Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Procedure

Detecting and Identifying Small Organisms Associated with Grains and Field Crops

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FOREWORD

This PI-003 supersedes the parts (Chapter 2) of the Plant Protection Manual on Export Inspection of Grains and Oilseeds (2.7) on detection methods.

Contact

The contact for the review will be an officer of the Grains and Field Crops Section (GFCS) of the Plant Health(PHD), as assigned by the National Manager of this section.

Review

The PHD of the Canadian Food Inspection Agency (CFIA) shall review PI-003 every second year or earlier if required. The next revision date is January 30, 2008. This document will be reviewed jointly with the Canadian Grain Commission (CGC), as per the requirement of the Memorandum of Understanding between these two organizations (2.9). This PI-003 has been jointly developed by the CFIA and CGC.

Endorsement

This PI-003 is hereby approved:

Greg Stubbings, Director, PHD, CFIA	Date
Quality System Procedures Committee Representative, PHD, CFIA	Date

Amendment Record

Amendments to this document will be given consecutive numbers. Insert all amendments, remove obsolete pages and ensure the record below is completed.

Number of amendment:	**Amended by:**	**Date of submission for approval of amendment:**	**Summary of amendment and number of amended section(s) or page(s):**

Distribution

The CFIA maintains, issues and distributes copies of this PI-003 to their central registry file (No. 3530-1D1) and the CGC chief inspector. The latest PI-003 is also be available on the CFIA internal and external websites*. The office of the Director of the PHPD keeps a controlled copy of this PI-003.

* CFIA internal website address: http://merlin/english/plaveg/grains/mane.asp

** CFIA external Website address: http://www.inspection.gc.ca/english/plaveg/grains/mane.shtml.

0 INTRODUCTION

Certain Canadian facilities (3.6) directly export grains and field crops (3.5). Grains and field crops exported may have to be certified free from quarantine pests (3.3), as per the requirements of plant health authorities in the importing countries. The CFIA issues this required certification by way of a phytosanitary certificate (3.7) based on inspections of facilities, transportation vehicles and/or lots of stored products to be exported. These inspections include sampling of residues, visible small organisms and stored products, and include detecting pests in these samples. This PI-003 specifies the procedures that inspectors (3.1) must follow to detect and identify small organisms (3.4) in samples taken during inspections. The CFIA will periodically audit inspectors against this PI-003 using PI-004 (2.10).

1 SCOPE

This PI-003 specifies the procedures that inspectors (3.1) must follow to detect and identify small organisms (3.4) sampled in facilities (3.6) or vehicles that export or transport grains and field crops (3.5). This PI-003 does not specify the general roles and responsibilities of inspectors. These roles and responsibilities shall be outlined in documents such as memoranda of understanding, internal work plans or other agreements between the CFIA and inspectors. This PI-003 may be used for the purpose of the standards PI-001 (2.24) and PI-002 (2.25).

2 REFERENCES

The legislative documents listed herein are available at the Canadian Justice Department website (http://Canada.justice.gc.ca).

2.1 *Canada Grain Act*, 1970-71-72, c. 7, s.1., R.S., 1985, c. G-10.

2.2 *Plant Protection Act*, S.C. 1990, C.22.

2.3 *Plant Protection Regulations*, SOR/95-212.

2.4 Food and Agriculture Organization of the United Nations (FAO), 1992. International Plant Protection Convention (IPPC) (AGPP/PQ/92/1). FAO, Rome. 17 pages.

2.5 ISO/TC 34, 1986. ISO 6639/3: Cereals and Pulses - Determination of Hidden Insect Infestation - Part 3: Reference Method. International Organization for Standardization (ISO), Switzerland, 6 pages.

2.6 ISO/TC 34, 1987. ISO 6639/4: Cereals and Pulses - Determination of Hidden Insect Infestation - Part 4: Rapid Methods. International Organization for Standardization (ISO), Switzerland, 20 pages.

2.7 The Plant Protection Division (PPD), 1989. Plant Protection Manual (Chapter 2). Export Inspection of Grains and Oilseeds. The PPD, Ottawa, Ontario.

2.8 Demianyk, C.J., White, N.D.G. and D.S. Jayas, 1997. Rapid Detection of Rusty Grain Beetles (Coleoptera: Cucujidae) from Wheat Samples Passing Through a Mechanical Dockage Tester. Canadian Journal of Plant Science 77: 717-719.

