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Great Lakes Forestry Centre
Insect Production Services

STANDARD OPERATING PROCEDURE

Number: IPS/022/001

Quality Control for Choristoneura pinus pinus

Effective Date: 6 May 2015

Canada

TITLE: Quality Control for <u>Choristoneura pinus pinus</u> (Cpp)

APPROVING OFFICIAL:

DD / MM / YY

*Manager, Insect Production Services (IPS)*_____ ___/___/___

SIGNIFICANT CHANGES FROM PREVIOUS VERSION:

NA

1.0 INTRODUCTION

1.1 Purpose

This Standard Operating Procedure (SOP) has been established to ensure that procedures are followed for the receipt and processing of *Cpp* samples for the detection of pathogens and to reduce the incidence and spread of pathogens and microbial contaminants in the Great Lakes Forestry Centre (GLFC) insect production facility.

1.2 Scope

This SOP shall be followed by all Quality Control Unit (QCU) personnel for the microbial screening and process control of *Cpp*.

1.3 Definitions

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.

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

Head Quality Control (QC) Technician – A member of IPS having authority over the daily operation of the QC lab and other QC personnel.

Insect Production Services (IPS) – A GLFC work team consisting of the Insect Production Unit (IPU), 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 Manager – The individual who has overall responsibility for activities of the IPS team.

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

Insect Rearing Advisory Group (IRAG) – A GLFC advisory board that monitors and advises IPS activities and initiatives, provides a mechanism for CFS client input, ensures that user needs and priorities are met, and acts as a liaison between IPS personnel, clients and management.

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 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 to perform staining operations.
1.4.2 Naphthalene Black staining procedure shall be conducted within a functioning chemical fume hood.
1.4.3 Personnel shall have access to, and be familiar with, the MSDS for all chemicals used for the staining procedures.

1.5 Materials

Materials required include:

1.5.1 Personal protective safety equipment:
(a) lab coat
(b) disposable chemical protective gloves
(c) chemical fume hood

1.5.2 Chemicals:
(a) Naphthalene Black 10B stain (CAS#1064-48-8)
(b) Bromophenol Blue stain
(c) glacial acetic acid
(d) methanol (99%)
(e) sodium dodecyl sulfate (1%)
(f) MSDS

1.5.3 Tools:
(a) hot plate with boiling water
(b) slide warmer
(c) ice-water bath
(d) staining racks/dishes
(e) glass microscope slides
(f) microscope with oil immersion capability
(g) Dino Lite Digital Microscope (Hoskin Scientific) connected to computer system

1.5.4 Forms:
(a) Microbial Screening of *Cpp* Samples (IPS Form Number 0170/001, Appendix 1)
(b) QC Report for *Cpp* Adults (IPS Form Number 0171/001, Appendix 2)
(c) Rearing Summary for *Cpp* - Colony Maintenance (IPS Form Number 0173/001, Appendix 3)
(d) *Cpp* Multi-generation Summary (IPS Form Number 0172/001, Appendix 5)
(e) QC Report for *Cpp* Non-Adult Samples (IPS Form Number 0174/001, Appendix 6)
(f) *Cpp* Pupa Case Size (IPS Form Number 0175/001, Appendix 7)

2.0 PROCEDURES

2.1 Types of Samples and Documentation from the IPU

2.1.1 Adults:
(a) Upon dismantling of mating chambers by the IPU, female adults will have been collected by IPU personnel and maintained separately by chamber number in screw cap vials (labeled with the ID code and mating chamber number).

2.1.2 Larvae:
(a) Cups of larvae found to have one or more cadavers at the time of thinning or at pupa checks will have been removed from the rearing process, labeled with the ID code and supplied to the QCU for examination.

(b) When batches of larvae are found to have poor development or symptoms of disease, representative samples will be labeled with the ID code and supplied to the QCU for immediate examination.
(c) Routine screening of apparently healthy larvae will not be conducted at this time.

2.1.3 Historical Samples:
(a) The QCU will periodically (i.e., every 10 generations) request the IPU to collect larvae for the maintenance of a historical DNA record (to determine genetic drift).
(b) Samples of each cohort of *Cpp* will have been labeled with the ID code and supplied to the QCU.

2.1.4 Tracking sheets:
(a) The IPU will have documented the rearing process of each generation of every cohort of *Cpp* on the current version of IPS Form Number 0132 (Tracking Sheet for *Cpp*) and will copy the completed form to the QCU once larval progeny enter the diapause stage.

