Second Instar (Overwintering) Larvae

Three methods are used to evaluate overwintering spruce budworm populations: (1) Soak out procedure, which involves washing foliage with hot sodium hydroxide (Miller and McDougall, 1968; Miller *et al*, 1971; Sanders 1980), (2) Forced emergence--paper cone method (Sanders, 1980), and (3) Forced emergence--enclosed box method (Miller, 1958). Because mean-variance relationships for determining numbers of sample branches are similar for each of the three methods described, a table giving required number of branch samples based on numbers of larvae found is included at the end of this section of overwintering larval survey techniques (Table 8). Likewise, a table predicting infestation level and expected percent defoliation (Table 9) follows the descriptions of the methods.

Objectives: 1.Provide a more reliable and more economical estimate of expected population levels than egg mass sampling because the sample is obtained after the effects of egg parasitism, adult dispersal, and initial larval dispersal have taken place.

2.Check results of egg mass surveys.

3.Assess overwintering mortality.

4.Gather data on tree damage expectations in areas where egg mass samples were not collected.

Time of Year: Samples may be collected from August through April, as long as young larvae are not active. In Canada, foliage collected during this period is processed using the soak-out method. Less commonly, foliage collected in March and April may be treated by bringing the branches indoors to warm up (forcing methods). Counts of the emerging larvae are made.

METHOD 1: Soak-out Procedure

Equipment Needed: 1. **Field:** Extension pole pruners, tape measurer, heavy paper sacks to hold branch samples, hand pruners, marking pen.

2. **Lab:** Refrigerator or cool storage space (<50°F), data sheets

a. **For washing foliage:** 10-liter plastic pails, 400 ml collecting jars, 2 sets of tags that indicate plot, tree, and branch number (so that washing pail and collecting jar can be tagged), sodium hydroxide (90 g per pail), plenty of hot water (50° C).

b. **For processing the foliage:** large sink or 90 x 150 x 9 cm deep tray, preferably with a corrugated bottom, fitted to a sewer outlet, two interlocking sieves (soil sieves)--top sieve should be about 0.8 mm mesh, bottom sieve 0.25 mm mesh, wire basket made of hardware cloth, 5 mm mesh, with a "false" bottom (wire basket should fit inside a 40 cm x 20 cm tub or container).

c. **For separating larvae from debris:** 500 ml separating funnel, hexane.

d. **For vacuum filtering:** 15 cm Buchner funnel, 5000 ml filtering flask, Filtervac (rubber diaphragm), filter pump, gridded filter paper, microscope.

Procedure [from Miller and McDougall, (1968) and Miller *et al*, (1971) as presented by Sanders, (1980)]:

1. **Foliage collections:** For extensive surveys to establish infestation classes, a single, mid-crown sample is collected from each of 4 dominant or codominant trees (5 in Quebec), or, for intensive studies, a branch may be taken from each of four crown zones on sample trees. In New Brunswick, operational sampling is conducted in natural mature stands, plantations, thinnings, and seed orchards. The standard procedure is to sample a mid-crown branch from each of 3 trees of one species. Inidividual trees selected for sampling are dominant or co-dominant. The species selected is the predominant species in the stand, whether it be balsam fir, red/black spruce (they hybridize in New Brunswick) or white spruce. Where fir and spruce are both abundant, fir is selected as the indicator species (L. Hartling, pers. comm.). Though whole-branch samples are taken by workers in some regions, others standardize sampling units

as 75-cm branch tips (eg., New Brunswick), and have found that population counts per 75-cm branch tip correlate very well to counts expressed per 10 sq. m of foliage. Samples may be collected in late fall, throughout the winter, and in the early spring (prior to 75 GDD). However, samples collected in early fall maximize recovery of larvae (Allen *et al*, 1984).