2.9 Agriculture and Agri-Food Canada (AAFC) and the CGC, 1994. Memorandum of Understanding between the CGC and the Department of Agriculture (Food Production and Inspection Branch) Concerning the Sampling and Inspection of Grain and Grain Handling Facilities to Meet Phytosanitary Export Market Requirements. AAFC, Ottawa, 6 pages.

2.10 PI-004: Auditing the Inspection of Facilities that Export Grains and Field Crops. PHD, CFIA.

2.11 Aitken, A.D., 1963. A Key to the Larvae of Some Species of Phycitinae (Lepidoptera, Pyralidae) Associated with Stored Products, and Some Related Species. Bulletin of Entomology Research 54(2): 175-188. [For Pyralid moths, e.g., *Anagasta kuehniella kuehniella* Zell. and *Plodia interpunctella* Hbn.]

2.12 Bousquet, Y., 1990. Beetles Associated with Stored Products in Canada. An Identification Guide. Agriculture Canada Publication 1837. [For Coleoptera, including flour beetles and meal worms.]

2.13 Gorham, J.R., 1991. Insect and Mite Pests in Food, an Illustrated Key. Volume 1. United States Department of Agriculture Handbook No. 655, U.S. Government Printing Office, Washington, D.C. 20402. [For Lepidoptera, including larvae, and Coleoptera, including flour beetles and meal worms.]

2.14 Hinton, H.E., 1956. The Larvae of the Species Tineidae of Economic Importance. Bulletin of Entomology Research 47(2): 251-346. [For *Nemapogon granella* L.]

2.15 Hodges, R.W., 1974. Gelechioidea: Oecophoridae *In* Moths of North America North of Mexico. Classey, London. [For *Endrosis sarcitrella* L. and *Hofmannophila pseudospretella* Staint.]

2.16 Stehr, F.W., 1987a. Immature Insects. Volume 1. Kendall/Hunt Publishing Co., Dubuque, Iowa. [For Lepidoptera, including larvae.]

2.17 Stehr, F.W., 1987b. Immature Insects. Volume 2. Kendall/Hunt Publishing Co., Dubuque, Iowa. [For Coleoptera, including flour beetles and meal worms.]

2.18 R-002: Lepidopterae, Psocopterae and Acaridae Associated with Grains and Field Crops. PHD, CFIA.

2.19 R-003: Coleopterae Associated with Grains and Field Crops. PHD, CFIA

2.20 Sinha, R.N. 1964. Mites of Stored Grain in Western Canada -- Ecology and Methods of Survey. Proceedings of the Entomological Society of Manitoba 20: 19-33.

2.21 Smith, L.B. 1977. Efficiency of Berlese-Tullgren Funnels for Removal of the Rusty Grain Beetle, *Cryptolestes ferrugineus*, from Wheat Samples. Canadian Entomologist 109: 503-509.

2.22 Gerber, G.H. 1996. Research Progress on Control of Crop Pests in Manitoba (Section B.6) -- Stored Product Pests: Rapid Detection of Rusty Grain Beetle in Stored Wheat by Demianyk and White . Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, Manitoba. Page 6.

2.23 R-001: Grains, Field Crops, and their Exporting Facilities. PHD, CIFA.

2.24 PI-001: Inspecting Facilities that Export Grains and Field Crops. PHD, CFIA.

2.25 PI-002: Sampling Grains and Field Crops, their Residues, and Associated Small Organisms. PHD, CFIA

2.26 The Canadian Standards Association (CSA), 1998. CSA Standard C22.1-1998: Canadian Electrical Code, Part I. Eighteenth Edition. The CSA, Etobicoke, Ontario, 779 pages. ISBN-0-921347-65-0.

2.27 Kitto, G.B., Thomas, P.W., Lemburg, J., Brader, B. And W. Burkholder. 1996. Immunoassays for Detecting Insect Contamination of Food Products (Chapter 21). *In* Immunoassays for Residue Analysis -- Food Safety. Beier and Stanker (Editors). American Chemical Society. Pages 282-291.

3 DEFINITIONS

For the purpose of this PI-003, the following definitions apply.

