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Report Title: Development of Tools for Integrated Pest Management of Eastern Hemlock Looper (Lambdina fiscellaria fiscellaria (Gn.)): Assessment & Refinement of Forecasting Methods for Predicting Populations & Defoliation

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Development of Tools for Integrated Pest Management of Eastern Hemlock Looper (*Lambdina fiscellaria fiscellaria* (Gn.)): Assessment & Refinement of Forecasting Methods for Predicting Populations & Defoliation

Final Report – March 28, 2002

by

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FOREWORD

Data from New Brunswick's pheromone trapping network and extensive egg sampling in 2000 suggested hemlock looper populations were increasing. Also significant were increases in moth activity noted in other eastern Provinces and Maine. Forecasts for 2001 predicted some pockets of defoliation were expected within a gross area of approximately 525 000 ha in southwestern New Brunswick. Aerial surveys in 2001 mapped 760 ha of detectable defoliation. At the time this project was initiated it was unclear whether the Province was facing the start of a new looper outbreak or simply a temporary spike in populations. If the former, there would be need to consider future controls. However, more recent DNRE operational surveys with pheromone traps and egg sampling from the autumn of 2001 have suggested hemlock looper populations in New Brunswick might merely be experiencing a temporary spike in populations rather than the commencement of a new outbreak.

During the outbreak of 1989-1993, control programs were aimed at treating the maximum-infested area. There was fruitful but somewhat limited opportunity to generate local data on population dynamics and test the relevance of forecast tables used in other jurisdictions. Since proper survey forecasting methods are an essential cornerstone of good Integrated Pest Management and overall Best Management Practices, it is essential to fill knowledge gaps. Current circumstances provided the opportunity to investigate and improve existing biological relationships and survey methods to better monitor and forecast hemlock looper outbreaks, and to aid in the decision-making process when contemplating controls.

EXECUTIVE SUMMARY

Hemlock looper (*Lambdina fiscellaria fiscellaria* (Gn.)) populations did not reach large outbreak levels, hence conclusions from this study pertain to the conditions encountered. However, the study did allow the opportunity to help refine existing methods for forecasting and monitoring, essential cornerstones of good Integrated Pest Management and overall Best Management Practices. Furthermore, this report integrated data from this study and previous DNRE studies, and compared these findings with that of other jurisdictions. Such information will be invaluable if future foliage protection programs become necessary.

The study was able to build on relationships between pheromone trap catch and egg density, egg and early instar larval counts, egg counts and defoliation, and larval numbers and defoliation. Evaluation of pheromone trap survey data showed that pheromone (10-μg strength lure) trap catches of <1200 moths/trap are unlikely to represent threatening populations because associated egg densities never exceeded 15 eggs/100-cm lower-crown branch. Furthermore, counts up to ~25 eggs/100-cm lower-crown branch seldom caused >10% loss of current-year foliage, and seldom exceeded 25% loss of the 3 older age classes of needles.

Most of the egg parasitism in not accounted for when sampling in the fall and winter months. Significant increases in egg parasitism were caused by spring-attacking parasitoids. Any population forecast derived solely from sampling in the fall and winter months could underestimate egg parasitism, and thus have the potential to overestimate the forecast. When planning control plans it is probably prudent to include spring egg surveys to see if treatment areas can be excluded.

A trial indicated that pheromone traps might be placed out 1 - 2 weeks earlier than 'normal', thus giving some logistic flexibility to surveys at that time. Furthermore, it may be possible to retrieve traps one or two weeks earlier, a significant advantage in areas of higher elevation where snowfall comes early in the season. Data should be collected over several years to strengthen these observations.

Degree-day relationships were further developed to aid in forecasting egg hatch and larval development, both essential to 'timing' and assessing control operations.

While not intended as a major component of this project, opportunities arose to evaluate methodology for sampling eggs and larvae, and assessing feeding damage of the current and older age classes of foliage. Likewise, techniques for measuring larval populations (e.g. branch sizes and location within trees), and assessing defoliation of current-year and previous-years' foliage on balsam fir were examined.

Results from this project are expected to be pertinent to various agencies, landowners and other forest management groups dealing with hemlock looper outbreaks in such jurisdictions as Nova Scotia, Québec, Ontario, Newfoundland and the State of Maine.

SOMMAIRE

Les populations d'arpenteuses de la pruche (*Lambdina fiscellaria fiscellaria* (Gn.)) n'ont pas atteint de grands niveaux de prolifération; les conclusions de la présente étude portent donc sur les conditions observées. Toutefois, l'étude a contribué à améliorer les méthodes actuelles de dépistage et de surveillance, qui constituent les fondements d'une bonne lutte antiparasitaire intégrée et de pratiques de gestion optimales. Le présent rapport fournit également des données provenant de l'étude actuelle et d'études précédentes effectuées par le MRNE, et il compare ces résultats avec ceux d'autres régions. Ces renseignements seront très utiles pour élaborer de futurs programmes de protection du feuillage le cas échéant.

L'étude a permis d'établir un rapport entre les prises avec des pièges à phéromone et la densité des œufs, entre le nombre d'œufs et le stade larvaire précoce, entre le nombre d'œufs et la défoliation, et entre le nombre de larves et la défoliation. L'évaluation des données relatives aux prises dans les pièges à phéromone (renfermant 10 μg de phéromone) indique que les prises enregistrées, qui s'établissaient à moins de 1 200 papillons par piège, sont peu susceptibles de représenter des populations menaçantes puisque la densité des œufs correspondante n'a jamais dépassé 15 oeufs/100 cm sur les branches du bas. De plus, les nombres ne dépassant pas 25 œufs/100 cm ont rarement causé plus de 10 % de perte sur le feuillage de l'année courante, et rarement plus de 25 % de perte dans les aiguilles de trois classes d'âge annuelles subséquentes.

La plupart du temps, le parasitisme des œufs n'est pas prise en considération quand l'échantillonnage a lieu durant les mois d'automne et d'hiver. La grande augmentation dans le parasitisme des œufs est causée par les parasitoïdes, qui sont actifs au printemps. Toute prévision de population qui découle exclusivement d'un échantillonnage à l'automne et à l'hiver pourrait sous-estimer le parasitisme des œufs, et l'on risquerait ainsi de surestimer la prévision. Dans l'établissement de plans de lutte, il serait probablement sage d'inclure le nombre d'œufs relevé au printemps pour déterminer s'il est possible d'exclure des zones du traitement.

Un essai a indiqué que les pièges à phéromone pourraient être placés une à deux semaines plus tôt que la « période normale », ce qui donnerait une certaine souplesse logistique pour les relevés effectués à cette période. De plus, on pourrait peut-être enlever les pièges une ou deux semaines plus tôt, ce qui constituerait un avantage important dans les zones élevées où les précipitations de neige sont précoces. Les données devraient être recueillies pendant plusieurs années pour confirmer ces observations.

