distribution to internal GLFC clients may be taken out of diapause at the discretion of IPU personnel (i.e., quantity and diapause duration of 20 to 32 weeks). Egg masses from several cohorts may be pooled.

2.3.4 Egg masses shall be surface sterilized one day after they are removed from diapause. They shall be placed in a glass pint jar fitted with a screened screw top lid and maintained within a chemical fume hood with the exhaust fan running. A magnetic stir bar shall be added and the jar shall be 1/3 filled with a 10% formaldehyde solution (refer to section 2.13) and gently stirred on magnetic stirrer (e.g., Stir Jack setting #5) for 90 minutes.

Adding formaldehyde solution to jar containing egg masses

Egg masses undergoing surface sterilization

2.3.5 With the fume hood running, the 10% formaldehyde solution shall be decanted from the pint jar through the screened lid into a waste bottle (stored under the fume hood).

2.3.6 The eggs remaining in the jar shall be rinsed by gently running cool tap water through the screened lid for 15 minutes in the sink within the operating fume hood.

Rinsing egg masses after surface sterilization

2.3.7 The tap water shall be drained through the screen, the screened lid removed from the jar and the eggs dumped onto a perforated metal tray lined with 4 layers of sterile paper towel, within the operating fume hood. Egg masses shall be separated using sterile forceps to facilitate drying.


Drying egg masses

2.3.8 The tray with eggs shall be left in the fume hood and allowed to dry for approximately 2 hours.

2.3.9 Using sterile forceps, the dried egg masses shall be transferred to a round, clear plastic dish (6" diameter X 2.5" high) lined with one layer of sterile paper towel (cut to a 6" disc) and then covered with a non-vented lid. The dish shall be placed into an environmental chamber dedicated to *OI* and maintained at 22±3°C, 50±10% RH and a 12:12 light:dark cycle until the eggs turn light grey in colour or until the start of eclosion (i.e., after about 15 days). Eggs shall be monitored daily for eclosion after 12 days.

2.4 Eggs Setup on Diet

2.4.1 As the eggs turn grey or larvae begin to emerge, both eggs and larvae shall be transferred, using sterile forceps within a BSC, to ten sweetheart brand cups containing Bell diet (maximum one week old; 1/3 full; containing raw linseed oil). Target 200-300 insects per cup.

Egg masses on diet

2.4.2 Cups shall be double capped (tabs at opposite ends), placed lid-down on perforated metal trays and returned to the environmental chamber for 1 week.

2.4.3 The date set up on diet, # of cups, diet ID and technician's initials shall be recorded on the *OI Tracking* form (IPS Form Number 0014/007, Appendix 3).

2.5 Thinning

2.5.1 Larvae destined for distribution shall be given out prior to thinning. Larvae destined for colony maintenance shall be thinned (i.e., transferred to fresh Bell diet in sweetheart cups; maximum two weeks old; 1/3 full; containing raw linseed oil) weekly for six weeks, as specified in 2.5.2 through 2.5.6. Thinning shall be conducted within a BSC. During each transfer, slow developers shall be removed from the rearing process. When more than five dead larvae are found within any

one cup during a thinning session, the dead larvae shall be collected and placed into a 50ml screw top vial, labeled with the ID code for the cohort and submitted to the QCU for immediate analysis. The "yes" box on the *OI Tracking* form (IPS Form Number 0014/007, Appendix 3) shall be checked indicating that dead larvae were collected for QC analysis. The "no" box shall be checked when five or fewer cadavers are found in one cup (i.e., QC analysis is not required). Cups with more than five dead larvae shall be removed from the rearing process (i.e., live larvae from these cups shall not be kept). Forceps shall be replaced with sterile ones whenever cadavers are handled, thereby preventing the potential spread of pathogens to healthy insects.

2.5.2 Larvae destined for colony maintenance shall be split (i.e., thinned) one week after being set up on diet. Larvae from each cup of one-week-old larvae shall be split onto two (typically) new cups containing Bell diet for a total of about 20 cups (i.e., each cup should contain about 100 larvae per cup). Cups shall be double capped, placed lid-up on perforated metal trays and returned to the environmental chamber for a second week. The date, # of cups, diet ID and technician's initials shall be recorded on the tracking form under the column heading "Split".

