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Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/019/003

Rearing Trichoplusia ni

Effective Date: 10 March 2015

Canada

TITLE: Rearing <u>Trichoplusia ni</u> (T ni)

APPROVING OFFICIALS:

Manager, Insect Production Services _____ DD / MM / YY ___/___/___

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 the *T ni* (*Trichoplusia ni)* 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 of *T ni*.

1.3 Definitions

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.

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.

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.

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

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.

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 2.1 through 2.6.
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, sodium hypochlorite, methyl paraben, ethanol and ascorbic acid.

1.5 Materials

1.5.1 Safety equipment:
(a) BSC
(b) lab coat
(c) disposable chemical protective gloves
(d) chemical fume hood
(e) laminar flow hood
(f) dissecting microscope

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: creamer cups, un-waxed lids, regular diet, sterile paper towel, absorbent towels (for mating cages), autoclaved RO water, plastic sample bags, paper trays, 250 ml Nalgene bottles, plastic garbage bags, paper bags, 6% bleach solution, 10% formaldehyde solution, feeding solution, plastic petri dishes, absorbent cotton.
(c) perforated metal trays
(d) funnels
(e) analytical balance
(f) glass crystallizing dish
(g) petri dishes
(h) gauze
(i) mating chambers
(j) refrigerator
(k) sterile scissors and forceps
(l) environmental chamber

1.5.3 Forms:
(a) *T ni Tracking and Distribution* (IPS Form Number 0015/005, Appendix 1)

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 Eggs on Diet (Day 0; Tuesday)

2.2.1 A *T ni Tracking and Distribution* form (IPS Form Number 0015/005, Appendix 1) shall be initiated for every generation of each family and shall be maintained in the immediate vicinity of the cohort (four separate families are being reared, one beginning on each Tuesday of the month).

2.2.2 A portion of one strip of eggs/gauze from the cohort shall be removed from the refrigerator (on Tuesday) and set-up on diet in order to maintain the colony. Cut the gauze into patches containing approximately 25-30 eggs and placed into creamer cups ¾ full of regular budworm diet. Thirty-two cups shall be set up to maintain the colony (i.e., this will yield enough adults to set up two mating cages); surplus eggs from the strip used for maintaining the colony shall be returned to refrigerated storage for potential distribution to clients.

2.2.3 The remaining 11^{+} strips of eggs/gauze (each containing approximately 2000eggs) which have been refrigerated for potential distribution to clients will only be taken out of storage (prior to the 10d discard date) and set-up on diet pending demand. Strips of gauze shall be cut into patches containing approximately 25-30 eggs and placed into creamer cups ¾ full of regular budworm diet.

Patches of eggs/gauze on diet

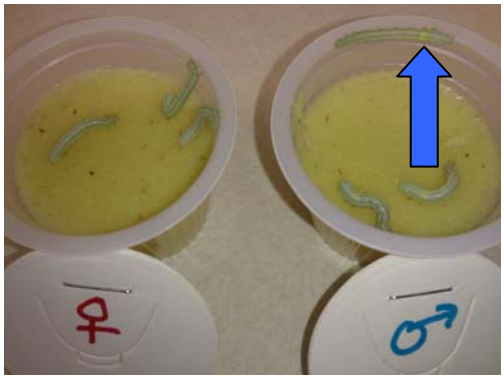
2.2.4 Cups shall be placed lid side down on a perforated metal tray and maintained at 27±3°C, 55±10%RH and 16:8 light:dark until yellow gonads are visible in the males (i.e., approximately 7-8 days).

2.2.5 The set-up date, # of cups set-up, diet ID and initials of technician shall be recorded on the *T ni Tracking and Distribution* form.