2.1.5 Gauze patches:
(a) Gauze patches from each weekly batch of *Cpp* will have been placed in a labeled (ID code) bag and supplied to the QCU.

2.1.6 Pupa cases:
(a) Representative samples of male and female pupa cases from each cohort will be provided.

2.1.7 Un-hatched eggs:
(a) When there is evidence of poor hatch, un-hatched eggs will have been placed in a labeled (ID code) bag and supplied to the QCU for examination.

2.2 Receipt of Samples from the IPU

Any sample received from the IPU shall be documented and tracked as specified in the current version of the SOP IPS/029 (Tracking QC Samples)

2.2.1 Adults: The QCU shall ensure that containers received (max. 10 per weekly cohort), are appropriately labeled and shall freeze samples immediately for microbial screening prior to the completion of diapause of progeny larvae.

2.2.2 Larvae, pupae or un-hatched eggs: The QCU shall ensure that samples received from the IPU are labeled with the ID code, collection date, and type of sample (i.e., dead at thinning, slow developers, suspected microbial infection, etc.) and shall freeze the samples immediately for subsequent microbial screening if deemed necessary by the QCU.

2.2.3 Historical samples: The QCU shall ensure that a larval sample (n=10) of each cohort is received periodically (i.e., every 10 generations) from the IPU and maintained at –70ºC for a historical DNA record.

2.2.4 Gauze patches: The QCU shall ensure that bags of patches received from the IPU are labeled with the ID code and will freeze the samples immediately for subsequent analysis.

2.3 Sample Preparation for Detection of Pathogens

2.3.1 Processing of vials of adults:

(a) Adults shall be maintained frozen in separate (labeled) containers until the time of processing; subsequent examination shall occur prior to the end of diapause of the resulting progeny.

(b) Adults from each mating chamber shall be macerated as described in the current version of SOP IPS/004 (Homogenizer).

(c) A 500µl subsample shall be taken and placed in a sterile 1.5ml microtube; 500µl of 1% SDS (refer to section 2.8) shall be added to the subsample (i.e., final concentration = 0.5% SDS) which shall then be placed on a tube rocker for 1-2h at room temperature.

2.3.2 Slide preparation of adults:

(a) Two preparations shall be made from each subsample for microscopic examination using different staining methods (i.e., Naphthalene Black and Bromophenol Blue).

(b) 5µl of each subsample shall be applied to each of two prelabeled glass slides (I.D. code, bag number, type of sample and staining method).

(c) Each 5µl subsample shall be spread over an area of approximately 1cm^2 using a new sterile pipette tip and shall be allowed to air dry before staining using one of the two methods; up to five samples can be applied to one slide.

2.3.3 Processing of samples of larvae, pupae or un-hatched eggs:

(a) Samples of larvae, pupae or un-hatched eggs shall be processed and examined for pathogens only if deemed necessary by the QCU (i.e., when significant numbers of dead larvae are found at emergence, thinning or pupa checks).

(b) A representative number of larvae, pupae or un-hatched eggs (to be determined by the IPU at the time of processing) from each group of samples shall be processed and examined in a timely manner (i.e., to reduce wasted effort in the rearing of contaminated batches). The number of insects examined shall be documented in the comments section on the screening form (IPS Form Number 0170/001, Appendix 1).

2.3.4 Slide preparation of samples of larvae, pupae or un-hatched eggs:

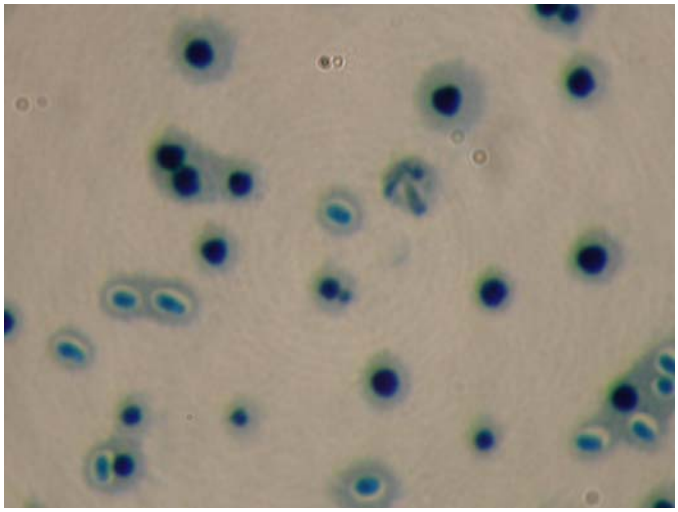
(a) Two preparations shall be made from each insect smear for microscopic examination using two staining methods (i.e., Naphthalene Black and Bromophenol Blue).
(b) Each insect shall be smeared (using a new toothpick for each) onto a prelabeled glass slide (I.D. code, date of collection, type of sample and staining method), ensuring that sufficient mid-gut tissue is obtained; slides shall be allowed to air dry before staining.