2.5.3 Two-week-old larvae shall be thinned to 50 larvae per cup of fresh diet (i.e., total of about 40 cups). Cups shall be single capped, placed lid-up on perforated metal trays and returned to the environmental chamber for a third week. The date, # of cups, # of larvae/cup, total larvae, diet ID and technician's initials shall be recorded on line 1 of the "Thinnings" box on the tracking form.

Thinning larvae

2.5.4 Three-week-old larvae shall be thinned to 25 larvae per cup of fresh diet. Pupae shall be harvested as per section 2.6. Approximately 5 trays of diet cups are required. Cups shall be single capped, placed lid-up on perforated metal trays and returned to the environmental chamber for a fourth week. The date, # of cups, # of larvae/cup, total larvae, diet ID and technician's initials shall be recorded on line 2 of the "Thinnings" box on the tracking form.

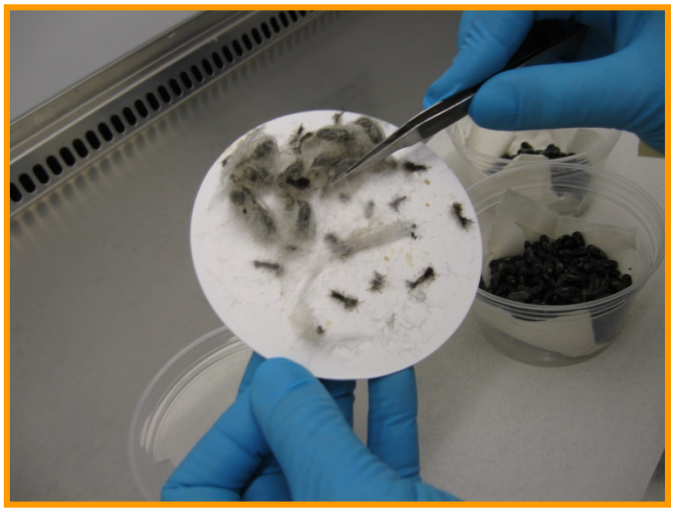
2.5.5 Four–week-old larvae shall be transferred onto fresh diet (i.e., 25 larvae per cup). Pupae shall be harvested as per section 2.6. Three to five trays of diet cups are required. Cups shall be single capped, placed lid-up on perforated metal trays and returned to the environmental chamber for a fifth week. The date, # of cups, # of larvae/cup, total larvae, Bell diet date and the technician's initials are recorded in the "Thinnings" box, line 3, on the *Ol Tracking.*

2.5.6 Five-week-old larvae shall be transferred onto fresh diet (i.e., 25 larvae per cup). Pupae will be harvested as per section 2.6. One to three trays of diet cups are required. Cups shall be single capped, placed lid-up on perforated metal trays and returned to the environmental chamber for a fifth week. The date, # of cups, # of larvae/cup, total larvae, Bell diet date and the technician's initials are recorded in the "Thinnings" box, line 4, on the *Ol Tracking* form.

2.6 Pupa Harvest

2.6.1 Pupae shall be harvested within a BSC on 2-3 occasions. The first pupa harvest will occur upon the first observance of pupae, typically during the second or third thinning (i.e., 3 to 4-week-old larvae). The second pupa harvest shall occur one week after the first. A third pupa harvest shall occur 4-7 days after the second, pending availability of sufficient quantities to justify the collection.

2.6.2 During each harvest session, pupae shall be removed from the majority of their webbing using forceps and placed in a labeled solid plastic crisper box (or empty sweetheart cup) until surface sterilization (refer to 2.7). Record the date on the *Ol Tracking* form (IPS Form Number 0014/007, Appendix 3) under the section for the applicable pupa harvest. When more than five dead pupae or larvae are found within any one cup during a pupa harvest session, the entire cup shall be labeled with the family and cohort number and submitted to the QCU for immediate analysis. The "yes" box on the tracking form shall be checked indicating that dead insects were collected for QC analysis. The "no" box shall be checked when five or fewer cadavers are found (i.e., QC analysis is not required). Cups with dead insects shall be removed from the rearing process (i.e., live insects from these cups shall not be kept). Forceps shall be replaced with sterile ones whenever cadavers are handled, thereby preventing the potential spread of pathogens to healthy insects. The number of cups with dead insects (and subsequently removed from the rearing process) shall be recorded on the tracking form. Pupae shall be maintained at 22±3°C, 50±10% RH and a 12:12 light:dark cycle.