2.3 Thinning (Day 7-8; Tuesday or Wednesday)

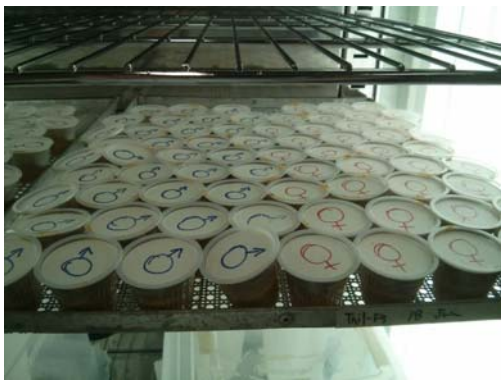
2.3.1 Only larvae destined for colony maintenance shall be thinned when yellow gonads are visible in the makes, 7-8 days after the eggs were placed on diet (i.e., larvae destined for distribution shall be given out prior to thinning).

2.3.2 Larvae shall be separated by gender during the thinning process. Males are easily distinguishable from females (starting at 3rd instar) by their characteristic yellow gonads. If time is limited, pupae can be separated by gender during harvest (refer to section 2.4) using the procedure specified in Appendix 3.



Gonads of male larvae

2.3.3 Larvae shall be thinned to three per cup of regular diet (¾ full). Approximately two trays of diet are required for thinning. Males shall be kept separate from females (if separated by gender as per 2.3.2) and each lid shall be labelled as either ♂ or ♀, as applicable. Cups shall be placed lid side up on a perforated metal tray and maintained at 27±3°C, 55±10%RH and 16:8 light:dark for eight days.



Thinned larvae separated by gender

2.3.4 Cups with dead larvae shall be removed from the rearing process (i.e., live larvae from these cups shall not be kept). After handling dead or contaminated larvae, forceps shall be exchanged for sterile ones and gloves shall be replaced. When three or more cups with dead larvae are present, they shall be maintained in a plastic bag, labeled with the ID code and frozen for subsequent QC analysis.

2.3.5 The thinning date, number of cups of males, number of cups of females, diet ID and initials shall be recorded on the *T ni Tracking and Distribution* form (IPS Form Number 0015/005).

2.4 Pupae Harvest (Day 16; Thursday)

2.4.1 Pupae shall be harvested within a BSC, 8d after thinning (i.e., 16d after egg set-up) and placed within open petri dishes lined with sterile paper towel. Deformed pupae shall be frozen and discarded. Only 200 pupae of each gender shall be kept for subsequent mating (i.e., enough for two mating cages) and remaining pupae and larvae shall be frozen and discarded. One hundred males shall be placed into each of two dishes (i.e., total of 200 males). One hundred females shall be placed in each of two additional dishes (i.e., total of 200 females).

2.4.2 Cups with dead larvae or pupae shall be removed from the rearing process (i.e., apparently healthy pupae from these cups shall not be kept). After handling dead or contaminated insects, forceps shall be exchanged for sterile ones and gloves shall be replaced. When three or more cups with dead insects are present, they shall be maintained in a plastic bag, labeled with the ID code and frozen for subsequent QC analysis.

2.4.3 The harvest date, number of male and female pupae in each petri dish, and initials of technician shall recorded on *T ni Tracking and Distribution* form (IPS Form Number 0015/005).

2.4.4 Dishes of pupae shall be placed in mating chambers (as per section 2.5) on the same day in which they were harvested.

2.5 Mating

2.5.1 Two mating chambers shall be prepared using an inverted, vented, Rubbermaid bin as per the photos below. Screened vents shall be covered with sterile paper towel to prevent the escape of wing scales which are a human health hazard and can also carry pathogens between mating cages. Each cage shall have 6 pieces of gauze (7cm x 22cm) taped to the inside so they will hang above (but not touch) the petri dishes of pupae once inserted. The lid of the Rubbermaid container will form the base of the mating chamber. The recess in the centre of the lid shall be fitted with 3 layers of absorbent pads and shall be saturated with 500ml autoclaved RO water. A petri dish containing an absorbent cotton pad saturated with 50ml feeding solution shall be placed within each mating chamber (refer to Appendix 2 for feeding solution recipe). One petri dish with 100 males and one dish with 100 females shall be placed within each of the two mating chambers.