On a davantage tenu compte des rapports avec les degrés-jours pour prévoir l'éclosion des œufs et le développement des larves, deux facteurs essentiels pour établir la bonne période et les mesures efficaces pour la lutte antiparasitaire.

On a pu évaluer des méthodes pour échantillonner les œufs et les larves et pour estimer les dégâts causés par l'alimentation des insectes sur le feuillage récent et celui faisant partie de classes d'âge subséquentes, même si cet aspect ne constituait pas un élément important du projet. On a aussi examiné des techniques pour déterminer les populations de larves (comme la grosseur des branches et leur emplacement dans l'arbre) et pour évaluer la défoliation de sapins baumiers pendant l'année courante et les années précédentes.

Les résultats du projet devraient s'avérer utiles pour les divers organismes, propriétaires fonciers et autres groupes de gestion forestière qui s'intéressent aux proliférations d'arpenteuses de la pruche dans diverses régions comme la Nouvelle-Écosse, le Québec, l'Ontario, Terre-Neuve et l'État du Maine.

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PREFACE

The eastern hemlock looper (*Lambdina fiscellaria fiscellaria* (Gn.)) has been historically a significant forest pest in Newfoundland and Québec, with more recent outbreaks in New Brunswick, Nova Scotia and Maine. Although it feeds primarily on hemlock and balsam fir, the latter species is of primary concern in New Brunswick.

The first hemlock looper outbreak ever recorded in New Brunswick occurred in the northern part of the Province in 1989 (Magasi, 1990). It is not known how large the outbreak could have become, how much additional fibre would have been lost [it was estimated some 650 000 m^3 were killed (MacLean and Ebert, 1999)] or how long the outbreak might have lasted without intervention. Aerial surveys annually detected 3 500-3 800 ha of defoliation from 1989-1991 and another 1 500 ha in 1992; no defoliation was detected in 1993 (Hartling and Carter, 1993). Spray programs were conducted in 1990, 1991 and 1993 to reduce expected levels of defoliation and to suppress populations. An area of 21 160 ha was treated with B.t. and fenitrothion in 1990; 16 975 ha was sprayed with B.t. in 1991; and 15 475 ha was treated with fenitrothion (6 950 ha) and B.t. (8 525 ha) in 1993 (Hartling and Carter, 1993). In addition, forest industry redirected some harvesting operations to remove dead and dying fir stands (Hartling and Carter, 1993).

The **New Brunswick Department of Natural Resources and Energy (DNRE)** conducts numerous operational pest surveys for forest protection to maximize sustainable wood supply for the Province. Consequently, it is necessary to forecast infestations, plan and conduct appropriate control programs, and assess their efficacy. At the time of the 1989 hemlock looper outbreak, DNRE had no direct experience with appropriate survey methods and control prescriptions for this insect. Information was obtained from scientific literature, technical reports and conversations with colleagues in Newfoundland who had direct experience from numerous looper outbreaks and control programs. DNRE gleaned what was available from these sources and adjusted for local circumstances. Nevertheless, it became apparent there was numerous information gaps concerning control; spray assessment methodology; population sampling and forecasting methodology; and parasitoids and their behaviour/ecology. Also, at the time of the first control program in 1990, only fenitrothion and one formulation of B.t. were registered for aerial control. Of necessity, DNRE began operational research to better understand how to manage looper outbreaks in New Brunswick.

From previous operational studies, DNRE was able to determine there were five larval instars present in New Brunswick (Hartling *et al*., 1991; Hartling and Carter 1991), and established rudimentary baseline relationships between larval development and accumulated degree-days (FPMS, unpublished data; Hartling and Carter 1991; Hartling *et al.,* 1991). Assorted, but limited, studies were also conducted to examine relationships between eggs and subsequent larval populations; eggs and larvae versus subsequent defoliation; and to assess other survey methods (e.g. egg extraction process) (FPMS, unpublished data; Hartling *et al.,* 1991).

Previous operational studies by DNRE also led to the confirmation of spring egg parasitism in 1994 by the Hymenoptera parasitoid *Telenomus* nr. *alsophilae* Viereck (Hartling *et al.,* 1999). This information has helped alert Québec to the impact of spring parasitism, and led them to modify their provincial spray programs. One of DNRE's own technicians also brought the presence of gregarine (protozoa) gametocysts in hemlock looper frass to the attention of the Canadian Forest Service in 1992. The Canadian Forest Service went on to describe a new species of gregarine parasite (Clopton and Lucarotti 1997; Lucarotti *et al.,* 1998). DNRE also helped stimulate research on the hemlock looper virus (e.g. past and present SERG projects by Dr. Lucarotti, CFS).

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1. OBJECTIVES

A first step in the sound management of any hemlock looper problem is having access to valid damage forecast curves, specific to ***local conditions***. None exist for New Brunswick. As already mentioned, DNRE had developed initial correlations between egg counts and subsequent larval populations, as well as a partial data set correlating egg and larval counts with subsequent defoliation. However, since forested areas with the highest populations were treated with insecticide during this last outbreak, correlations of egg or larval counts with subsequent defoliation only exist at lower pest densities. An accurate damage forecast curve over a broad range of populations was needed. This was a key impetus for the present study. More specifically, the objectives of this study were to investigate the following:

Objective 1: Relationship between winter and spring egg counts;
Objective 2: Relationship between egg counts and numbers of early instar larvae;
Objective 3: Relationship between egg counts and numbers of late instar larvae;
Objective 4: Relationship between egg counts and defoliation;
Objective 5: Relationship between larval numbers and defoliation;
Objective 6: Relationship between fall and spring egg parasitism;
Objective 7: Relationship between degree days and various life stages; and
Objective 8: Relationship between pheromone trap catches and any of the above

While not intended as a major component of this project, data were also collected to evaluate methodology for sampling eggs and larvae, and assessing feeding damage of the current and older age classes of foliage.

Results from this project are expected to be pertinent to various agencies, landowners and other forest management groups dealing with hemlock looper outbreaks in such jurisdictions as Nova Scotia, Québec, Ontario, Newfoundland and the State of Maine. In New Brunswick, DNRE will be the primary user, on behalf of its clients (e.g. throughout forest industry and Fundy Model Forest Partners), through its Provincial mandate of forest management and protection. Nevertheless, depending on results, any landowners can use an early detection tool like pheromone traps to become aware of impending threats to their own woodlots, and hence the need for more detailed surveys and/or control.