3.1	Inspectors	Includes the inspectors of the CFIA and any other inspector authorized to inspect on behalf of the CFIA.

3.2	Pest	Any thing that is injurious or potentially injurious, whether directly or indirectly, to plants or to products or by-products of plants, and includes any plant prescribed as a pest (2.2). See also 3.3.
3.3	Quarantine pest	A pest (3.2) of potential national economic importance to the country endangered thereby and not yet present there, or present but not widely distributed and being actively controlled (2.4).
3.4	Small organisms	Small living beings. Includes insects, mites, bacteria and fungi.
3.5	Grains and field crops	See R-001 (2.23).
3.6	Facilities that export grains and field crops	Means facilities that directly export grains and field crops (3.5), including elevators as defined in the *Canada Grain Act* (2.1), other elevators, malting plants, pellet plants and other facilities.
3.7	Phytosanitary certificate	See Part IV, Section 55.1 of the *Plant Protection Regulations* (2.3) for complete definition. Briefly, it is a document, issued by an inspector designated under the *Plant Protection Act* (2.2), that attests to the phytosanitary status of any thing exported from Canada.

4 DETECTING AND IDENTIFYING SMALL ORGANISMS

Inspectors (3.1) shall detect small organisms (3.4) associated to grains and field crops (3.5), or their residues, as specified in Sections 4.1 and 4.2, and identify small organisms as specified in Sections 4.2 and 4.3.

Note that other methods are under investigation but have not yet been approved by the CFIA. Some of these methods are described in *ISO 6639/3* (2.5), *ISO 6639/4* (2.6), Demianyk *et al* (1997) (2.8) and Kitto *et al* (1996) (2.27). These methods include the carbon dioxide, ninhydrin (paper dip to detect insect protein), whole grain flotation, acoustic, x-ray, immunoassay (ELISA) and dockage tester methods.

The CFIA only recommends methods that are efficient to detect small live organisms, either based on sufficient practical experience or sufficient scientific literature, that are available for use in large scale, that are rapid, and that are cost-efficient. Presently, the CFIA deems that, for insects and mites, only the Berlese funnel method meets most of these requirements.

4.1 Detecting Insects and Mites -- Berlese Funnel Method

The Berlese funnel method is based on the fact that insects and mites will move away from a source of heat and light. During pest extraction by the Berlese method, steep gradients of temperature and humidity will be established in the sample. These gradients will stimulate the organisms to leave the sample.

This method can extract numbers of mites and adults and larvae of insects, even insects that feed inside grain kernels, e.g., *Cryptolestes ferrugineus*. The CFIA recommends this method based on Smith's tests (2.21) and the CGC's and CFIA's positive experience of several years. Note, however, that the method may not extract all internal grain feeders (e.g., legless grubs that have limited mobility) which can be killed by heat stress long before reaching the collection jar.

Inspectors shall calibrate their Berlese funnel device when newly installed and as required. Calibration shall be done with at least one species of internal feeder insect (including adults and larvae), e.g., *Cryptolestes ferrugineus*. Calibration is useful to verify the efficiency of the device and procedures at extracting various species of organisms.

Calibration can be done by artificially infesting grains or field crops by known quantities and species of organisms, and by subsequently extracting the known organisms. Inspectors may artificially infest samples as in Smith (2.21), and extract the known organisms as specified in Section 4.1.1. Inspectors may maintain stock cultures of organisms by keeping and rearing specimens found during inspections, or inspectors may contact other offices or organizations (e.g., the CGC, CFIA or Agriculture and Agri-Food Canada, e.g., the Cereal Research Center in Winnipeg) for cultures.

Devices are calibrated, i.e., ready for use, when the majority of the test specimens are extracted. Inspectors shall contact the Grains and Field Crops Section of the CFIA for calibration problems.

4.1.1 Equipment Required

See Appendix A for device assembly*. The proposed material and procedures are based on those described by Sinha (1964) (2.20) with some differences. The following equipment is sufficient to install one device that will test only one sample at a time. If more devices are required, multiply the following equipment by the required number of devices.

a. A funnel big enough to hold a 1 kg sample (see Appendix A for example). This size is acceptable: 18 cm wide by 11 cm deep. The funnel shall be made of metal (e.g., galvanized iron, stainless steel or polished stainless steel).

b. A reflector built to match with the funnel (see Appendix A for example). The base diameter of the reflector shall be almost equal to the top diameter of the funnel. The reflector shall be mounted on a frame (bank). This frame can have adjustable legs to adjust the space between the bottom of the reflector and the top of the funnel-jar assembly, as required. If the frame does not have adjustable legs, the funnel-jar assembly can be mounted on blocks of wood that can be removed and placed easily under the jar, as required. The reflector will concentrate heat and light on the sample as well as enclose the sample. There should be a distance of 5 to 7 cm between the light bulb and the sample or top of the funnel. The reflector shall be made of metal (see examples listed for funnels).