2.3.5 Examination of gauze patches:
(a) Patches shall be examined in a timely manner to reduce wasted effort in the rearing of contaminated batches.
(b) Patches shall be examined under the Dino Lite digital microscope and a determination made of the total number of larvae remaining within the patches (i.e., those that did not emerge from their hibernacula); larvae that were cut in two during the preparation of the patches by the IPU shall be counted only when the portion with the head attached is in the patch being examined (to avoid counting an insect twice).
(c) A calculation of the percentage of larvae lost (i.e., number of larvae counted ÷ number of larvae taken out of diapause x 100) shall be determined and recorded on IPS Form Number 0170/001 (Microbial Screening of *Cpp* Samples, Appendix 1); the date of examination shall also be recorded.
(d) When numbers of non-emerging larvae are greater than 10%, a subsample of 120 larvae shall be smeared and stained as identified in sections 2.3.4 and 2.4

2.4 Staining and Examination of Slides

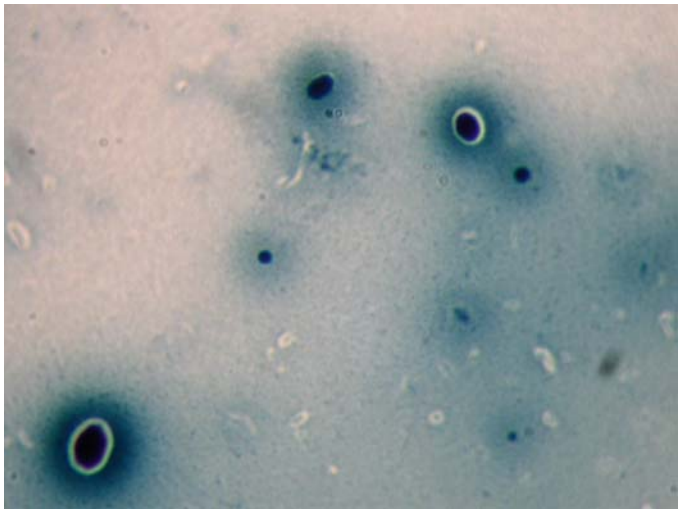
2.4.1 Staining with Naphthalene Black:
(a) One of the two sets of slides shall be stained with Naphthalene Black 10B and examined for the presence of pathogens (i.e., viral occlusion bodies, microsporidia, other).
(b) The staining solution shall be prepared by dissolving 2.4 g Naphthalene Black 10B in 130 ml distilled water and 70 ml glacial acetic acid (use a magnetic stirrer with gentle heat); replace the stain monthly or when the volume becomes depleted with use (i.e., slides are no longer covered); Naphthalene Black 12B may be substituted for 10B.
(c) Slides shall be immersed in the pre-heated stain (40-45ºC) for 10 minutes, removed and rinsed gently in tap water.
(d) Slides shall be air dried or dried on a slide warmer before examination.
(e) A minimum of 20 fields of view of each sample shall be examined at 1000x magnification using oil immersion (bright field optics).

(f) Note that protein occlusion bodies (i.e., CPV and NPV) stain deep blue-black with a light blue background and cannot be differentiated using this stain (Bromophenol Blue staining will be used to distinguish between these two types of occlusions when detected using the Naphthalene Black stain); microsporidia stain a distinctive dark blue at one end and light blue at the other (no attempt will be made to distinguish between species of microsporidia):



CPV, NPV and microsporidia in 10B stain

EPV also stains a deep blue-black but can be differentiated from CPV and NPV by their oval shape and larger size:



EPV and CPV in 10B stain

(g) Observations and preliminary determination of microbial contamination shall be recorded on IPS Form Number 0170/001

(Microbial Screening of *Cpp* Samples, Appendix 1). Pathogens shall be quantified by class ranges defined as:

- high (+++) = more than 50 occlusions per field of view
- medium (++) = average of 5 to 50 occlusions per field of view
- low (+) = less than an average of 5 occlusions per field of view, but more than 3 in 20 fields
- trace (T) = total of 3 or fewer occlusions in 20 fields of view
- negative (-) = no pathogens observed in 20 fields of view
-

2.4.2 Staining with Bromophenol Blue:

(a) The second set of slides shall be stained with 0.1% Bromophenol Blue and examined for the presence of CPV occlusion bodies.

