Recommendations for sampling and extracting the eggs of the western hemlock looper, *Lambdina fiscellaria lugubrosa*, (Lepidoptera: Geometridae)

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ABSTRACT

No significant differences were found in the numbers of both new and old western hemlock looper eggs per 100 grams of lichen between three crown levels. Representative samples may be collected from lower crown levels with pole pruners, rather than from upper crown levels, which usually involves tree felling.

Hot water is more efficient than 2% chlorine bleach for extracting the eggs from the lichen on which they are laid. However, the bleach method is non-destructive and allows the eggs to be reared in order to assess parasitism and fertility. These characteristics can also be identified through egg color using the hot water method, but the parasitoid species cannot be identified. It is recommended that the hot water method be used for forecasting population trends and the bleach method for specific information about parasitoids.

RÉSUMÉ

Aucune différence notable n'a été trouvée entre le nombre d'oeufs d'arpenteuses présents, par 100 grammes de lichen, sur les spécimens jeunes et sur les spécimens âgés de pruches de l'Ouest, les échantillons ayant été prélevés à trois niveaux de la cime. Pour éviter l'abattage, l'échantillonnage se fait non pas au faîte mais au bas de la cime, au moyen d'un échenilloir-élagueur.

Pour extraire les oeufs de leur support de lichen, l'eau chaude donne de meilleurs résultats que l'emploi d'une solution chlorée à 2%. Mais la méthode de la solution chlorée est non destructive et permet de maintenir en vie les oeufs pour en dégager les caractéristiques parasitologiques et la fertilité. Précisons toutefois que si la méthode de l'eau chaude permet de caractériser les parasitoïdes par l'observation de la coloration des oeufs, elle ne permet pas d'en identifier les espèces. On recommande donc la méthode de l'eau chaude pour étudier les tendances générales des populations de parasitoïdes et celle de la solution chlorée pour dégager des données spécifiques sur celles-ci.

INTRODUCTION

The western hemlock looper (WHL), *Lambdina fiscellaria lugobrosa* (Hulst) (Lepidoptera: Geometridae), is periodically a destructive defoliator of western hemlock, *Tsuga heterophylla* (Raf.) Sarg., and to a lesser extent other associated coniferous tree species (Harris *et al.* 1982). A reliable population index is required to predict an approaching epidemic of western hemlock looper. Estimates of frass quantity (Thomson 1949), and numbers of eggs (Kinghorn 1952; Thomson 1958; Carolin *et al.* 1964), larvae (Harris *et al.* 1982) and pupae (Shore 1989), have all been examined as population indicators. The egg is the preferred stage for sampling WHL because it is the overwintering stage, and is relatively stable in numbers, position and time through the fall and winter months.

Most of the early research on the WHL was done in coastal forests (e.g., Hopping 1934; Richmond 1947; Wyatt 1946; Thomson 1949, 1957; Kinghorn 1954; Carolin *et al.* 1964), but since the 1950s most infestations in British Columbia have occurred in interior forests (Harris *et al.* 1982). This change in outbreak location is probably due to a reduction in area of mature western hemlock forest on the coast because of a longer history of logging. On the coast it was found that the WHL laid most ot its eggs in moss on tree trunks, limbs, logs and on the forest floor (Hopping 1934; Carolin *et al.* 1964). However, in interior forests the preferred oviposition site is on lichens (*Alectoria* spp.) which grow mainly on the branches of trees (Thomson 1958). The Forest Insect and

Disease Survey (FIDS) of Forestry Canada developed a sampling index based on the number of WHL eggs per 100 grams dry weight of these lichens (Shore 1985, FIDS General Instruction Manual, Forestry Canada, unpub.).

A study was conducted comparing the number of WHL eggs per 100 grams of lichen between three crown levels of hemlock trees. If the number of eggs found in lichen from the lower crown is the same, or can be related to the number of eggs in lichen in the mid- and upper crown levels then sampling can be simplified; lichen can usually be collected from the lower crown using pole pruners, whereas sampling from other crown levels often necessitates felling the tree.

Removing insect eggs by hand from the substrate to which they have been attached is extremely time consuming and inefficient (Carolin *et al.* 1964, Condrashoff 1967, Otvos and Bryant 1972). Several methods have been presented for washing eggs from the substrate including NaOH solution (Condrashoff 1967, Shepherd and Gray 1972), hot water (Eidt and Cameron 1970, Gray *et al.* 1973) and chlorine bleach solution (Otvos and Bryant 1972). The hot water method was adapted by FIDS for removing western hemlock looper eggs from the lichen (Shore 1985). However, the chlorine bleach method has the advantage of being non-destructive to live eggs (Otvos and Bryant 1972), unlike the hot water and NaOH methods, and therefore it can be used for biological studies to determine viability and parasitism. An experiment was conducted in which the bleach method was compared with the hot water method for WHL egg extraction from lichen samples.