c. A 60 watt incandescent light bulb fixed inside the reflector with a lamp holder. The lamp holder shall be a CSA approved phenolic shell lamp holder (e.g., the *Leviton* No. 95080, keyless 660W-250V). Installation of lighting equipment shall be done as prescribed by the *Canadian Electric Code* (2.26), Rules 26 and 30, and any other applicable Rule.

d. A wide-mouth glass jar that fits snugly to the bottom of the funnel. The jar should be big (tall) enough to contain at least 227 ml (8 oz) (i.e., large enough to contain 25 ml of liquid and fallen organisms, and large enough to provide a barrier wall for insects trying to escape from the jar).

e. Water or 70% ethyl alcohol (25 ml or 2 oz).

f. A 4 mesh/cm (1680 micron) screen platform (circular and of metal) to rest in the groove of the funnel to allow organisms through without allowing the sample through.

g. A timer to control the electric power for the device (optional).

* Equipment can be obtained from AMS Industries Ltd, 1575 Franklin St., Vancouver, B.C., V5L 1P3, Tel.: 604-251-3591, Fax: 604-251-6422. In 1997, for 126 units ordered in bulk, the cost was $22 per unit. Additional sources will be listed as available.

4.1.2 Procedures

Follow these procedures for each sample to test.

a. Assemble the device as shown in Appendix A, using the equipment listed in Section 4.1.1.

b. Clean jars, screen platforms and funnels, brushing them thoroughly under hot water and drying them before use. Then for each sample to be tested, do as follows.

c. Rest the screen platform in the groove of the funnel, ensuring that the platform is positioned horizontal.

d. Place the empty funnel, wide end up, on a piece of clean paper. The paper is to facilitate recuperating the sample particles that will pass through the funnel during pouring (see Section 4.1.2.i).

e. Pour 1 kg (or less if the sample is smaller) of the sample into the top of the funnel. If particles of the sample are finer than the openings of the screen platform, place enough screening material (e.g., clean and larger grains) on the platform before placing the sample to prevent the particles from falling through the screen. Samples that are composed of fine particles, e.g., flour, may also be blent with a small quantity (e.g., 300 g or other quantity necessary based on practical experience) of coarser and pest free grain to allow effective heat penetration in the sample. The inspector can determine the exact quantity of screening material which will work best with practical experience or calibration.

f. Pour about 25 ml (2 oz) (i.e., just enough to enclose all organisms that might be in the sample) of 70% ethyl alcohol or water into a jar. Do not pour too much liquid because the insects will be able to crawl out of the jar. Water can be used providing the examination is made within 24 hours.

g. Place a label on the outside of the jar to identify the sample tested. The label originally put in/on the bag of the sample can be re-used. This label can be put on or underneath the jar, held in place by the weight of the jar, or by an elastic or adhesive tape.

h. Insert the funnel, in an upright position, gently into the mouth of the jar.

i. Transfer the particles of the sample that have fallen through the funnel (while performing Section 4.1.2.e), from the paper to the funnel.

j. Place the jar and funnel loaded with the sample below the 60 watt light bulb fitted in the reflector.

k. Turn the power on immediately or set the timer to turn the power on later, as convenient.

l. Keep the funnel under the light for 6 hours*. The organisms in the sample will try to move away from the heat and light and will drop into the jar.

m. Identify the organisms in the jar as specified in Section 4.3.

* The CFIA does not approve of shorter periods (e.g., 4 hours) because they would unnecessarily compromise (decrease) the efficiency of the method, especially since the CFIA recommends a 60 watt light bulb, even though shorter periods will allow more tests to be run per single work shift (each of approximately 7.5 hours). The CFIA, based on practical experience, recommends that 60 watt light bulbs and 1 kg samples (instead of 100 watt light bulbs and 300g samples as in Smith, 2.21) be used to ensure that samples do not burn and that less mobile small larvae are allowed more time to escape from the hot zone in the sample by reducing their mortality from desiccation. **The 6 hours period is already a compromise between the 16 hours used in Smith (1977) (2.21) and the regular 7.5 hours single work shift.** This compromise is deemed acceptable since previous inspection reports indicate that the presence of most species of organisms (including internal feeders) is detected using a 6 hours period. AAFC's Cereal Research Center also states that a minimum of 6 hours is required (2.22). Smith (1977) (2.21) took 16 hours (with a 100 watt light bulb and samples of 300g each) to virtually remove from samples all adults and large larvae of *Cryptolestes ferrugineus*, a species that feeds inside grain kernels.