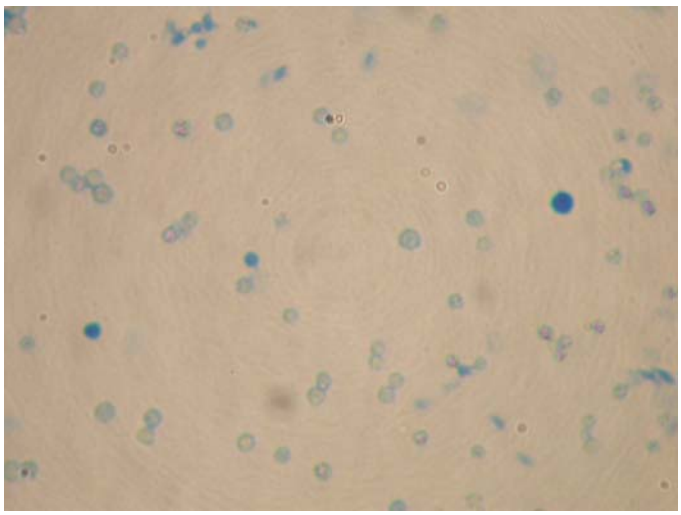
(b) The staining solution will be prepared by dissolving 0.2g Bromophenol Blue in 199.8ml distilled water and shall be stored in the dark; replace the stain monthly or when the volume becomes depleted with use (i.e., slides are no longer covered).

(c) Slides shall be fixed by immersion in 99% methanol for 90 sec., followed immediately by immersion in boiling water for 5 sec., ice-water (less than 5ºC) for 5 sec., 0.1% Bromophenol Blue stain for 15 min., then rinsed gently in tap water.

(d) Slides shall be air dried before examination.

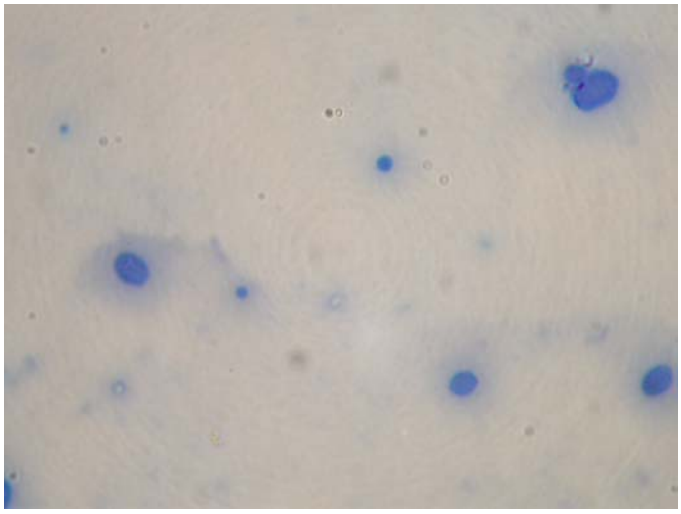
(e) A minimum of 20 fields of view of each sample shall be examined at 1000x magnification using oil immersion (bright field optics).

(f) Note that CPV stains a medium blue, whereas NPV, microsporidia and the background remain relatively unstained:



CPV, NPV and microsporidia in Bromophenol Blue stain

EPV stains medium to dark blue, is oval in shape and is considerably larger than CPV:



EPV and CPV in Bromophenol Blue stain

(g) Observations and preliminary determination of CPV contamination shall be recorded on IPS Form Number 0170/001 (Microbial Screening of *Cpp* Samples, Appendix 1). CPV contamination shall be quantified by class ranges defined as:

- *high (+++) = more than 50 occlusions per field of view*
- *medium (++) = average of 5 to 50 occlusions per field of view*
- *low (+) = less than an average of 5 occlusions per field of view, but more than 3 in 20 fields*
- *trace (T) = total of 3 or fewer occlusions in 20 fields of view*
- *negative (-) = no CPV occlusions observed in 20 fields of view*