Branches (L x W) are measured (see options for determining area of foliage under egg survey section, page 6), clipped into small pieces, and put into paper bags. Bags are labelled with plot, tree, and branch number. Branch measurements should be written on bag labels for later association with branch samples. If foliage must be stored before washing, it should be kept at 0° C. In Quebec, whole-branch samples are brought to the laboratory, where clipping and foliage evaluations (defoliation, length and width measurements) are made.

2. **Lab procedure:**

a. **Washing the samples:** The samples to be washed on one day are left in a warm room overnight to thaw. Make two sets of tags showing plot, tree, and branch number, and tag washing pail and collecting jar. Transfer branch measurements to data recording sheet. Add 90 g of sodium hydroxide per pail and fill to the 9 liter mark with hot water (50°C) to make a 1% solution. Put foliage in pail, usually one branch per pail. Do not pack tightly, and keep foliage completely submerged with a weighted screen top. The foliage should remain in the pails for 5 hours if stirred manually. If automatic agitation or stirring of all pails is done, the foliage washing process can be reduced to 2 hours (pers. comm. with Auger, Direction de la conservation des forets, Quebec). Trial (1985) found that constant agitation with a vibrating box improved extractions.

b. **Processing the foliage:** Pour the liquid contents of the pail through the two sieves. Place the wire basket in the tub. Pour the remaining contents of the pail into the wire basket. Rinse the pail and pour the rinse through the sieves. With a flexible hose, wash the foliage thoroughly in the wire basket inside the tub and discard. Pour the contents of the tub through the sieves, being careful not to overflow the sieves. Rinse the tub and pour through the sieves. Wash the contents of the coarse sieve into the fine sieve. Wash

the contents of the fine sieve into the tagged collecting jar.

Note: Foliage my yield a good deal of debris, and there is a danger of plugging the fine sieve, overflowing both sieves, and losing larvae. This has to be checked carefully and it may be necessary to transfer the debris from a "dirty" sample to the collecting jar on a step by step basis.

c. **Separating Larvae from Debris:** Pour the contents of the collecting jar into the separating funnel. Rinse jar to remove larvae that may stick to the sides. Add hexane to the separating funnel to create a 3 mm layer on top of the aqueous solution. Shake the water-hexane mixture vigorously to obtain a thorough mixing and allow 5 minutes to settle. About 70 to 90 percent of the plant debris settles to the bottom of the funnel and 99 percent of the larvae collect at the oil-water interface. Draw off the plant debris that has settled at the bottom of the funnel and discard. Draw off the oil-water fraction into 400 ml beakers to be vacuum filtered. Too much plant debris hinders the separation process; therefore only 100 ml of debris should be processed at one time.

Note: In both New Brunswick and Quebec, hexane is added to the bottom of the separating funnel. When this is done, shaking of the funnel is not necessary, and there is no delay in drawing off the plant debris.

d. **Vacuum Filtering:** Fit the Buchner funnel to the 500 ml filtering flask using a molded rubber diaphragm (Filtervac) and connect the filter pump. Water may also be used to create a vacumn. Pour debris onto the wetted filter paper. It is important not to overload the filter paper because small larvae could be missed when one is checking under the microscope. Addition of methyl blue helps in detecting the larvae. It is also helpful to have the filter paper gridded to a size that is visible under the microscope. The "washed" budworm larvae have black head capsules and very light bodies. Note: Careless handling of foliage samples can result in large counting errors with this technique.

In New Brunswick, the NaOH method is used to process foliage from approximately 950 to 1,600 plots during the initial forecast survey conducted each year from August to October. An additional 300 to 1,500 samples are processed during supplementary sampling in the winter months in order to fine-tune the initial forecast. The

most significant departures from the procedure described above are (1) seives with 850 and 212 micron openings are used, but wire baskets are not used; (2) water temperature is 60°C; (3)soaking time in 1% sodium hydroxide solution is only 2 hours; and (4) a double-rinsing proceudre is used which greatly enhances the estraction rates of larvae. A copy of the laboratory procedure used in New Brunswick for extracting overwintering spruce budworm larvae from foliage is included as an appendix. (Part 3: Appendix 1).