METHODS AND MATERIALS

Comparison of number of eggs at three tree crown levels

To examine the effect of tree crown level on the number of eggs per 100 g lichen, recently felled western hemlock trees were examined at four locations in B.C.: Kingfisher Creek (10 trees) in the Kamloops Forest Region, Cranberry Creek (4 trees) and Red Rock Harbour (5 trees) in the Nelson Forest Region, and Abbott Creek (7 trees) in the Cariboo Forest Region. Average tree diameter at breast height was 43.0 cm (standard error 11.6). Each tree crown was divided into thirds and lichen samples were collected from each third. The lichen samples were processed using the bleach extraction method and the numbers of eggs found were standardized per 100 g dry weight of lichen. Numbers of healthy (h), parasitized (p), infertile (i), new (h + p + i), and old eggs per 100 g dry weight of lichen were transformed to log10(x + 1) and compared for three crown levels across the four locations by repeated measures analysis of variance (SAS 1985). Old eggs are those from previous years whereas new eggs include healthy, parasitized and infertile eggs from the current year.

Comparison of egg washing methods

Lichen was collected, using pole pruners, from 10 western hemlock trees in each of three locations in the Nelson Forest District of B.C. Trees were a minimum of 25 cm diameter at breast height, and enough lichen was collected to fill a 5 × 10 × 25 cm polyethylene bag. The lichen from each tree was divided approximately in half and air dried in the laboratory. One half of each lichen sample was processed using the hot water egg extraction method and the other half was processed using the chlorine bleach extraction method, both of which are described below. Egg counts were standardized by converting them to per 100 g dry weight of lichen sample. Color, confirmed by other physical characteristics (Thomson 1958), was used to classify the eggs as to type: healthy, infertile, parasitized, or old (Table 1). For the non-destructive chlorine bleach extraction method, a sample of these eggs was reared to confirm the classification. Rearing was conducted on a moistened blotter in a screen vial which was kept at 0°C for two months and then at 20°C until hatching was complete. Based on the rearing results the numbers of healthy and parasitized eggs removed by the bleach method were corrected. The numbers of WHL eggs of each type extracted by the two methods, were compared using a paired t-test. The relationship between the number of healthy eggs extracted by the hot water method and the number extracted by the bleach method was described by linear regression.

Table 1
Color characteristics of western hemlock looper egg types removed from lichen by the bleach or hot water methods

Type of egg	Bleach Method[1]	Hot water method
Healthy	Brown	Bronze
Parasitized	Black	Black
Infertile	Green	Yellow
Old	Opaque	Opaque

1. From Otvos and Bryant (1972).

Hot Water Extraction Method:

Each lichen sample was placed in a 2 l plastic bucket. Water, heated to 100°C, was poured over the sample until the lichen was immersed. The sample was swirled with tongs to shake the eggs free of the lichen. The contents were then poured through nested strainers consisting of a large meshed (1000 micron) top strainer to remove the debris but allow the eggs through, and a close-meshed (250 micron) bottom strainer which retained the eggs. The contents of the top strainer were rewashed to remove eggs which remained attached during the first rinse. The bottom strainer was then inverted in a funnel and the contents rinsed into a glass jar. Finally, the contents of the glass jar were extracted onto filter paper using a vacuum filter. Egg counts were made by examining the filter paper with a dissecting microscope.

Chlorine Bleach Extraction Method:

Lichen samples were teased apart, placed into a 2 l plastic bucket and covered with a solution of 2% chlorine bleach in water. The buckets were mechanically shaken at the lowest setting for 45 minutes. Each sample was then processed as described for the hot water method with the additional step of rinsing the contents of the bottom strainer with tap water for 10 minutes to halt the corrosive action of the bleach.

RESULTS

Comparison of number of eggs at three tree crown levels

There were no significant differences among crown levels across the four locations for either new (h + p + i) or old WHL eggs (Table 2). When the new eggs were analyzed separately by type, no significant differences were found among crown levels for healthy ($P > .69$), parasitized ($P > .67$), or infertile ($P > .24$) eggs. There were no significant interactions between crown levels and location for any of the egg types (new: $P > .89$, old: $> .94$, h: $> .44$, p:$> .47$, i: $> .09$).