2. METHODS

Plot selection

Five hundred and ninety-two operational egg survey plots sampled throughout northern and southern New Brunswick in the fall and early winter months of 2000 were considered for suitability as potential project sites. These plots included 557 sampled by DNRE on crown, private and large industrial freehold forestlands, and 35 sampled by J.D. Irving Ltd. on its own industrial freehold. Since the highest egg counts were concentrated in southwestern New Brunswick, it was decided to conduct ***most*** of the project in that geographic area. Supplemental sampling then followed in these areas of highest egg counts, ***if*** they met certain other criteria. Firstly, plots had to be winter accessible by truck (supplementary sampling was conducted in March) and secondly, any stands which might be considered for pesticide treatment[2] in a

[2] Based on the Canadian Forest Service's own stated requirements for its aerial spray trials.

planned research trial by the Canadian Forest Service were excluded. This led to 98 supplemental plots being sampled and the foliage processed for egg counts.

While most components of the study were conducted at a small subset of the entire 690 plots, some components utilized every plot. A summary of the components of the project is as follows:

- For each of the 690 plots, all hemlock looper eggs were removed from the foliage during the lab wash and kept for rearing and parasitoid identification [Objectives 1 and 6(a)].
- A subset of 80 plots in southwest New Brunswick were selected as the primary project sites for the majority of the project [Objectives 2-5, 6(b) and 7] (Figure 1). All plots contained a balsam fir component. At these 80 plots the following work was conducted:
  - Foliage was collected in the fall and winter months, and again just prior to egg hatch in spring. Samples were processed in the DNRE lab and looper eggs counted and retained for rearing.
  - All eggs were reared for parasitoid emergence and identification.
  - Larvae were sampled at an early instar development stage (i.e. representing a "pre-spray sample").
  - Branches were collected and assessed for defoliation of the older age classes of foliage. This was done on two occasions, both before and after larval feeding. Likewise, defoliation was assessed on the current year's age class of foliage.
  - Looper development was monitored from egg hatch to pupation.
- At 41 plots monitored with pheromone traps (DNRE's 2001 operational trapping survey or this project), the relationship between moth captures in pheromone traps (*10-μg lure*[3]) and egg densities was compared [Objective 8(a)]. To increase sample size, data from additional 28 plots sampled by DNRE the previous year were incorporated into the analysis.
- At 32 plots (from the pool of 80), pheromone traps were placed one and two weeks ahead of the usual placement date and moth captures compared to traps hung at the usual time [Objective 8(b)].
- Finally, a pheromone trap was hung at each of 21 plots and monitored at *weekly* (or more frequent) intervals to evaluate the cumulative capture rate of male moths in traps over time [Objective 8(c)]. Furthermore, another 34 traps were *periodically* monitored for supplemental information. Traps were hung at many of the same 80 project plots in the southwest, while others were hung throughout the Province. Collectively, the 55 weekly and periodically monitored traps were distributed across 6 of the 7 *phenological zones of the Province* (Figure 2) that are used to predict the relative development of spring during years of *spruce budworm* control programs (MacDonald, 1963; Webb, 1958).

Objective 1. Relationship between winter and spring egg counts

Combined with Objective 6.

Objective 2(a). Relationship between egg counts and numbers of early instar larvae

Egg sampling was conducted at 80 plots in the fall and winter months. A single 100-cm lower-crown branch was removed from each of 3 trees. All but one plot were resampled the following spring on May 14-22, just prior to anticipated egg hatch. Egg hatch was estimated from a

[3] DNRE switched from a 200-μg lure to a 10-μg lure in 2001. This was undertaken after 3 years of paired testing which consistently indicated a strong relationship between the two lure concentrations.

BioSIM model (Régnière *et al*.,1995a and b) that utilized local historical DNRE data from southwestern New Brunswick for 1990-92. Secondly, egg hatch was estimated by simply comparing DNRE's historical "degree-days–looper development curves" with the available temperature information. Dates of actual first egg hatch were later verified with field observations. After the project, model runs of BioSIM at 19 larval development sites estimated accumulated degree-days (threshold of 3^0C) to be 146-169 to 199-231 at the time of egg sampling from May 14 to 22, respectively. **Previous DNRE studies** suggested 234-279 accumulated degree-days are needed for first egg hatch (Hartling *et al.*, 1991).

Branches were returned to the lab and stored in an unheated building until processed one or two days later. All foliage was processed according to DNRE's "standard wash method", involving a 6% Javex® solution (600-ml bleach to 9.4 litres of cold tap water). Each branch, cut into small pieces (~8- cm) were placed in the Javex® solution and lightly stirred every 15 minutes for 45 minutes before rinsing. The solution was then poured through a series of 3 sieves, run through a Buckner funnel and the eggs collected on gridded filter paper for tallying. Details are reported in Hartling *et al*. (1991) and Hartling and Carter (1993)[4].

Each egg was examined under a microscope to determine if it was sound or parasitized. All were kept for lab rearing. Since the eggs collected in the fall had not experienced adequate cold temperature conditions necessary for successful diapause, they were initially kept in a cooler for 3 months. When rearing began, all eggs were routinely monitored for embryo development, larval hatch and emergence of any parasitoids. An experienced technician (Danny O'Shea) with expertise in the taxonomy of parasitoid Hymenoptera identified all emerged parasitoids. Secondly, world expert Dr. L. Masner (Agriculture and Agri-Food Canada) was sent representative specimens of *Telenomus* nr. *alsophilae* for *taxonomic confirmation*, who in turn placed them in the National Collection as a matter of record.

Larval density was estimated at the same 80 plots by removing a 45-cm mid-crown branch from each of the same 3 trees. Larval sampling was conducted at an appropriate timing to coincide with pre-spray sampling of a hemlock looper foliage protection program (larval index ~1.5 and fir shoots flushed). The actual larval index at time of sampling was calculated to average 1.7 (range of 1.3 to 2.0 per plot). All plots had an Auger Shoot Development Index of 5.0; that is, fir shoots were fully flushed (refer to Dorais and Kettela, 1982). Every attempt was made to carefully cut each branch, and drop it to a tarp laid on the forest floor below. If the branch was struck, hit other branches on its descent to the tarp, or missed the tarp it was discarded and another branch cut. Whenever a branch fell outside the boundary limits of 89-140% of the desired length (i.e. 40-63-cm length), it was not used and another branch was cut. *All* larvae on the branch and tarp were counted, and the *actual* branch length and width were recorded so that larval counts could be expressed in a standardized format of "larvae/sq. m of foliage" and "larvae/100-cm branch length".

[4] The egg extraction process consisted of a 6% Javex® solution rather than a 2% solution as reported in Hartling *et al*. (1991). The concentration was changed following lab wash trials on 450 balsam fir branches over the winter of 1991-92. Parameters that were tested included Javex® concentration, length of branch soaking time, plus hot and cold solutions (Hartling and Carter, 1993).