Data Sheets: Data sheets are provided on page 41.

Interpretation: Counts are related to branch size and expressed as larvae/100 ft^2 of foliage (Table 9 at end of L2 section).

Comments: 1.Information recieved from New Brunswick (L. Hartling, pers. comm., indicates that the cost to conduct an operational L2 survey is 70% that of an egg mass survey. (See Part 3: Appendix 2). According to Miller *et al* (1971), cost is 1/3 to 1/2 that of egg mass surveys when examined on a per plot basis, but the cost of washing larvae is higher than that of beating for large larvae (see subsequent survey methods).

2.Examination time for the method described here includes 5 hours of soaking with manual stirring and 30 minutes per branch for preparation and examination. With agitation, soaking time can be reduced to 2 hours.

3.Overwintering larval survey can be conducted over a 7 month period rather than in the month or a few weeks available during most life stages.

4.Counting overwintering larvae eliminates the problem of distinguishing old and new egg masses.

5.Spruce can be evaluated as easily as fir with the soak-out procedure. By contrast, evaluating populations on spruce by egg-mass sampling is difficult (Trial, 1985).

6.The liquid residual from the soak-out process is hazordous waste in most states. The pH of the residue is likely to be 13 or so, and liquid usually has to be neutralized prior to disposal.

METHOD 2. Forcing "Enclosed Box Method" [from Miller (1958) as presented by Sanders (1980)].

Equipment Needed: 1. **Field:** Extension pole pruners, tape measurer, heavy paper sacks to hold branch samples, hand pruners, marking pen.

2. **Lab:** Emergence cage which may consist of any sealed container with a transparent collecting vial attached, light source, masking tape, data sheets.

Procedure: 1. **Foliage Collections:** See foliage collections, pg. 31.

2. **Lab Procedure:** Diapause must be satisfied before larvae will emerge from hibernaculae. This can be achieved by chilling clipped branches in the laboratory, or by delaying sampling until diapause is completed in the field (early March). The emergence cages are loosely filled with the foliage samples and the covers are sealed with masking tape. They are then placed on their sides in a holding rack with the vials uppermost, and are pointed toward a bank of lights that serve as a light source. The lights are in operation approximately 10 hours per day. The larvae, attracted into the vials by the light, are collected and counted periodically during emergence period.

Note: A modification of this method has been used in Quebec (see Montgomery *et al*, 1982) to check pre-spray budworm population in April. It involves placing the sample unit (1 45 cm branch tip) in a small polystyrene ice bucket with the top closed. The bucket is painted black on the outside to reduce light transmission, and a transparent plastic vial is fitted into the bottom of the bucket at the center. All buckets are placed horizontally on a wire fence with the vials facing the light. (The buckets are protected from direct sunlight, however.) Emerging larvae are attracted to the light, enter the vial, and are counted daily with a minimum of manipulation. About 50% leave the bucket within a few days. The branch is kept in the bucket for a total of 10 days, after which it is examined visually. At that time, the larvae have grown to third or fourth instar and are easily recovered. Alternatively, the branches can be left in the boxes until the larvae have developed into the third (and fourth) instars, when they can be removed by beating the branches in a drum as in large larval sampling.

Interpretation: Table 9 is used to interpret counts of overwintering larvae.

Data Sheets: An example of a data sheet is reproduced on page 41.

Comments: 1. Processing time is not a serious factor with this technique since it requires only a few minutes to set up each sample and a few minutes to check each day for emerged larvae. The provision of suitable space and lighting is a consideration since the cages have to be in place for several weeks.

2. Forcing is not practical until larval diapause requirements have been satisfied.

3. The emergence period of the larvae during forcing is related to the length of the cold period spent in hibernaculae. The longer the cold period, the more rapid the emergence period. Since all these techniques require considerable space, the time required for emergence may be a serious consideration.