Table 2
A comparison of the mean number of western hemlock looper eggs per 100 gram lichen sample from three tree crown levels

Location	Number of trees	Mean number of new eggs[1] (±SEM)[2]			Mean number of old eggs (±SEM)[2]		
		Upper crown	Mid crown	Lower crown	Upper crown	Mid crown	Lower crown
Kingfisher	10	12.9± 5.1	3.6± 1.4	7.1± 3.5	23.7± 6.1	26.6± 6.9	27.7±14.9
Cranberry	4	23.9± 5.3	25.1± 5.1	23.2± 7.0	198.0±32.6	125.4±28.2	111.0±22.2
Red Rock	5	34.5±13.7	28.8± 7.7	49.5± 9.7	184.2±89.0	197.2±21.1	179.5±15.5
Abbott	7	75.5±10.5	61.6± 9.9	46.8± 9.9	49.2±12.2	50.3±10.0	59.1±18.4

1. New eggs includes healthy, infertile and parasitized eggs of the current year.
2. No significant differences among crown levels were found within egg types across locations, Repeated Measures ANOVA on data transformed to $\log_{10}(x+1)$; new: $F = .43$; $P > .65$, old: $F = .25$, $P > .78$.

Table 3
A comparison of the mean number of western hemlock looper eggs per 100 gram lichen sample extracted from lichen by the bleach and hot water methods

	Bleach method		Hot water method	
Type of Egg	Mean[1]	S.E.M.	Mean[1]	S.E.M.
Healthy	74.0	12.7	122.6	17.3
Parasitized	28.1	2.6	44.6	6.1
Infertile	29.8	4.2	53.9	6.5
Old[2]	66.8	7.8	118.7	16.1
New[2]	131.9	18.4	221.1	26.5
Total	198.8	25.2	339.8	41.0

1. Mean numbers of all types of eggs were significantly greater for the hot water method than the bleach method, paired t-test, $P < 0.01$, $n = 30$.
2. Old eggs were those from previous years, new eggs included healthy, parasitized and infertile eggs from the current year.

Comparison of egg extraction methods

There was no significant difference between the weights of the lichen sample halves assigned to the bleach or hot water treatment (paired t-test, $t = 1.17$, $n = 30$).

Significantly more of all types of WHL eggs (healthy, parasitized, infertile, old, new and total) were removed from the lichen substrate by the hot water method than by the bleach method (Table 3).

The number of healthy eggs removed by the hot water method was regressed on the number removed by the bleach method to provide an equation for converting egg numbers derived from one extraction method to the other. A linear model was used and three points were identified as outliers (Freund and Littell 1986) and removed from the regression. As the intercept was not significantly different from zero ($p > 0.2$) the regression was forced through the origin, producing the following relationship ($R^2 = 0.89$; s.e. slope 0.01):

$$\text{No. healthy eggs (hot water)} = 1.473 \times \text{No. healthy eggs (bleach)}$$

The difference in color between healthy and parasitized eggs removed by the bleach method is not so distinctive as for the hot water method. Of 180 "healthy" eggs reared, parasitoids emerged from 26 indicating that 14.4% of the "healthy" eggs were misclassified. Misclassification cannot be quantified for the destructive hot water method; however, because the color of the healthy egg type is more distinctive it is assumed that misclassification is minimal. Support for this assumption can be found by comparing the percentage of total eggs in each egg type extracted by the two methods. Initially, healthy eggs represented a higher percentage and parasitized eggs represented a lower percentage of total eggs for the bleach method than for the hot water method (Fig. 1). However, when the numbers of healthy and parasitized eggs extracted by bleach were corrected for the 14.4% misclassification found in the rearing study, all egg types represented similar percentages of the total number of eggs for both extraction methods (Fig. 1).

DISCUSSION

The finding that there were no significant differences between tree crown levels for number of WHL eggs per 100 g lichen should simplify egg sampling procedures for this insect species. In the past, trees frequently have been felled to obtain egg samples for WHL from the upper part of the crown. Lower crown samples, which can usually be collected with pole pruners, should provide reliable estimates of WHL egg density.