Objective 2(b). Relationship between egg counts and numbers of early instar larvae: Expressing egg density by branch length vs foliar area

DNRE's operational forecast is based on an interpretation of mean egg densities at the plot level. For this study, mean egg densities were calculated as per the method of expression used in operational egg surveys in New Brunswick, that is "eggs/branch" and then compared to egg density expressed "per sq. m of foliage". While only sound eggs are used for a population forecast, both sound and parasitized eggs were included in this test to broaden the range of egg densities. The data set consisted of 159 plots (80 sampled in the fall-winter and 79 resampled in the spring), as per details in Objective 2(a).

Objective 2(c). Relationship between egg counts and numbers of early instar larvae: Larval distribution in the tree crown and within different sampling units of the mid-crown

Thirty balsam fir trees were sampled at a single plot that had the highest larval counts of the project. Larval sampling was conducted on June 14, corresponding to a larval index of 1.7. A single branch, approximately 45-cm in length was removed from the lower, mid and upper thirds of the living crown of each tree. As well, a longer branch (~100-cm in length) was removed from the mid-crown of each tree.

Every attempt was made to carefully cut each branch, and drop it to a tarp laid on the forest floor below. If the branch was struck, hit other branches on its descent to the tarp, or missed the tarp it was discarded and another branch cut. Whenever a branch fell outside the boundary limits of 89-140% of the desired length (i.e. 40-63-cm for a 45-cm branch; 89-140-cm for a 100-cm length), it was not used and another branch was cut. *All* larvae on the branch and tarp were counted. *Actual* branch length and width were recorded so larval counts on the 45-cm and 100-cm branches could be expressed in a standardized format of "per sq. m of foliage" and "per 100-cm branch length".

Objective 3. Relationship between egg counts and numbers of late instar larvae

Natural mortality appeared to be very high. Populations declined steadily from the egg stage to L4 and L5. Older larval instars were very low at most plots and so no data were collected.

Objective 4. Relationship between egg counts and defoliation

The same egg counts used to compare egg density to larval populations [Objective 2(a)] were utilized to compare with subsequent defoliation levels (i.e. 80 plots sampled in the fall-winter months). Details on egg sampling methodology are provided under Objective 2(a).

Assessing hemlock looper defoliation required modifications to the methods commonly used to assess damage by defoliators feeding primarily on the current year's needles (e.g. spruce budworm). Hemlock looper initially feeds on the current year's age class of foliage and then moves to the previous years' age classes of foliage (Martineau, 1984). To simply assess defoliation after looper feeding *erroneously assumes all the needles were present on the tree prior*

to this event. In fact there could be missing foliage from defoliation in previous years by other insects, diseases or abiotic factors. Indeed balsam fir throughout southwest New Brunswick experienced needle loss *for several years prior to 2001* from balsam gall midge. The implication is that a single defoliation estimate in any given year only reflects *cumulative feeding to date*. While this is an adequate approach to compare the pattern of cumulative defoliation with tree mortality over time (e.g. MacLean and Ebert, 1999), it does not reflect damage caused in a single year. A second potential pit-fall when assessing looper damage assessments happens if total branch condition is evaluated in spring *prior to shoot flushing* and larval feeding, and then compared to a second assessment *after shoot flushing* and larval feeding. This approach only measures the *change in tree condition* since the same foliage compliment is not actually compared in both assessments. A more sound but time-consuming way to evaluate efficacy of a protection program or defoliation at research plots is to *separately assess* defoliation on the current year's needles (year "n") and the older needle growth (years "n-1", "n-2", "n-3"). Furthermore, both pre and post-feeding assessments are necessary for the older needle growth.

The specific sampling protocol for this project was to remove a single 45-cm mid-crown branch from each of 3 marked trees at 80 plots *prior* to looper feeding. *After* larval feeding the same trees were resampled, as per the same method as the pre-feeding assessment. The first set of branches (sampled *prior* to larval feeding) was assessed for *pre-feeding damage levels* on 3 years of old needle growth (2000, 1999 and 1998 age classes). Each branch was evaluated by 3 technicians and placed into a single needle loss category based on 10% damage classes (i.e. ocular method). The second set of branches (sampled *after* larval feeding) was assessed for *post-feeding damage levels* on the same 3 years of old needle growth (2000, 1999 and 1998 age classes), as per the method for the pre-feeding assessment. For both assessments, the 10% damage classes were converted to mid-point values to arrive at a numerical rating for each branch. The difference between the pre- and post-feeding ratings approximated the actual amount of *old foliage* removed by looper feeding in 2001. Finally, a plot average was calculated from the rating for each of the 3 plot trees.

The same 3 branches sampled *after* larval feeding were again evaluated, this time for damage to the *current year's age class of needles (2001 age class).* The assessment was based on the ocular method, as described for the pre-feeding assessment. The more detailed Fettes shoot assessment method (Fettes, 1950) was not used. The Fettes method requires each shoot to be individually rated and then a branch value calculated, and so is more manpower intensive. As it became apparent defoliation was very low at most plots, it was felt this more detailed assessment was not justified.

Objective 5(a). Relationship between larval numbers and defoliation

Larval density was estimated at each of the 80 project plots by removing a single 45-cm mid-crown branch from each of 3 trees. This was the same data set used to compare eggs to larvae [Objective 2(a)]. Actual larval sampling protocol was explained under that objective. Furthermore, defoliation data already collected to compare egg counts to defoliation was utilized for this analysis (Objective 4). Defoliation had been assessed on a single 45-cm mid-crown branch removed from each of the same 3 trees sampled for larval counts. Defoliation on the current year's needles and on the older foliage was assessed separately, as per details explained under Objective 4.

Objective 5(b). Relationship between larval numbers and defoliation: Assessing defoliation

Supplemental defoliation data were collected at two plots having the highest looper populations. This enabled branch samples to span the broadest possible range of damage levels. A single 45-cm branch was removed from each of three crown levels on a total of 27 marked trees. Defoliation was assessed on the *current* year's foliage, using both the ocular method, as per details explained under Objective 4 and the Fettes method (Fettes, 1950). The Fettes method is considered a more detailed assessment since individual defoliation assessments are made on each shoot and a branch defoliation rating then calculated. With the ocular method a single evaluation is determined after examining the entire branch. For both methods, the 10% damage categories were converted to mid-point values to arrive at a numerical rating for each branch. The *older* age classes of foliage were rated by the ocular method, as per details explained under Objective 4. From the data collected an analysis compared (1) ocular and Fettes methods, (2) distribution of defoliation in the upper, mid and lower crown of the trees, and (3) defoliation on the 45-cm mid-crown branch versus mean defoliation for the whole tree.