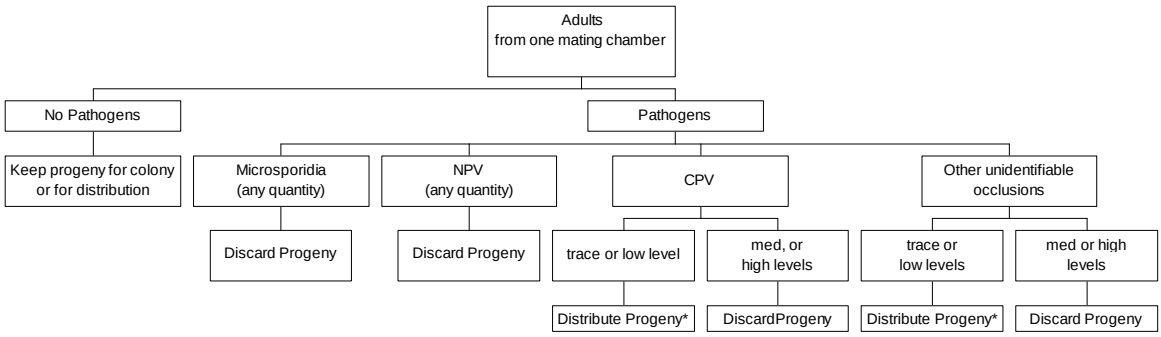
2.4.3 The QCU may solicit the assistance of other GLFC (or external) personnel in the determination of the presence of pathogens using whatever means are deemed necessary; results of screening by others shall be maintained with QC records for the applicable cohort.

2.4.4 Adults will not routinely be examined for the presence of NPV using Naphthalene Black, since adults contain numerous protein occlusions that stain similarly to NPV and cannot be distinguished; it is assumed that the presence of NPV in the insect population would cause high larval mortality that would be easily be recognized/detected at an earlier life stage.

2.4.5 The QCU shall retain all samples and derivative slides as specified in the current version of SOP IPS/029, Tracking of QC Samples.

2.5 Dissemination of Results to IPU

2.5.1 Upon examination of female adults obtained from mating chambers, the QCU shall use the following flow chart to determine instructions to be given to the IPU with regard to the fate of the progeny from each chamber:

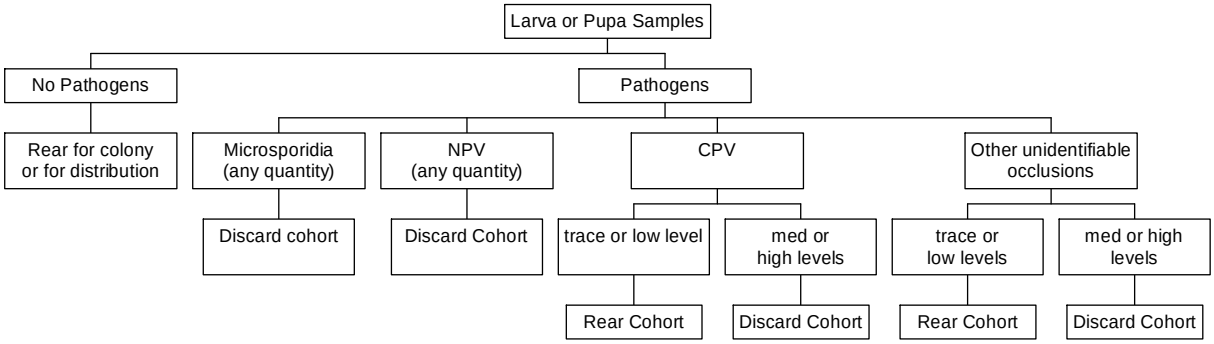


*Do not keep for colony unless there are insufficient numbers of insects from the other mating chambers.

2.5.2 Results of QC analysis of adults and a decision on the fate of the progeny from each mating chamber shall be documented on IPS Form Number 0170/001 (Microbial Screening of *Cpp* Samples, Appendix 1) as well as on IPS Form 0171/001 (QC Report for *Cpp* Adults, Appendix 2).

2.5.3 IPS Form 0171/001 shall be sent electronically to the IPU and the dissemination of results documented as specified in the current version of SOP IPS/029 (Tracking QC Samples).

2.5.4 Upon examination of larva or pupa samples obtained during the rearing process, the QCU shall use the following flow chart to determine instructions to be given to the IPU with regard to the fate of the cohort:



2.5.5 Results of QC analysis of samples of larvae, pupae, or un-hatched eggs and a decision on the fate of the cohort shall be documented on IPS Form Number 0170/001 (Microbial Screening of *Cpp* Samples, Appendix 1) as well as on IPS Form 0174/001 (QC Report for *Cpp* Non-Adult Samples, Appendix 6).