Rubbermaid bin with gauze strips

Completed mating chamber

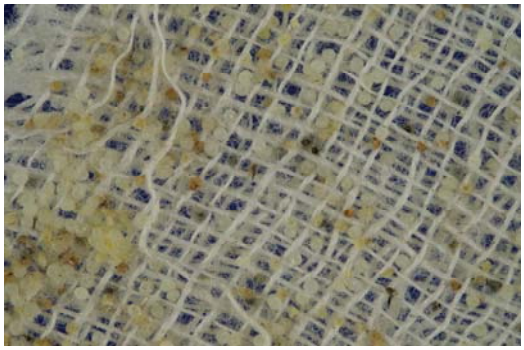
2.5.2 Mating chambers shall be maintained at 27±3°C, 55±10%RH and 16:8 light:dark and shall be monitored daily for the appearance adults, which usually occurs 2-3 days after pupae harvest. The date of the appearance of the first adults shall be recorded on the *T ni Tracking and Distribution* form (IPS Form Number 0015/005) as the mating date (typically day 19, Sunday).

Environmental chamber with three cohorts in different stages of development

2.5.3 If the absorbent towel on the base of the mating chamber dries out, lift the corner of the screened vent and mist the chamber with autoclaved RO water.

2.6 Egg Harvest and Surface Sterilization (Day 23-24; Thursday or Friday)

2.6.1 Eggs shall be harvested five days after the initiation of mating.
2.6.2 Mating cages shall first be placed in a refrigerator for 10-15 minutes to immobilize the moths.
2.6.3 The chilled cages shall be transferred to a running chemical fume hood where they shall be disassembled and the gauze strips containing the eggs removed. Eggs/gauze strips may be pooled from the two mating cages of the same cohort.



Gauze strip containing eggs

2.6.4 Adults from both mating cages shall be pooled and placed into a 250ml Nalgene sample bottle, labeled with the ID code, generation and egg harvest date (cages may be frozen and adults harvested at a later date). The bottle shall be placed into the freezer located in the IPU distribution room for subsequent pick-up by QCU personnel for quality control screening. The date when the QC sample was collected for QC shall be recorded on the *T ni Tracking and Distribution* form (IPS Form Number 0015/005).

Collecting adults for QC screening

2.6.5 Other contents of the mating cage shall be discarded. When time permits, the cage shall be washed with hot soapy water, sterilized by soaking in a bleach working solution (refer 2.9.2) and given a final rinse with water.

2.6.6 Eggs shall be surface sterilized on the day of harvest (i.e., Thursday or Friday), when time permits, otherwise the eggs/gauze may be stored in a refrigerator and sterilized on the following day. Within a running chemical fume hood, the gauze strips containing the eggs shall be placed in a glass crystallizing dish pre-filled with 750ml 10% formaldehyde solution (refer to 2.9.1) and allowed to soak for 45 minutes. Forceps should be used occasionally to ensure that the strips remain submerged.

Eggs/gauze in formaldehyde solution

2.6.7 With the fume hood running, the 10% formaldehyde solution shall be decanted from the crystallizing dish into a waste bottle (stored under the fume hood). A small amount of autoclaved RO water shall be added to dish, then decanted into the waste bottle. The dish shall be

filled with autoclaved RO water and eggs/gauze shall be allowed to soak for 45 minutes. The water shall be decanted (and discarded down the drain) 2-3 times during this soaking period and the gauze dipped up-and-down prior to each water change in order to assist with dislodging wing scales.

2.6.8 Eggs/gauze strips shall be blotted briefly by placing them (using sterile forceps or gloved hands) on at least 4 layers of sterile paper towel (placed on a perforated metal tray) and covering them with another 2 layers of sterile paper towel).