Objective 6 (a) (and 1). Relationship between fall and spring egg parasitism; Relationship between winter and spring egg counts

Egg parasitism was evaluated to see what impact it might have on the population forecast. *Parasitism from autumn-attacking parasitoids* was evaluated by rearing all eggs found at every positive plot sampled for DNRE's 2000 operational egg survey (592 plots) and supplemental sampling conducted specifically for planning this project (98 plots). In all, eggs were collected and reared from 484 of the 690 plots sampled. (Ultimately a subset of 80 plots was selected as sites for many components of this project). Secondly, to evaluate *parasitism of eggs attacked in spring, just prior to looper hatch,* 79 plots were re-sampled on May 14-22. Foliage collected from both egg-sampling periods was processed (and eggs reared) in an identical fashion, as per details described under Objective 2(a).

The decision on when to conduct spring egg sampling was based on estimates of egg hatch from (1) a BioSIM model utilizing local historical DNRE data from southwestern New Brunswick for 1990-92 and (2) DNRE historical relationship curves of accumulated degree-days to larval development. All predictions were verified with on-site observations. **Previous DNRE studies** suggested 234-279 accumulated degree-days are needed for first egg hatch (Hartling *et al.*, 1991). In the present study, subsequent runs (after egg sampling) with the BioSIM model estimated accumulated degree-days to be 146-169 (May 14) to 199-231 (May 22) during the period of spring egg sampling.

Objective 6(b) (and 1). Relationship between fall and spring egg parasitism; Relationship between winter and spring egg counts: Sentinel placement to monitor for spring parasitism

Sentinel eggs were placed in four stands to replicate a **previously** successful DNRE experiment that first proved the existence of spring attack by looper egg parasitoids (Hartling *et al*., 1999). The present experiment was designed to see if the technique could verify spring parasitism at low populations (and possibly at the beginning of an ensuing outbreak). It was recognized that a small sample size (few sentinel traps at a few locations) might yield a negative

result even if spring parasitism was taking place at this stage of the looper population dynamics.

Hemlock looper eggs were purchased from the Insect Production Unit of the Canadian Forest Service (Sault Ste. Marie, Ontario). Sentinel eggs were from laboratory-reared material laid on cheesecloth, in diapause (>5 months) and free of parasitoids and disease. Sections of cheesecloth were placed in each of 2 sentinel traps suspended from trees at 1.5-2.5 m above the forest floor in 4 stands containing a component of fir (fir stand to a mixed-wood stand of fir and hardwoods). Sentinel traps were made from non-sticky, green, Delta® design pheromone traps with ends removed. Each cheesecloth section (2 x 2 cm) contained a mean (±SD; range) of 176 eggs (±19; 158-195), based on counts from 3 representative samples.

Once eggs were taken out of cold storage, 10-12 days were required before egg hatch at room temperature (B. McCron, Canadian Forest Service, pers. comm. March 7/01). To maintain fresh sentinel eggs, traps placed on May 2 were replaced with fresh sentinels on May 9. These in turn were replaced with a 3rd set of sentinels on May 16. This final set was removed on May 24 and coincided with the actual 1st detectable egg hatch of ***naturally occurring*** looper at several monitored plots. All retrieved sentinel eggs were first examined under a dissecting microscope and then placed in petri dishes to complete development at room temperature (~20^0C). Several times a week all egg in petri dishes were examined for possible signs of parasitism (i.e. darkening of part of chorion, obvious embryo development or parasitoid emergence).

Objective 7. Relationship between degree-days and various life stages

Larval and shoot development was monitored on a regular or occasional basis at 19 plots throughout the spring and summer months. Two plots in particular, having high population counts were evaluated approximately every 2-3 days. Insects were collected from mid-crown branches cut and dropped to a tarp. Larvae were returned to the lab and examined under a dissecting microscope with a micrometer eyepiece. The larval instar of each insect was determined by measuring the width of each headcapsule and comparing to the established range of values for each instar as reported by Hartling *et al.* (1991).

As if to "biologically time" a hemlock looper spray program, egg hatch and early larval development (L1 to L3) were compared to regressions of historical larval development and corrected (using BioSIM) and uncorrected accumulated degree-days. The historical regressions originated from hemlock looper data collected in southwestern New Brunswick from 1990 to 1992. ***Uncorrected*** accumulated degree-days (threshold of 3^0C) were calculated from maximum-minimum temperatures at the nearest weather station. Conversely, ***corrected*** accumulated degree-days (threshold of 3^0C) were calculated from BioSIM predictions of maximum-minimum temperatures corrected for latitude and elevation. All BioSIM predictions were run using New Brunswick historical data. Predictions using Québec data for the hemlock looper development model within BioSIM were not used because it predicted biological events 10-14 days sooner than predictions based on accumulated degree-days thresholds developed in New Brunswick[5]. Details on the BioSIM model are found in such reports as Régnière *et al.* (1995a and b).

However, the primary thrust of Objective 7 was not ***real time*** comparisons of larval development

[5] Dan Lavigne (DNRE), pers. comm. to L. Hartling, N. Carter and D. O'Shea on May 17, 2001. Pierre Therrien (Ministère des Ressources naturelles du Québec) had recently informed Lavigne that the Québec data originated from Anticosti Island where hemlock looper have only 4 larval instars (compared to 5 in New Brunswick). Furthermore, he felt the Québec larval development model needed more validation.

to historical regression lines or to BioSIM predictions. Rather, the objective was to augment data to enhance existing biological relationships between hemlock looper larval development and accumulated degree-days. This project allowed DNRE's historical data to be enhanced by re-plotting new regressions of larval development and accumulated degree-days (threshold of 3^0C).

Objective 8. Relationship between pheromone trap catches and any of the above

Three components were specifically addressed. The relationship between pheromone trap catches and subsequent egg counts was examined. Secondly, the effect of early trap placement on total moths captured was evaluated. Thirdly, the pattern of trap catches over time was assessed to determine optimal timing of trap retrieval. "Time" was based on both calendar dates and accumulated degree-days (threshold of 3^0C), with the latter derived from site-specific projections generated by runs of the BioSIM model.

Objective 8(a). Pheromone trap catches: Relationship between pheromone trap catches and subsequent egg counts

Moth captures in Multi-Pher I ® pheromone traps (*10-μg lure*[6]) were compared to egg counts to better improve on the Province and forest industry's ability to accurately forecast populations. At 41 plots where a pheromone trap had been hung as part of DNRE's 2001 operational trapping network or this project, egg sampling was conducted. Plots were carefully selected to include those with highest moth catches. Egg sampling was conducted by removing a single 100-cm lower crown branch from each of 3 balsam fir trees. Foliage was processed to remove eggs, as per details described under Objective 2(a). Sample size was increased by also using a 2nd data set from DNRE's 2000 operational survey. This 2nd data set consisted of 12 plots with *actual* moth counts from *10-μg lure traps* and 16 plots with *estimates* derived from a regression equation of actual moth counts in *200-μg lure traps*[7]. Egg counts from this 2nd data set had also been based on similar methodology as described above.