2.5.6 IPS Form 0174/001 shall be sent electronically to the IPU and the dissemination of results documented as specified in the current version of SOP IPS/029 (Tracking QC Samples).

2.6 Analysis of Pupa Cases

2.6.1 Ten pupa cases of each gender shall be measured and recorded on IPS Form Number 0175/001 (*Cpp* Pupa case size, Appendix 7).

2.6.2 The first 10 undamaged pupa cases observed shall be measured using the Dino Lite digital microscope. Linear measurements shall be taken along the dorsoventral plane of the first segment of the pupa case posterior to the wing pads, as shown on the diagram on the reporting form. Data may be sent to an Excel spread sheet then copied to the reporting form.

2.6.3 Pupa case measurements shall be used in statistical process control monitoring as specified in Section 2.7.

2.7 Statistical Process Control

2.7.1 Statistical process control monitoring shall be documented by the QCU as specified in Appendix 4 (Procedure for Maintaining *Cpp* Process Control Records) upon each receipt of a cohort tracking form from the IPU.

2.7.2 The QCU shall maintain IPS Form Number 0173/001 (Rearing Summary for *Cpp*, Appendix 3) which is part of the Procedure for Maintaining *Cpp* Process Control Records.

2.7.3 The QCU shall maintain a *Cpp* Multi-generation Summary (IPS Form Number 0172/001, Appendix 5).

2.8 Calculations

2.8.1 1% SDS working solution shall be prepared by adding 10ml of 10% SDS stock solution to 90ml dH_20.

2.8.2 10% SDS stock solution shall be prepared by dissolving 10g of sodium dodecylsulfate ($C_{12}H_{25}O_4SNa$; FW 288.4) in approximately 90ml dH_20 (heat to 68ºC to assist dissolution); after dissolution, adjust final volume to 100ml with dH_20 and store at room temperature in an air-tight bottle.

2.9 Documentation and Reporting

2.9.1 Compliance to this SOP shall include the completion of the following forms:

(a) Tracking Sheet for *Cpp* (current version of IPS Form Number 0132).

(b) Microbial Screening of *Cpp* Samples (IPS Form Number 0170/001, Appendix 1).

(c) QC Report for *Cpp* Adults (IPS Form Number 0171/001, Appendix 2).

(d) Rearing Summary for *Cpp* (IPS Form Number 0173/001, Appendix 3).
(e) *Cpp* Multi-generation Summary (IPS Form Number 0172/001, Appendix 5).
(f) *Cpp* pupa case size (IPS Form Number 0175/001, Appendix 7)

2.9.2 Compliance to this SOP shall include completion of IPS Form Number 0174/001 when non-adult samples are provided by the IPU.
2.9.3 The QCU shall maintain files of all forms identified above.
2.9.4 The QCU shall provide the IPU with electronic copies of QC Reports as specified in section 2.5.
2.9.5 The QCU shall prepare and maintain current *Process Control Charts* as specified in Appendix 4.
2.9.6. The QCU shall make all records available to the Insect Rearing Advisory Group.

3.0 DISTRIBUTION AND ARCHIVING

3.1 Distribution

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

3.2 Archiving

3.2.1 The IPS manager shall maintain a historical file of this SOP when it is replaced by a new version.
3.2.2 The QCU shall ensure that files of all documentation identified in section 2.9 are maintained for expedient retrieval.
3.2.3 The QCU shall retain all samples and derivative slides as specified in the current version of SOP IPS/029, Tracking QC Samples.

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 that 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 head QC technician is responsible for assuring that this SOP is valid.
4.1.2 The head QC technician is responsible for assuring that this SOP is followed by QCU personnel and that these persons have been appropriately trained in its use.
4.1.3 QCU 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 head QC technician is responsible for assuring that this SOP is current. If necessary, the head QC technician 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 QCU personnel find circumstances that do not permit compliance with this SOP, the head QC technician 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 the following SOPS:
a) SOP IPS/004 (Homogenizer)
b) SOP IPS/029 (Tracking QC Samples)
Current version of the following form:
a) IPS Form Number 0132 (Tracking Sheet for *Cpp*)