4.2 Detecting and Identifying Bacteria, Fungi or Small Organisms other than Insects and Mites

Inspectors shall submit the samples (50-200g each) to be examined, preferably untreated, directly to the Canadian Food Inspection Agency (CFIA) laboratory (CPQP), 3851 Fallowfield Rd., Nepean, Ontario, K2H 8P9. The samples shall be individually enclosed in plastic bags, with additional protection against damage to the bag, and properly labeled regarding its origin. The CPQP will provide detection and identification results to the inspectors upon analysis.

4.3 Identifying Insects and Mites

4.3.1 Equipment Required

a. Stereo-microscope with a resolution of about 300x and suitable lamp (preferably a fibre-optic light source).

b. Watch glasses and/or petri dishes.

c. Needles, fine brushes or scalpels to handle insects.

d. A heat source (e.g., stove or plate) to kill larvae.

e. Pyrex beakers to kill larvae on the heat source.

f. Covered garbage container to dispose of analyzed samples.

g. Alcohol (i.e., 70% ethyl, isopropyl or methyl) to kill and preserve specimens.

h. Identification keys.

i. Preserved insect specimens or reference insect collection.

j. Vials (1, 2 and 3 drams) to preserve specimens.

k. An illuminated magnifier (optional).

l. Black and white paper of about 23 cm x 23 cm to facilitate identification (optional).

m. Plastic wash bottles to contain alcohol or water (to kill and to prevent insects from drying under the microscope).

4.3.2 Procedures

a. For specimens detected using the Berlese funnel method, place the jar with liquid on a white paper. Look down through the jar, identify species, and count the number of specimens per species. Use black paper for the larvae of insects and mites.

b. For specimens visually detected without the Berlese funnel method, or isolated after using the Berlese funnel method, do as follows.

c. Immobilize the specimen if identification cannot be done without immobilization. For most specimens, immobilization can be done by placing specimens in a glass or petri dish and pouring alcohol in the dish. For adult moths, kill the specimens by using a killing bottle as soon as possible after detection, and place them in a petri dish; this will limit the handling of the moths which would damage their scales and make them difficult to identify. Larvae should preferably be placed in a beaker of water, heated to boiling point (to the start of bubbling) for one minute, and removed from heat before they are placed in the dish; the larvae will swell slightly and their distinguishing features will stand out.

d. Place the petri dish, containing the specimens, under the microscope, if necessary.

e. Place the plate on black or white background and examine it under a stereo-microscope, if necessary. To count specimens of different species move the petri plate with fingers in a circular motion while examining under the microscope. If necessary, isolate specimens, using a needle, fine brush, a scalpel or another tool, and place them in separate petri dishes to facilitate examination.

f. Identify the specimens using keys in reference material. The CFIA recommends the following reference material: Aitken (1963) (2.11), Bousquet (1990) (2.12), Gorham (1991) (2.13), Hinton (1956) (2.14), Hodges (1974) (2.15), and Stehr (1987a and b) (2.16 and 2.17). The CGC and the CFIA will provide their employees with identification keys to identify the most common insect pests associated to grains and field crops, for example, the standards R-002 (2.18) and R-003 (2.19). For training on identification, inspectors should contact their supervisors. The manager of facilities may contact the CGC or the CFIA to discuss training for their employees.

g. Record the findings in writing. Larvae should preferably be identified to the species, but if impossible, they shall be identified to the Order level at the minimum*.

* If the identification cannot be done or if the specimen found is uncommon, send the specimen to the Centre of Expertise for Plant Quarantine Pests (CPQP) to: D. Parker or B. Gill, Entomology, CPQP, 4th floor, Neatby Building, Central Experimental Farm, Ottawa, Ontario, K1A 0C6. In case of emergency, indicate "rush" on the request for identification sent with the specimens to the CPQP. The CPQP will provide inspectors with the identification results.