Objective 8(b). Pheromone trap catches: Effect of early trap placement on total moths captured

Thirty-two plots were selected to evaluate the effect of earlier trap placement on the ultimate number of moths captured in a pheromone trap. The experimental design approximated a randomized block design. Each plot consisted of a 3-trap configuration, separated by a minimum of 40 m in any direction. The 1st trap was hung at the usual operational placement time, that is, the 10-μg lure was aged approximately one week prior to estimated moth eclosion (later verified by weekly monitoring traps). A 2nd trap was hung one week in advance of the usual operational time and a 3rd trap was placed two weeks in advance.

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[6] DNRE switched from a 200-μg lure to a 10-μg lure in 2001. This was undertaken after 3 years of paired testing which consistently indicated a strong relationship between the two lure concentrations.

[7] Before switching from a 200-μg lure to a 10-μg lure in 2001 DNRE undertook 3 years of paired testing to compare the relationship between the two lure concentrations. That is why DNRE data from the 2000 survey includes both actual moth catches in 10-μg lure traps, and estimated moth counts derived by equation from moth counts in "200-μg lure traps".

Objective 8(c). Pheromone trap catches: Assessing the optimal timing of trap retrieval by evaluating the pattern of moth catches over time

New Brunswick's current practice is to leave hemlock looper pheromone traps in the woods until mid-October, when moth activity has largely tapered off, and before (hopefully) snowfall occurs in the areas of higher elevation. It is not clear if leaving traps out until mid-October actually provides forest managers with a more accurate picture than if the traps had been retrieved somewhat earlier. To address this, a single trap was hung at each of 21 sites distributed throughout 5 of the 6 spring phenological zones of the Province (i.e. low to high elevations). These phenological zones were historically developed to predict the relative development of spring during spruce budworm control programs. These 21 traps were monitored at weekly (or more frequent) intervals to evaluate the cumulative capture rate of male moths over time. These data were supplemented with 34 additional traps that were periodically monitored.

Data were then plotted against calendar dates and accumulated degree-days (threshold of 3^0C). Real temperature data was obtained from New Brunswick weather stations (and adjacent Maine stations) and degree-days at each trap site was derived from *estimated* maximum-minimum temperatures using the BioSIM model that adjusted for variations in elevation and latitude.

3. RESULTS AND DISCUSSION

Objective 1. Relationship between winter and spring egg counts

Combined with Objective 6.

Objective 2(a). Relationship between egg counts and numbers of early instar larvae

The relationship between egg counts and larval populations was evaluated in **previous DNRE studies.** Some data sets gave a strong position association between the two variables; other data sets did not. For example, 1989-90 egg versus 1990 larval counts indicates a good relationship between egg densities and subsequent larval populations (Hartling *et al.*, 1991). Those regression equations suggest a ***trend*** of "≤25 eggs/100-cm lower-crown branch" represented larval populations not exceeding "94 larvae/sq. m of foliage" or "34 larvae/100-cm branch length", based on 5-tree plots[8]. In another example, there was no relationship between egg density (1991-92) and subsequent larval density (1992) (Hartling and Carter, 1993). However, the data did indicate that with "≤25 eggs/100-cm lower-crown branch", larval density did not exceed "69 larvae/sq. m of foliage" or "27 larvae/100-cm branch length", based on 3-tree plots[9].

In the present study, there was not a strong relationship between egg density at 80 plots sampled in the fall-winter and larval density, whether the latter was expressed "per 100-cm branch length" or "per sq. m foliage". This also held when plots were re-sampled (n=79) in spring, just prior to egg hatch. However, decision boxes can be drawn around the data for management decisions. **For mean egg densities "≤22 eggs/100-cm lower-crown branch", larval density did not exceed "93 larvae/sq. m of foliage"** (Figure 3A) **or in 99% of the cases, "21 larvae/100-cm branch length"** (Figure 3B).

Objective 2(b). Relationship between egg counts and numbers of early instar larvae: Expressing egg density by branch length vs foliar area:

In the present study the relationship between egg density expressed "per branch" and "per sq. m of foliage" was extremely good. When mean egg density at plots sampled in the fall-winter (n=80) and spring (n=79) was plotted there was a strong positive association (r^2=0.9103) between the "mean number of eggs/sq. m of foliage" and "mean number of eggs/branch" (Figure 4A). Once again, when data were re-plotted by individual branches (n=477) rather than by plot averages there was a strong positive association (r^2=0.9060) between "number of eggs/sq. m of foliage" and "number of eggs/branch" (Figure 4B). Given the strong relationship between the two methods of expression, it is suggested the two have equal validity.

[8] Note the titles for the two Y-axis of the graph on page 18 of Hartling *et al.*, 1991 are incorrectly reversed.
[9] Internal memo from P. MacNutt to N. Carter and L. Hartling dated November 23, 1992 and entitled "Hemlock looper egg counts/larval population levels/defoliation – 1992" .

Objective 2(c). Relationship between egg counts and numbers of early instar larvae: Larval distribution in the tree crown, and within different sampling units of the mid-crown

It is a well-established fact the distribution of hemlock looper life stages on the branch, and between crown levels changes over the seasons. For example, Dobesberger (1989) reported that the distribution of *eggs* varied between crown levels, with the upper-third of the balsam fir crown having the lowest concentration of eggs, increasing as you move down the tree. **DNRE** examined the distribution of *eggs* on 56 fir branches sampled in the winter of 1989-1990 and found eggs were not evenly distributed along the 100-cm branch. More were found on the basal portion, with fewer on the 45-cm tip (Hartling *et. al.*, 1991). Finally, it is well established that hemlock looper *larvae* initially feed on the current year's age class of foliage and then move to the previous years' age classes of foliage (Martineau, 1984). These and other examples suggest it would not be unexpected if the current larval distribution study (30 trees) reported differences between crown levels or within different sampling units within a single crown.

(a) ***Larval distribution:***
The present study found no statistically significant difference in distribution of early instar larvae (larval index $\approx$1.5) in the upper, mid and lower crown levels of the tree, based on sampling 45-cm branches on 30 trees. Larval counts averaged "152-160 larvae/sq. m foliage", regardless of crown levels (ANOVA-single classification; F=0.05, P=0.95) (Figure 5). Although larval density expressed "per 100-cm branch length" was somewhat less in the upper-crown (mean of 34 larvae/100-cm branch length) than in the middle and lower crowns (44-45 larvae/100-cm branch length) (Figure 6), this difference was not statistically significant (ANOVA-single classification; F=1.55, P=0.22).