Eggs/gauze strips being blotted dry

2.6.9 The eggs/gauze strips shall be hung within an operational fume hood to dry for 2-3h.

Eggs/gauze hanging to dry

2.6.10 One strip of eggs/gauze shall be placed in a zip-lock bag identified as "colony" and labelled with the family ID and generation number. The

remaining 11 strips of eggs/gauze shall be placed in a separate zip-lock bag identified as "distribution" and labelled with the Family ID, generation number, egg harvest date and 10-day discard date (i.e., 10d after they are harvested). Both zip-lock bags shall be stored in a refrigerator until set-up for colony or distribution to clients, as applicable. Visual estimates shall be made of the total number of eggs produced, number dedicated to colony maintenance and number available for distribution, and shall be recorded on the zip-lock bags as well as on the *T ni Tracking and Distribution* form (IPS Form Number 0015/005, Appendix 1). Typically, females yield about 120 eggs thus each of the 12 gauze strips would have about 2000 eggs. Also, record the 10d discard date on the form.

2.6.11 A new ID code shall be generated for the progeny eggs. The new code shall include the next successive filial number than that of the parental adults.

2.7 Distribution of Insects

2.7.1 *T ni* shall be distributed to clients as eggs on gauze or may be set up on diet and distributed prior to thinning (i.e., the IPU will not conduct diet changes for clients) and shall be documented on the *T ni Tracking and Distribution* form (IPS Form Number 0015/005, Appendix 1). Documentation shall include:

(a) date distributed
(b) name of recipient
(c) # eggs/larvae requested
(d) internal or external
(e) # eggs/larvae distributed
(f) diet ID
(g) initials of distributer

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

2.7.3 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 by applicable personnel 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 applicable 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.

2.7.4 Eggs shall not be distributed after 10-days storage in the refrigerator.

2.7.5 Upon distributing or discarding all insects from the cohort as stated in 2.7.1 through 2.7.4, review the *T ni Tracking and Distribution* form to ensure that the sum of distributions and discards is equal to the number of eggs that were available. Calculate the percentage of eggs that were discarded and record on the form.

2.8 Quality Control

2.8.1 QC screening of adults will be conducted by the QCU and results sent electronically to the IPU. Instructions provided by the QCU shall be followed by IPU personnel. The QC report shall be printed and maintained with IPU records.

2.9 Calculations

2.9.1 10% Formaldehyde shall be prepared in a chemical fume hood by adding 75 ml Formaldehyde (37% active ingredient concentration from supplier) to 675 ml of autoclaved RO water to yield a 3.7% active ingredient concentration of Formaldehyde.

2.9.2 The bleach working solution for general cleaning shall have a final sodium hypochlorite concentration of 0.3%. Bleach stock material with a 5.25% sodium hypochlorite concentration (e.g., Javex®) shall be diluted by combining 60ml bleach and 940ml water (i.e., 6% dilution). Bleach stock material with a 6.0% sodium hypochlorite concentration (e.g., Ultra Javex®) shall be diluted by adding 53ml bleach and 947ml water (i.e., 5.25% dilution). If another brand of bleach is used, volumes may need to be adjusted to provide a 0.3% sodium hypochlorite working solution.
[Note: minimum contact time of 10 minutes is required for effective sanitation]

2.10 Documentation and Reporting

2.10.1 Compliance to this SOP shall include the completion and maintenance of the *T ni Tracking and Distribution* form (IPS Form Number 0015/005; Appendix 1).

2.10.2 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 applicable tracking form.