4. Forcing method is not practical for predictive surveys, but is more likely to be used in control project population work.

METHOD 3. Forcing "Paper Cone Method" (from Sanders (1980). This technique was first devised in the 1950s at the Forest Insect Laboratory in Sault Ste. Marie.

Equipment Needed: 1. **Field:** Extension pole pruners, tape measurer, heavy paper sacks to hold branch samples, marking pen.

2.**Lab:** Paper toweling or brown paper to wrap whole-branch samples, string, strong light source, tanglefoot, data sheets.

Procedure: 1.**Foliage Collections:** See foliage collections, pg. 31.
Samples are taken after diapause has been satisfied. Branches (L x W) are measured (see options for determining area of foliage under egg survey section, page 6), and put into paper bags. Bags are labelled with plot, tree, and branch number. Branch measurements should be written on bag labels for later association with budworm counts.

2.**Lab Procedure:** Branches are suspended, wrapped in paper toweling, brown wrapping paper, or paper bags to make a cone-shaped covering with the open apex at the butt end of the branches. The wrapped sample is them suspended under a strong light. The larvae are collected as they crawl on the paper; those that drop are caught on a sheet of paper placed beneath the sample ringed with tanglefoot. The samples are sprayed periodically with water during the emergence period.

Interpretation: See Table 9 to determine relationship between number of overwintering spruce budworm larvae per branch and expected infestation level. Allen *et al* (1984) comment that the relationship between overwintering population density and expected infestation levels may vary between regions and that this association is probably influenced by age of infestation, stand characteristics, and user bias.

Data Sheets: An example of a data sheet is reproduced on page 41.

Comments: 1.Examination time is similar to that required for the enclosed box method. However, because tanglefoot is used, this method is somewhat messier than the enclosed box method.

2.This method requires slightly more time for examination, and more space than the enclosed box method.

3.Like the enclosed box method, diapause must be satisfied.

4. Forcing method is not practical for predictive surveys, but is more likely to be used in control project population work.

Table 8. Tentative estimates of variance-mean relationships for overwintering second-instar larvae on mid-crown branches of balsam fir, and required sample size for 20% precision. [Data in this table were prepared by Miller (unpub.) and presented by Sanders (1980)].

Mean	Variance	Required Number of Branches
2	2.3	18
4	5.4	8
6	10.5	7
8	17.1	7
10	25.0	6
>10		5

Table 9. Relationships between number of overwintering spruce budworm larvae per branch and expected infestation level (from Dorais and Kettela, 1982).

Geographic Region	Number of Larvae per Whole Branch	Number of Larvae per 100 ft^2 (9.3 m^2) of foliage	Forested Infestation
Maritimes	1 to 6 7 to 21 21 to 40 >40		Low Medium High Extreme
Ontario	1 to 25 26 to 65 >66		Low Medium High
Newfoundland		1 to 100 101 to 300 301 to 650 >651	Low Medium High Extreme
Maine, Quebec		0 to 175 176 to 500 502 to 1,100 >1,100	Low Medium High Extreme

SPRUCE BUDWORM SURVEY MAP AREA _____

FIR SPRUCE POINT NO._____

Collectors:_____
_____

Date: _____ Year: _____ Town: _____-
_____

Location:_____
_____

Stage: Early larval___ Late larval___ Pupal___ Egg___Overwintering larval___
Adult_____

PRE-SPRAY_____ POST-SPRAY _____ DEVELOPMENT_____
PARASITE_____
No. Units Searched _____ Total No. of Egg Masses or Larvae_____

EGG MASS OR OVERWINTERING LARVAL SURVEY

Branch #	Length	Width	Sq. Ft.	#Larvae or Egg Masses	#/Sq. Foot	#/100 Sq. Ft.

Egg Mass No. Old _____ Par._____ D.O.C. _____