(b) ***Unit of measurement for expressing larval density/branch:***
The present study suggests **it has equal validity to express larval density by a standardized unit of "per 100-cm branch" or "per sq. m foliage", whether using a sampling unit of a 100-cm or 45-cm branch from the mid-crown.** There was a strong positive association between the two expressions, whether based on a 100-cm branch (r^2=0.7649) (Figure 7A) or a 45-cm branch (r^2=0.7481) (Figure 7B).

This further substantiates **past conclusions** reported by DNRE during the 1989-1993 outbreak. Data from several hundred plots over several years indicate there is an extremely good correlation between "larvae per metre of branch length" and "larvae per sq. metre of foliage" (Hartling and Carter, 1993). However as was stated at that time, "whether either expression adequately describes the true plot population density is not so apparent".

(c) ***Unit of measurement for expressing larval density/branch (Tree mean):***
Furthermore, when sampling 45-cm branch tips at all 3 crown levels to calculate a *mean density for the tree*, it would appear equally valid to express larval density "per 100-cm branch" or "per sq. m foliage. Based on the same 30 trees assessed above, there was a strong positive association (r^2=0.7907) in mean larval densities expressed "per 100-cm branch" or "per sq. m foliage" (Figure 8) **.**

(d) ***Sampling 45-cm vs 100-cm branches:***
There was no relationship between a 100-cm and 45-cm mid-crown branch when larval density was expressed "per 100-cm branch" (r^2= 0.0997) or "per sq. m foliage" (r^2=0.1554). Thus, while both methods of expressing larval density ("per 100-cm branch length" and "per sq. m foliage") seem equally valid (see above), what is the optimal branch length?

(e) ***Branch vs whole tree assessment of larval density:***
A single 45-cm branch from the upper, mid or lower portion of the crown did not generally have a strong positive association with the mean larval density of 3 crown levels. When larval density was expressed "per sq. m foliage" (Figure 9A) or "per 100-cm branch" (Figure 9B), r^2's were respectively, (1) 0.6931 and 0.4641 for upper-crown, (2) 0.4926 and 0.3538 for mid-crown and (3) 0.4792 and 0.5155 for the lower-crown. The strength of the relationships was somewhat better (upper and mid-crown) or approximately equal (lower-crown) when larval density was expressed "per sq. m foliage" compared to "per 100-cm branch".

Furthermore, there was **not a strong positive association between larval density on a single 100-cm mid-crown branch and the mean density from 3 crown levels** (although the latter was based on a mean of 45-cm branches), whether expressed "per 100-cm branch" (r^2= 0.3680) or "per sq. m foliage" (r^2 = 0.3879).

Objective 3. Relationship between egg counts and numbers of late instar larvae

No data were collected because population levels of L4 and L5 instar larvae were very low at most plots.

Objective 4. Relationship between egg counts and defoliation

From the 1989-1993 outbreak **DNRE reported** that sampling 3 trees/plot (one, 100-cm lower-crown branch/tree) gave similar results to 5 or 10-tree plots (Hartling *et. al.*, 1991). Whether this sampling protocol gave an accurate prediction of defoliation remained to be seen. Indications from DNRE's earliest data suggested a plot average (5 trees/plot) "≤ 9 eggs/100-cm branch" gave <10% loss of the current year's needles and <17% needle loss of *all age classes* of foliage (Hartling *et. al*, 1991). Subsequent data (3 trees/plot) suggested "≤ 27 eggs/100-cm branch" generally caused <10% loss of the current year's foliage (with a maximum loss of 34%) and ≤27% loss to the *older* years' age classes of foliage (Hartling and Carter, 1993). It is not known how these data were influenced by population dynamics.

In the present study, there was only a poor relationship between eggs/branch and defoliation of the *older* age classes of foliage. This was true whether plotting regressions of defoliation and eggs sampled in the fall-winter (n=80) or re-sampled (n=79) in spring, just prior to egg hatch. Likewise the relationship was poor between egg counts and defoliation of the *current* age class of foliage. Since results reflected non-outbreak populations, there may not have been a wide enough range in larval densities to see patterns emerge. However, decision boxes can be drawn around the data for management decisions. **For populations of "≤22 eggs/branch" there was <25% needle loss of the older age classes of foliage in 99% of the cases** (Figure 10), **and <10% needle loss of the current year's needles for 97% of the same cases** (Figure 11). It would appear results are in keeping with other DNRE data generated during the 1989-1993 outbreak.

Note that some plots actually showed "negative defoliation" on the older age classes of needles. This was a sampling artifact. Different branches were rated for the pre and post-feeding assessments, and there is always inherent variability between branches. Most likely this would not be detectable with higher population levels capable of causing higher levels of defoliation.

Objective 5(a). Relationship between larval numbers and defoliation

In *Québec*, larval sampling from *branch beatings* failed to correlate with subsequent defoliation (Berthiaume and Hébert, 2001). In *New Brunswick*, **DNRE studies** from 1989-1993 suggested a correlation between early instar larval counts from *branch sampling* and defoliation on the current year's foliage. At densities as high as 27 larvae/m (69 larvae/sq. m foliage), defoliation of the current year's foliage never exceeded 34% (Hartling and Carter, 1993). The same relationship was not evident between larval densities and the older age classes of foliage. Nevertheless for populations as high as "27 larvae/m of branch" ("69 larvae/sq. m foliage"), defoliation of the older age classes of foliage never exceed 27% (Hartling and Carter, 1993).

In the present study, there was not a good mathematical relationship between larval density and defoliation on the *older* or *current* age classes of foliage, whether larval density was expressed "per sq. m foliage" or "per 100-cm branch length. Since populations were at non-outbreak conditions, larval counts may have been too low to give a broad enough range for a good regression analysis. However, decision boxes can be drawn around the data for management decisions. **Populations of "<95 larvae/sq. m foliage"** (Figure 12A) **or "≤30 larvae /100-cm branch length"** (Figure 12B) **caused <25% needle loss of the old age classes of foliage at 99% of the plots. Secondly, these larval densities caused <10% needle loss of the current year's age class of foliage at 97% of the same plots** (Figure 13A & B). These results are in keeping with other DNRE data from the 1989-1993 outbreak.

As was the case for the egg to defoliation relationships (Objective 4), some plots actually showed "negative defoliation" on the older age classes of needles. Again, this was a sampling artifact (different branches were rated for the pre and post-feeding assessments, and there is always inherent variability between branches). Most likely this would not be detectable with higher population levels capable of causing higher levels of defoliation

Objective 5(b). Relationship between larval numbers and defoliation: Assessing defoliation

An evaluation was made of the ocular and Fettes defoliation assessment methods from a data set generated by sampling 27 trees. Secondly, relationships between branch assessments from the mid-crown were compared to those made from all 3 crown levels of the tree. Thirdly, the distribution of damage between the 3 crown levels of the trees was assessed. Results are summarized as follows:

(a) ***Ocular vs Fettes (Defoliation on current year's needles):***
There was a strong positive association between the ocular and Fettes methods for rating defoliation of the *current year's* age class of needles. From an assessment of 81 branches (3 branches/each of 27 trees) there was a strong positive association (r^2=0.8930) between the two methods (Figure 14A). Similarly, when an average defoliation rating for the whole tree was calculated (mean of 1 branch/3 crown levels) for these same 27 trees, there was a strong positive association (r^2=0.8965) between the two rating systems (Figure 14B). However, both the branch and whole tree analysis indicted the Fettes method generally rated defoliation at least one 10% category higher than the ocular method.