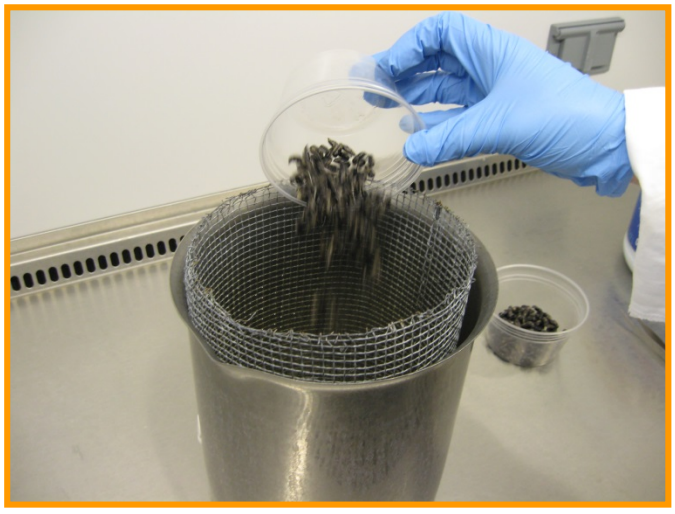
Harvesting pupae

2.6.3 Any larvae remaining after the last pupa harvest shall be frozen and discarded. Record the number of remaining larvae on the tracking form.

2.6.4 Pupae shall be surface sterilized (as per 2.7) after each harvest session.

2.7 Surface Sterilization of Pupae

2.7.1 Pupae shall be surface sterilized within two days of each harvest session. Place the pupae into a cylindrical 20 cm x 15 cm wire basket (galvanized hardware cloth with 5mm openings) and immerse into a 2L stainless steel beaker 1/3 filled with pure bleach (i.e., 5.25% sodium hypochlorite) for 1-2 minutes. The bleach will dissolve any remaining webbing and will separate the pupae from cast skins and any diet debris.

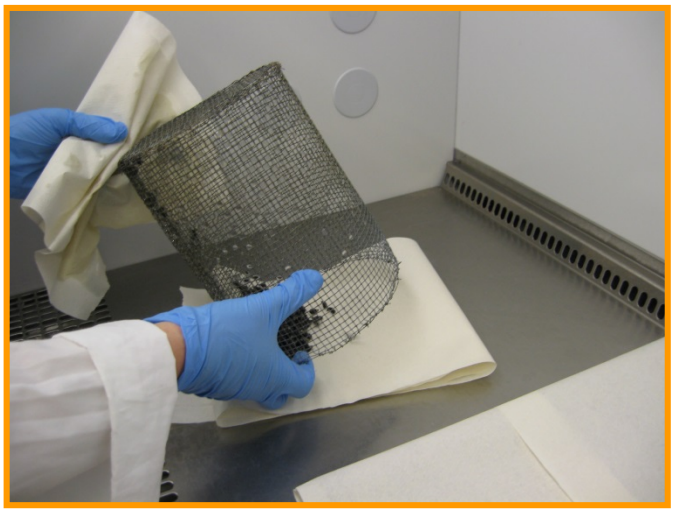


Adding pupae to bleach for surface sterilization

2.7.2 Separate the wire basket containing the pupae from the stainless steel beaker, dispose of the Javex®, nest the two containers back together, add cool tap and swish the wire basket up and down (or stir using clean forceps) for 1-2 minutes to separate pupae from debris. Repeat this process 3-4 times until the pupae are relatively free from debris.

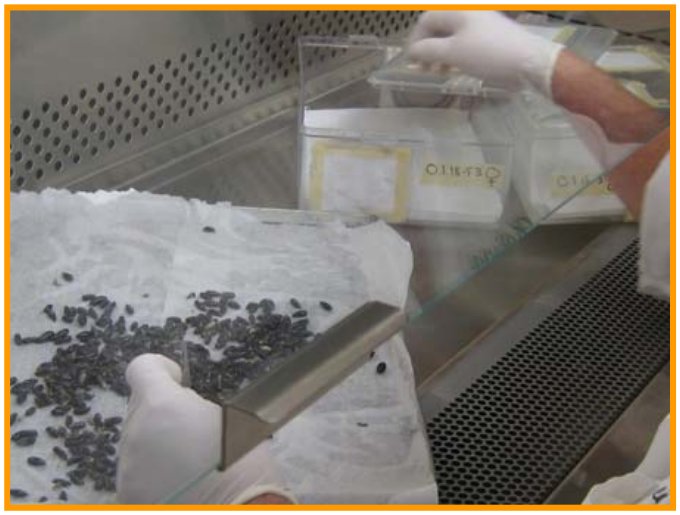
Washing pupae

2.7.3 Remove the wire basket and dump the pupae onto sterile paper toweling within a BSC. Transfer the pupae onto a sterilized metal tray (lined with sterile paper towel) and remove any remaining debris using sterile forceps. Discard any deformed pupae. Allow the pupae to dry within the BSC for approximately 2 hours.