The hot water method was more efficient at removing WHL eggs from the lichen than was the bleach method. As a result, when the insect is at low population densities, egg counts obtained by the hot water method should be more sensitive than those obtained by the bleach method. Also, the colors of the egg classes (h, p, i) were more distinctive with

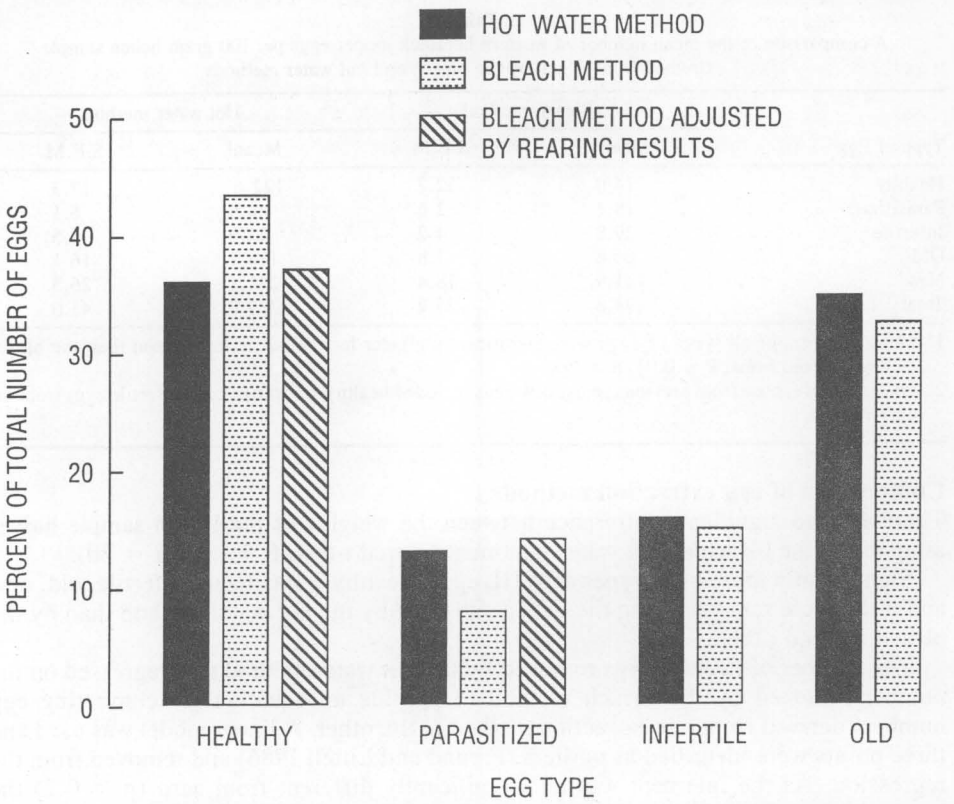

Fig. 1. A comparison of the percentage of total western hemlock looper eggs that were healthy, parasitized, infertile, or old, extracted by the bleach or hot water methods from lichen substrate.

hot water than with bleach and it seemed that misclassification was lower. For these reasons, it is recommended that the hot water method be used when the objective is relative population density estimation for damage prediction.

If the objective is to evaluate WHL egg mortality attributable to parasitism, and species specific information about parasitoids is required, the bleach method should be used. This method is non-destructive and therefore permits the user to rear the eggs to confirm classification and to identify parasite species.

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A review of mosquito collecting in the Yukon

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The first formal record of a mosquito collected in the Yukon was in 1904 when J. Keele caught *Anopheles occidentalis* in the Mayo River valley (Dyar 1921). In 1916 three females of *Aedes nearcticus* were collected on Herschel Island, off the north coast of the Yukon, by Frits Johansen of the Canadian Arctic Expedition (Dyar 1919). These species, now known as *An. earlei* and *Ae. impiger* respectively, were identified at the time by Dr. Harrison Dyar at the United States National Museum in Washington.

Dyar, himself, visited the Yukon in June and July of 1919. He travelled from Carcross in the south, along the Yukon valley to Dawson which is less than half way to Herschel Island. He recorded 16 species (Dyar 1920, 1921) including nearly 2,000 specimens of *Ae. cataphylla* which he found to be the dominant species with *Ae. campestris, Ae. communis* and *Ae. punctor* also common (Table 1). He described three new species from his Yukon material: *Ae. nearcticus* from Herschel Island, the Northwest Territories and Alaska (Dyar 1919); *Ae. callithotrys* from Whitehorse and Takheena River in the Yukon and from Alaska; and *Ae. mercurator* from 65 specimens collected around Dawson (Dyar 1920). He later synonymised *Ae. callithotrys* with *Ae. campestris* (Dyar 1928).