9.0 APPENDICES

Appendix1: IPS Form Number 0170/001 (Microbial Screening of *Cpp* Samples)
Appendix 2: IPS Form Number 0171/001 (QC Report for *Cpp* Adults)
Appendix 3: IPS Form Number 0173/001 (Rearing Summary for *Cpp*)
Appendix 4: Procedure for Maintaining *Cpp* Process Control Records
Appendix 5: IPS Form Number 0172/001 (*Cpp* Multi-generation Summary)
Appendix 6: IPS Form Number 0174/001 (QC Report for *Cpp* Non-Adult Samples)
Appendix 7: IPS Form Number 0175/001 (*Cpp* Pupa case size)

Appendix 1

Microbial Screening of *Cpp* Samples

ID Code:

Adult Screening

Bag No.	10B Stain			BPB Stain			Comments
	Date examined (DD/MM/YY)	Results		Date examined (DD/MM/YY)	Results		
		Micro-spordia	Other		CPV	Other	
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
	Conducted by:			Conducted by:			

Gauze Patches

L2 Counts					Diagnostic Results				
Date Examined (DD/MM/YY)	# out of diapause	# in gauze	% Loss	Initials	Date Examined (DD/MM/YY)	CPV	Micro-sporidia	Other	Initials

Screening of Other Stages

Sample Date (DD/MM/YY)	Stage	Date Examined (DD/MM/YY)	CPV	Micro-sporidia	Other	Comments	Initials

IPS Form Number 0170/001

Appendix 2

QC Report for *Cpp* Adults

ID Code:

Report Date: DD / MM / YY

Bag No.	Microbial Contaminants (adult screening)			Instructions to IPU		
	CPV	Micro-sporidia	Other	Maintain progeny for colony	Distribute progeny [1]	Discard progeny

[1] progeny with potential pathogens are to be distributed during diapause (i.e., do not rear in the insect production facility); clients must be informed by IPU of potential pathogens.

Additional instructions for IPU:

Completed by:

IPS Form Number 0171/001

Appendix 3

Rearing Summary for *Cpp*
(Colony Maintenance)

ID code	#L2s out	length of diapause (wks+d)	# cups	#L2s per cup	#L2s left in gauze	% loss of L2s	# cups at thin	# larvae at thin	% loss (L2 survivors to thin)	# pupae	% loss (thin to pupation)	male pupa size (mm)	female pupa size (mm)	% male pupa discard	% female pupa discard	% pooled pupa discard	% loss of L1 in pans	% loss (diapause to pupation)	Develop Time (dia. to dia.)	Mean # L2s per bag	# bags	# L2s per female	# L2s into diapause	Comments

Sum
Targeted Mean
Targeted Limits
Actual Mean

IPS Form Number 0173/001

Appendix 4 (Part 1 of 2)

4 May 2015

Procedure for Maintaining *Cpp* Process Control Records

Upon receipt of a *Tracking Sheet for Cpp* from the IPU:

1. Ensure that form is complete, legible and sequential to the last one received.
2. Stamp, date and initial the form.
3. Use a coloured marker to highlight data that is unusual or significant (e.g., sheets of L2s that are contaminated with fungus).
4. Calculate the following records using a calculator and document directly on the *Tracking Sheet for Cpp*:
   a) Sum of male pupae and % discard
   b) Sum of female pupae and % discard
   c) Pooled sum of pupae and % discard
   d) Number of L2s entering diapause from each bag (i.e., the sum of L2s from the top and bottom sheets; do not include the number of L2s lost)
   e) Sum of L2s from all bags (add the total for number of L2s derived from each bag calculated in 4d; include mean and standard deviation (list the number of L2s derived from each bag onto a Microsoft Excel worksheet; refer to 7a-i for calculation instructions)
   f) Sum of L2s lost from all bags (add the total number of L2s lost from each bag)
   g) % lost from all bags; i.e., [f ÷ (e + f)] x 100
   h) Development time in days (i.e., number of days between the date in which the cohort is taken out of diapause and the date in which progeny larvae enter diapause). Development time in days can be calculated using the following website: http://www.timeanddate.com/date/duration.html
5. Transcribe the following data onto the Excel *Rearing Summary for Cpp*:
   a) #L2s taken out of diapause (from *Tracking Sheet for Cpp*)
   b) Length of diapause (from *Tracking Sheet for Cpp*)
   c) #cups set up with patches (from *Tracking Sheet for Cpp*)
   d) #L2s left in gauze (transcribed from QC records)
   e) # cups at thin (from *Tracking Sheet for Cpp*)
   f) # pupae (from *Tracking Sheet for Cpp*)
   g) Male pupa size in mm (transcribed from QC Records)
   h) Female pupa size in mm (transcribed from QC Records)
   i) % male pupa discard (from *Tracking Sheet for Cpp*)
   j) % female pupa discard (from *Tracking Sheet for Cpp*)
   k) % pooled discard (from *Tracking Sheet for Cpp*)
   l) % loss of L1 in pans (from *Tracking Sheet for Cpp*)
   m) Development time (from *Tracking Sheet for Cpp*)
   n) Mean number of L2s per bag (from *Tracking Sheet for Cpp, see #4e*)
   o) # bags (from *Tracking Sheet for Cpp*)
   p) #L2s into diapause (from *Tracking Sheet for Cpp*).
6. The Excel *Rearing Summary for Cpp* spread sheet will automatically calculate:
   a) #L2s per cup
   b) % loss of L2s
   c) # larvae at thin
   d) % loss (L2 survivors to thin)
   e) % loss (thin to pupation)
   f) % loss (diapause to pupation)
   g) #L2s per female
7. Use the statistical analysis capability of Microsoft Excel to calculate mean #L2s per bag by:

Appendix 4 (Part 2 of 2)

a) List (in a column) the #L2s for each mating bag, into a Microsoft Excel worksheet
b) Select the *Tools* tab
c) Select *Data Analysis*
d) Select *Descriptive Statistics*
e) Highlight the *Input Range* (the #L2s per bag)
f) Highlight the *Output Range* (the location where the results will appear on the worksheet)
g) Select *Summary Statistics*
h) Click *OK*
i) Copy the mean #L2s per bag onto *Rearing Summary for Cpp* for the current generation. The #L2s per female will be automatically calculated by the excel spreadsheet.

8. The Excel program will automatically mark data on the *Rearing Summary for Cpp* (with red text) that is outside of the upper and lower targeted limits. Attempt to identify the source of the problem by discussion with IPU personnel, from notations on the tracking form, or by examination of production control records; document the source of the problem in the comments section of the rearing summary as well as on the tracking sheet for each affected cohort; take corrective action where possible to avoid future occurrences.
9. The data for the mean pupa sizes and the mean #L2s per female is then entered onto two *Process Control Charts* sheets (one for the current generation of *Cpp* and one for each *Cpp* family; both located on the QC/MD Drive). Microsoft Excel will automatically generate/update process control charts for male pupa size, female pupa size and #L2s per female for each cohort in the current generation, and for each family. (means, upper and lower control limits have been calculated using 2 standard deviations) Print all charts on a colour printer and maintain them in a file along with the tracking sheets for the current *Cpp* generation. Maintain process control charts for each *Cpp* family in their respective folder.
10. Attempt to identify the cause of production going out of control (i.e., data outside of upper or lower control limits) by discussion with IPU personnel, from notations on the tracking form, or by examination of production control records; document the cause in the comments section of the *Rearing Summary for Cpp* as well as on the *Tracking Sheet for Cpp* for each affected cohort; take corrective action where possible to avoid future occurrences.
11. Enter comments onto the Excel *Rearing Summary for Cpp* for the current generation when production or process control abnormalities occur (e.g., rearing room breakdown).
12. The Excel program will automatically record today's date on the spread sheet. Print the *Rearing Summary for Cpp* on a colour printer and maintain it in the file along with the *Tracking Sheet for Cpp* and the *Process Control Charts* for the current *Cpp* generation.

Appendix 5

***Cpp* Multi-generation summary**

Generation	L2/cup	%L2 loss	%loss L2-thin	%loss Thin-P	Pupa size M (mm)	Pupa size F (mm)	%M discard	%F discard	%Pupa discard	%L1 loss	%loss dia-P	Devel. Time (d)	L2/bag	L2/F	mean #L2/wk	total #L2
										0.5		63.9		102.8		

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Appendix 6

QC Report for *Cpp* Non-Adult Samples

Report Date:
DD / MM / YY

Type of Sample:

Sample Description or ID:

Diagnostic Results:

Instructions to IPU:

Completed by:

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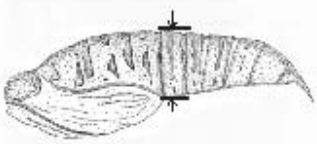
Appendix 7

Cpp Pupa Case Size

ID Code:

Male (mm)	Female (mm)
Initials:	Initials:



Linear measurement is taken along the dorsoventral plane of the first segment of the pupa case posterior to the wing pads.

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