Removing pupae from washing basket

2.7.4 Within the BSC, separate the pupae by gender (females are much larger than males). Record the number of male (♂) and female (♀) pupae collected on the *OI Tracking* form (IPS Form Number 0014/007, Appendix 3) under the section for the applicable pupa harvest session.



Separating pupae by gender

2.7.5 Place male and female pupae in separate plastic emergence boxes having screened/vented sides and lids, both of which are covered with sterile paper toweling taped into place with masking tape. Target a maximum of 200 pupae per box. (Paper toweling prevents the potential spread of microbial contaminants carried by wing scales which would otherwise pass through the screened vents). Maintain the pupae at 22±3°C, 50±10% RH and a 12:12 light:dark cycle and monitor daily until adult eclosion.

2.8 Mating

2.8.1 Prepare five mating chambers in advance of adult eclosion. Each chamber shall consist of a plastic crisper box (having vented sides and lids covered with sterile paper toweling taped into place with masking tape) lined with two strips of sterile paper towel which extend out of the box and are taped to the outside and to the bottom (refer to the photo below).

2.8.2 Mating chambers may need to be set up over a period of 2-4 days due to the staggered eclosion of adults. Chambers shall be set up within a BSC. Once female adults appear in the emergence boxes (approx. 32d post egg hatch), those boxes containing males shall be chilled in a refrigerator (approx. 4°C) for about 20 minutes to immobilize them prior to being transferred to mating chambers. Females do not require chilling since they are not able to fly.

2.8.3 Add available females prior to males by gently placing them on the inside walls of the mating chamber using forceps (those that cling to the wall will yield more eggs). Do not place one female on top of another as this will impede mating and subsequent oviposition. Do not add more males than there are female mating partners available. All females should be added since unlaid eggs harden off and become unusable within 1-2 days. More females than males in a mating chamber is acceptable since males can mate with more than one

female. Surplus males shall be kept in their eclosion boxes until a female mating partner becomes available. Males may be kept for 2-3 days.

2.8.4 Refrigerated males shall be added quickly (using forceps) to the mating chamber since they will become active as soon as they warm up. There are only 5-10 minutes available for this process. A maximum of 50 mating pairs shall be added to each mating chamber.

2.8.5 Label each mating chamber with the ID code for the cohort, box number (i.e., 1-5), "start" date, and quantity of each gender.

2.8.6 Add males and females daily as they become available from the adult eclosion boxes. Once 50 mating pairs have been added to each chamber, record the date when "last adults" were added. When adding adults to a chamber that was started on a previous day, it shall first be chilled in the refrigerator (approx. 4°C, about 20 minutes) to immobilize the males and prevent them from escaping when removing the lid.

2.8.7 Do not set up more than five mating chambers per family.

Crisper boxes with females, males and mating pairs

2.9 Egg Harvest

2.9.1 Eggs shall be harvested five days from the last addition of adults to the mating chamber (i.e., egg harvest will occur over several days due to staggered set-up dates).

2.9.2 Remove the two paper towel strips from the mating chamber within a BSC. Using forceps or clean gloved hands, gently peel the egg masses from the paper towel and place them on a sheet of paper. Separate them into groups weighing 10 grams (do not combine eggs from different mating chambers) and place them into plastic bags labeled with the cohort ID (i.e., family number and new generation number), mating chamber number, date when "last adults" were added to the mating chamber, number of grams of egg masses in the bag (i.e., all bags will have 10 grams of egg masses except the at last bag which may have fewer) and the date when the eggs are to be placed

into cold storage for diapause (i.e., one month after the last egg harvest).

Harvested egg masses

2.9.3 Upon completion of egg mass harvesting, adults from each mating chamber shall be collected and placed into 50ml centrifuge tubes (maintained separately by mating chamber), labeled with the insect ID and chamber number, then placed into a clear zip-lock bag and stored in the freezer for pick-up by QCU personnel for analysis.