2.10.3 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 2.10 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*

9.0 APPENDICES

Appendix 1: IPS Form Number 0015/005, *T ni* Tracking and Distribution
Appendix 2: Feeding Solution Recipe
Appendix 3: Sexing Pupae

Appendix 1

Tni Tracking and Distribution

ID Code for egg setup: _____ Created from: □ Previous generation □ Other _____

Egg Set-Up

Date DD/MM/YY	# of Cups (@ 25 insects/cup)	Diet ID DD/MM/YY	Initials

Thinning (Sexing)

Date DD/MM/YY	# of Cups (@ 3 ♀ insects/cup)	# of Cups (@ 3 ♂ insects/cup)	Diet ID DD/MM/YY	Initials

Pupae Harvest

Date DD/MM/YY	# of ♀	# of ♂	Initials

Mating

Container	Date DD/MM/YY	# of ♀	# of ♂	Egg Harvest (5 days) DD/MM/YY	Adults frozen for QC DD/MM/YY	Initials

ID Code for progeny eggs: _____

eggs produced: _____
eggs kept for colony: _____
eggs available for distribution: _____

Distribution of progeny eggs

Date DD/MM/YY	Name/Affiliation	# Requested	internal or external	# distributed		Diet ID DD/MM/YY	Initials
				Larvae	Eggs		

total distributed: _____
eggs discarded: _____
Mod discard date: _____ DD/MM/YY

% discarded: _____

IPS Form Number 0015/005

IPS Form Number 0015/005

Appendix 2

Feeding Solution Recipe

5g methyl paraben
5ml ethanol
0.5g ascorbic acid
250g table sugar
25ml honey
500ml autoclaved RO water

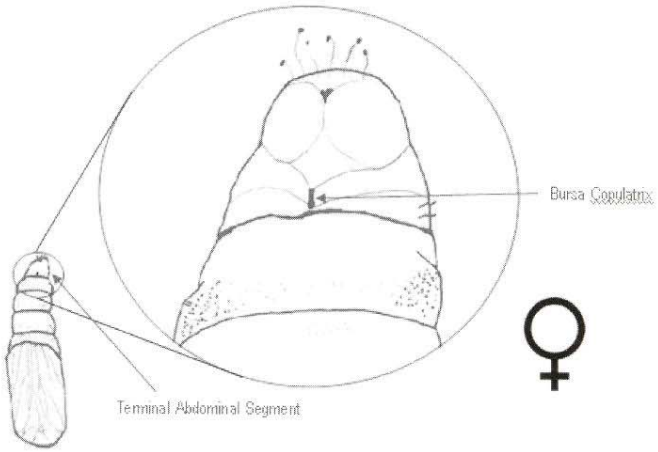
Dissolve the methyl paraben in the ethanol. Add all ingredients to the autoclaved RO water within a sealed bottle. Shake until all ingredients are dissolved. Label and date the solution and store in a refrigerator for not more than 3 months.

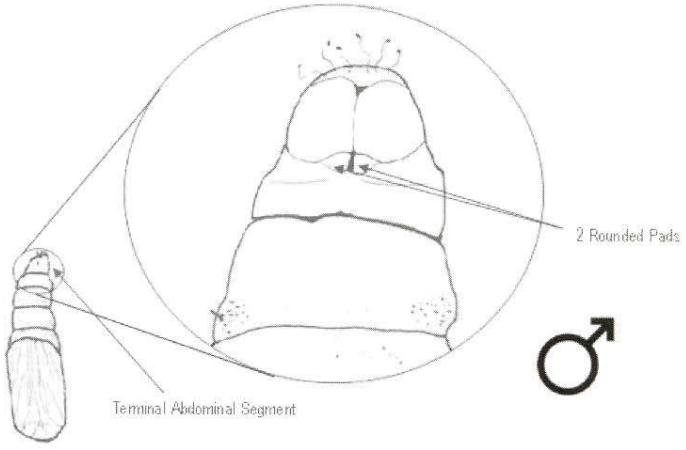
Appendix 3

6

Sexing pupae

Male and female pupae are separated based on differences found on the terminal abdominal segments. A compound microscope (at approx. 6.4X) is needed to observe these. The eighth segment of the female pupa is divided by the ninth segment on the ventral median and the opening of the bursa copulatrix is visible in this segment. The eighth segment of the male pupa is not divided and the ninth segment has two rounded pads, one on each side of the middle ventral line.





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