APPENDIX A. Berlese Funnel Device Assembly

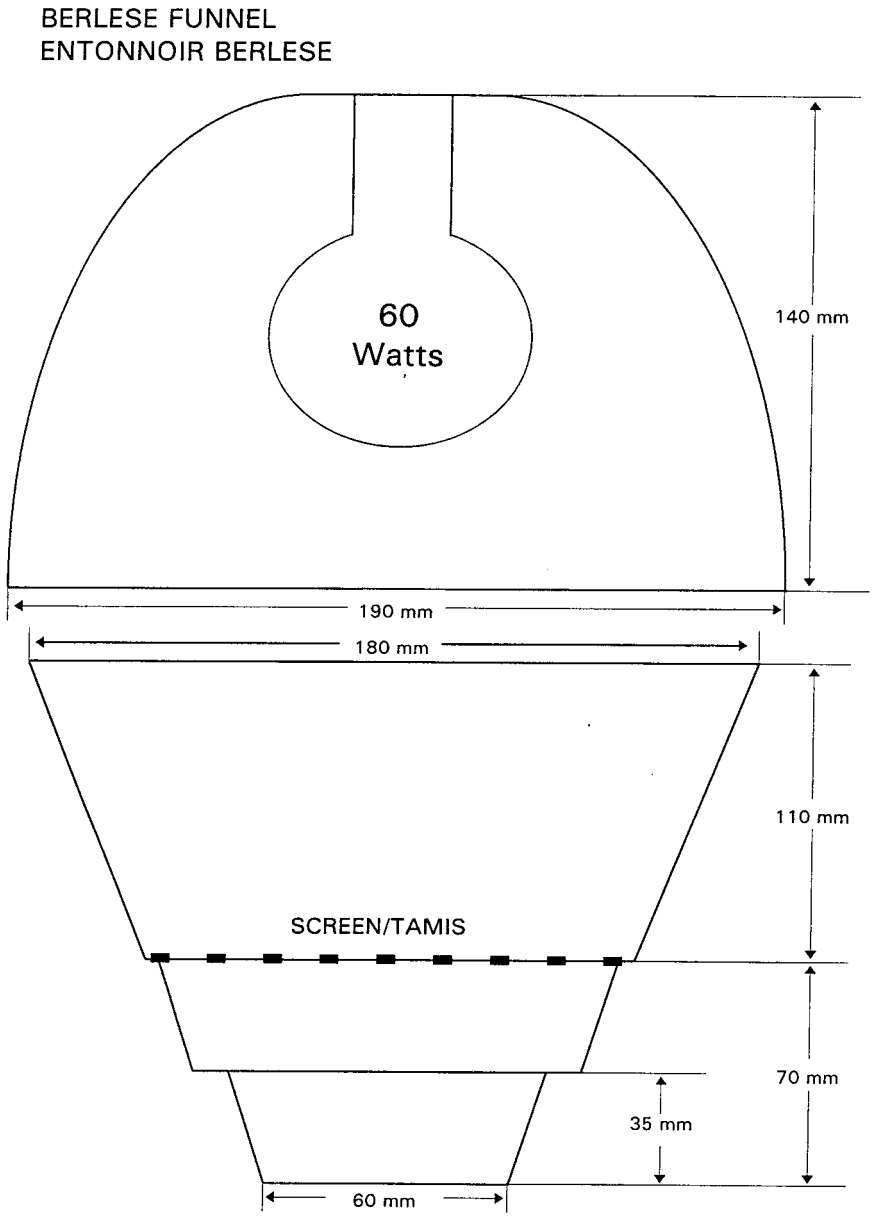

Figure 1. Berlese funnel device assembly. Provided by the CGC, April 1998.

Figure 2. Berlese funnel bank (multiple, not to scale). Provided by the CGC, April 1998. Lamp holders are *Leviton Phenolic Shell* No. 95080. Banks may be configured with 6, 12 or 24 lamps and Berlese funnels.

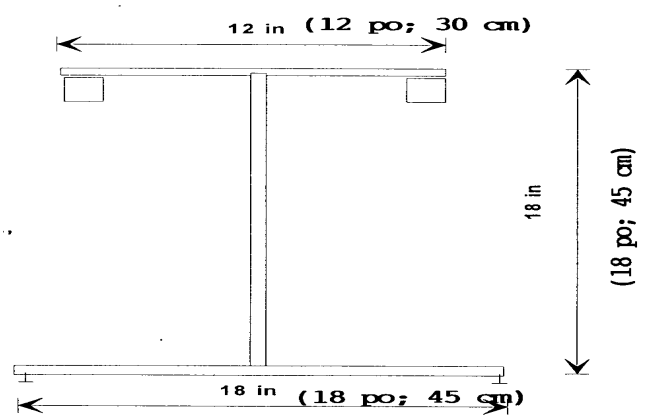

Figure 3. Berlese funnel bank (double, not to scale). Provided by the CGC, April 1998.