2.10 Pre-diapause

2.10.1 Sealed storage containers with eggs shall be maintained in an environmental chamber (13±3°C, 16:8 L:D, n/a RH) for one month pre-diapause.

Egg masses in pre-diapause

2.11 Diapause

2.11.1 During the one month pre-diapause storage period, the QCU will assess the quality of parental adults from each mating box and provide a written report instructing the IPU with regards to the fate of egg masses derived from each box. At the end of pre-diapause, IPU personnel shall discard those specified by the QCU and shall label each remaining bag of egg masses either "maintain for colony" (in red ink) or "for distribution" (in black ink), as instructed.

2.11.2 Bags of egg masses for colony or distribution shall be placed sequentially by date in similarly labelled bins and shall be transferred to the 2.5±1°C cold room for 20-32 weeks diapause (optimum 24 weeks).

Egg masses in diapause

2.11.3 If the eggs are to be discarded, then an "×" shall be placed in the final column of the "Mating Boxes" section of the *OI* Tracking form labeled "QC ✓/×". If the eggs are cleared for use, then a "✓" is to be placed there. If the QC for mating box #1 has been identified as "colony", then the first bag of eggs from that box shall be used for the next generation. If the adults have been identified as "for distribution" or "discard" the pre-labeled eggs will need to be replaced with the eggs from another mating box identified by the QCU as "maintain for colony". The QC report shall be printed and placed in the *OI* Tracking, Distribution and QC binder (consecutively by generation and family).

2.11.4 A copy of the *OI Tracking* form (IPS Form Number 0014/007, Appendix 3) shall be given to the QCU once the egg masses enter diapause. The date on which the eggs are placed into diapause shall be transcribed onto the multiple generation summary for *OI Discards* on the IPU computer system.

2.12 Rearing Schedule

2.12.1 The *Ol Rearing Schedule* (IPS Form Number 0025/002; Appendix 2) shall be maintained electronically on the Insect Production drive and shall be predetermined by discussion between the IPU supervisor and the technician having responsibility for the colony. Typically, eggs shall be taken out of diapause every 4 weeks (typically on a Thursday) to meet demand and to sustain 12 cohorts for each generation.

2.13 Calculations

2.13.1 10% formaldehyde shall be prepared in a chemical fume hood by adding 100ml formaldehyde (37% active ingredient concentration from supplier) to 900ml of tap water to yield a 3.7% active ingredient concentration of formaldehyde. This solution may be stored as needed.

2.14 Documentation and Reporting

2.14.1 Compliance to this SOP shall include the completion and maintenance of the following forms:
(a) IPS Form Number 0013/005, *Ol Distribution* (Appendix 1)
(b) IPS Form Number 0014/007, *Ol Tracking* (Appendix 3)
(c) IPS Form Number 0140/004, *Ol Discards* (Appendix 4)

2.14.2 Any other pertinent information (e.g., malfunction of environmental chamber, justification for deviating from procedures identified in this SOP, observation of unusual occurrences, etc.) shall be documented on the back of the applicable tracking form.

2.14.3 IPS Form Number 0014/007, *Ol Tracking* (Appendix 3) shall be copied to the QCU once larval progeny enter pre-diapause.

2.14.4 The IPU shall make all records available to the QCU.

3.0 DISTRIBUTION AND ARCHIVING

3.1 Distribution

This SOP shall be distributed by the IPS manager to all IPU personnel.

3.2 Archiving

3.2.1 The IPS manager shall maintain a historical copy of this SOP when it is replaced by a new version.

3.2.2 The IPU supervisor shall ensure that files of all documentation identified in 2.14 are maintained for expedient retrieval.

3.3 Destruction of Outdated SOPs

When new versions of this SOP are available for distribution, all persons in possession of a controlled copy shall ensure the retired version is returned to the IPS manager upon request.

4.0 ASSURING SOP VALIDATION AND COMPLIANCE

4.1 Responsible Individual

4.1.1 The IPU supervisor is responsible for assuring that this SOP is valid.
4.1.2 The IPU supervisor is responsible for assuring that this SOP is followed by IPU personnel and that these persons have been appropriately trained in its use.
4.1.3 IPU personnel are responsible for complying with procedures specified on a *Controlled Copy* of this SOP and shall never use non-controlled copies which could be outdated.

5.0 REVISION OF THE SOP

5.1 Responsible Individual

The IPU supervisor is responsible for assuring that this SOP is current. If necessary, the IPU supervisor shall initiate the revision process.

5.2 Revision Schedule

This SOP shall be revised when its provisions no longer agree with current practices or GLFC policies, and shall be approved by the IPS manager.

6.0 CONTINGENCIES

When IPU personnel find circumstances that do not permit compliance with this SOP, the IPU supervisor shall be consulted.

7.0 CONFIDENTIALITY

IPS SOPs are not considered to be confidential documents and may be distributed to outside parties. *Controlled Copies* shall not be reproduced.

8.0 REFERENCES

Current version of SOP Number IPS/009, *IPU Personnel Responsibilities*
Current version of IPS Form Number 0052, *QC Report for Ol Adults*

9.0 APPENDICES

Appendix 1: IPS Form Number 0013/005, *Ol Distribution*
Appendix 2: IPS Form Number 0025/002, *Ol Rearing Schedule*
Appendix 3: IPS Form Number 0014/007, *Ol Tracking*
Appendix 4: IPS Form Number 0140/004, *Ol Discards*

Appendix 1

***Ol* Distribution**

ID Code: _____

Date into Diapause: _____ (DD/MM/YY)

24wk date: _____ (DD/MM/YY)

Total grams egg masses in diapause: _____

grams kept for colony maintenance: _____

Box ID for colony maintenance: _____

grams discarded for QC: _____

grams available for distribution: _____

Distribution during diapause

Date Distributed (DD/MM/YY)	# Eggs requested	# grams Egg masses Distributed (1 mass=200 eggs)	Recipient name and affiliation				
			Internal GLFC			External (Eggs only)	Initials
			Eggs only	Eggs on diet	Diet ID (DD/MM/YY)		
		∑=					

grams egg masses discarded (after 32wks): _____

Discard Date: _____ (DD/MM/YY)

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Appendix 2

***Ol* Rearing Schedule**

ID Code	Date into Diapause (DD/MM/YY)	Date out of Diapause (DD/MM/YY)	#days In storage 24wk=168days	32 week Discard date
Ol 1-F_				
Ol 2-F_				
Ol 3-F_				
Ol 4-F_				
Ol 5-F_				
Ol 6-F_				
Ol 7-F_				
Ol 8-F_				
Ol 9-F_				
Ol 10-F_				
Ol 11-F_				
Ol 12-F_				
Ol 13-F_				
Ol 14-F_				
Ol 15-F_				
Ol 16-F_				
Ol 17-F_				
Ol 18-F_				
Ol 19-F_				
Ol 20-F_				
Ol 21-F_				
Ol 22-F_				
Ol 23-F_				
Ol 24-F_				
Ol 25-F_				

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Appendix 3

***OI* Tracking**

I.D. Code _____ Create from: □ Previous generation □ Other _____

Date into diapause_____

Set-up

Date out of diapause (DD/MM/YY)	# days in diapause	# grams egg masses out	Date set-up (DD/MM/YY)	Egg Mass set-up		Initials	Split			Initials
				# lg cups	Diet ID (DD/MM/YY)		Date (DD/MM/YY)	# Cups	Diet ID (DD/MM/YY)	

Thinnings

Date (DD/MM/YY)	# cups	#of larvae/cup	Total larvae	Diet ID (DD/MM/YY)	QC √/x	Initials
1.						
2.						
3.						
4.						

QC Results_____

Pupae Harvest

	1st		2nd		3rd		Total		
	Date:		Date:		Date:				
	♂	♀	♂	♀	♂	♀	♂	♀	Pooled
# kept									
# cups with dead							# of larvae remaining and discarded		
cups to QC	□Yes □No		□Yes □No		□Yes □No				

QC Results _____ _____ _____

Mating Boxes

Box #	Date Started (DD/MM/YY)	Initials	Date Last Adults (DD/MM/YY)	Initials	5-day egg harvest into pre-diapause (DD/MM/YY)	# grams Egg masses	New I.D. Code	Adults for QC Y/N	Initials	Date into diapause (DD/MM/YY)	24 weeks optimum storage (DD/MM/YY)	QC √/x
1												
2												
3												
4												
5												

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Appendix 4

OI Discards (multi-generation summary)

ID Code of eggs	Date into Diapause (DDMMYY)	Date out of Diapause (DDMMYY)	# days in storage (24 wk=168d)	32wk discard date (DDMMYY)	# grams egg masses in diapause	Distribution					# grams egg masses discarded (after 32wks)	% Discarded
						colony	QC Discard	internal (GLFC)	external	external to GLFC		

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