(b) ***Defoliation on mid-crown vs whole tree (current year's needles):***
A comparison of mid-crown and mean tree defoliation of 27 balsam fir trees indicated there was a strong positive association when 45-cm branches were assessed for

defoliation of the ***current year's* age class of needles.** The Fettes method produced a good regression (r^2=0.7501), with the mid-crown branch being slightly higher than the mean of the 3 crown levels (Figure 15A). The ocular method also produced a good regression (r^2=0.8235), with the mid-crown branch being higher than the mean of the 3 crown levels (Figure 15B). **This suggests that the mid-crown branch will give a reasonable but higher index of defoliation than the mean of 3 crown levels.**

(c) ***Defoliation on mid-crown vs whole tree (Older age classes of needles):***
The *3 older age classes* of foliage were assessed by the ocular method, using the same branches used to assess the current year's needles (see above). **There was a significant positive association (r^2=0.6136) between a mid-crown branch sample and the average value for the tree.** The mid-crown branch was assessed at a slightly higher rating than the mean of the 3 crown levels, at least for the lower portion of the curve (Figure 16). As with the current year's needles, it appears **the mid-crown gave a reasonable but perhaps higher index of defoliation than the mean of 3 crown levels.**

(d) ***Distribution of defoliation between crown levels:***
Throughout ***all* project plots, whenever defoliation was noted it was more evident in the lower crown, within approximately 6-7 m of the ground**. For this component of the project, defoliation was studied in greater detail on 27 trees (2 plots) to evaluate the distribution of damage at 3 crown levels. Results clearly indicated **the most damage to the ***current year's*** needles was in the lower crown** (Figure 17A). For 74% of the 27 trees assessed, damage in the lower crown was greater than either of the other two crown levels. Furthermore, another 15% had damage levels that were at least as high as any of the other two crown levels. This may be a consequence of wind and rain dislodging larvae downwards in the tree crowns (a phenomenon frequently observed in the field), so that ultimately a higher percentage of surviving larvae are exerting feeding pressure in the lower crown.

On the ***older age classes* of foliage, defoliation was greater in the lower-crown but any difference was less pronounced than on the current year's needles** (Figure 17B). While damage in the lower-crown branches was greatest in only 37% of the trees, damage was as high (or higher) in the lower-crown than in the other crown levels for 70% of the trees, compared to 41% for the upper-crown and 37% for the mid-crown. Why the less pronounced difference in damage between crown levels on the older age classes compared to the current year's needles? It may be partly explained by the looper's feeding behaviour. Early instar larvae feed on the new needles and then move back onto the older foliage as they mature. Antidotal observations and ongoing larval sampling over the summer suggest mortality from early to late instar was quite high. It is quite possible that the survival rates of the older larvae (that feed on the older foliage) was insufficient to cause enough defoliation to detect major differences in feeding damage between crown levels.

Objective 6(a) (and 1). Relationship between fall and spring egg parasitism; relationship between winter and spring egg counts

In 1994, DNRE first proved the existence of spring egg parasitism by the Hymenoptera parasitoid *Telenomus* nr. *alsophilae* (Hartling *et al.,* 1999). Although it has yet to be documented in Nova Scotia[10] or (to the best of our knowledge) in Newfoundland, spring parasitism was later

[10] L. Hartling in personal communications with E. Georgeson (Nova Scotia Department of Natural Resources) on March 18, 2002.

corroborated in Québec (Hébert *et al*., 2001). These studies suggested any population forecast derived solely from sampling in the fall and winter months could underestimate egg parasitism, and thus have the potential to overestimate the forecast.

This conclusion was further demonstrated in the present study. **There was a significant increase in detectable parasitism from eggs sampled in the autumn and those resampled in spring, due to attack by spring egg parasitoids.** All eggs collected from fall and winter sampling were reared in the lab and monitored for hatch or parasitoid emergence. Egg parasitism at 484 plots (northern and southern New Brunswick) with looper eggs only averaged 4.6% ±14.4 (±SD). At a subset of 79 plots sampled in the fall-winter months and re-sampled just prior to egg hatch, mean parasitism (±SD) rose from 4.8% (±9.3) to 14.6% (±14.6). If one looks at the data another way, 81% of the plots (n=64) had detectable egg parasitism, and 78% (n=50) of those had higher egg parasitism when sampled in the spring, just prior to egg hatch (Figure 18). Another 5% (n=3) had almost identical parasitism levels for the two sampling periods. Only 17% (n=11) had higher levels of parasitism detected when sampled in the fall-winter months. **This study further underscores the significance of spring egg parasitism.**

Whether egg sampling was conducted in the fall and winter months or in spring, just prior to looper egg hatch, most of the parasitism was attributed to one species of egg parasitoid. At the 484 "looper-positive" plots sampled in the fall-winter months, almost all egg parasitism was attributed to *Telenomus* nr. *alsophilae* (93%). As with previous operational egg surveys, parasitism by *Trichogramma minutum* was only incidental, with 7% of the parasitized eggs attributed to this species. Secondly, most parasitism (96%) of eggs ***sampled in spring*** (from fall and spring-attacking parasitoids) was attributed to *Telenomus* nr. *alsophilae* and only 4% due to fall-attacking *Trichogramma minutum.*

Regardless of the species of parasitoid, any adjustment in parasitism rates was insignificant after the lab rearing of all ***fall-winter sampled*** looper eggs and had no impact on the original population forecast. Indeed, a plotted regression line of average "***sound*** eggs/branch/plot", as determined before and after lab rearing had a r^2 of 0.9967 and a 1:1 relationship (Figure 19A). That is not to say all parasitized eggs were identified prior to lab rearing. Detectable parasitism rose from a mean of 3.3% ± 12.6 (±SD) when eggs were initially examined to 4.6% ±14.4 (±SD) after rearing. After adjusting for "corrected egg parasitism", the estimated mean number of sound eggs/branch/plot did decline at some plots. **However, since parasitism of fall-winter sampled eggs was so low to begin with, any detectable change in parasitism after egg rearing was insignificant and so had no impact on the original population forecast.**

A subset of 79 plots were re-plotted and ***compared to egg counts when resampled in spring, just prior to looper hatch.*** The r^2's for this latter data set were 0.9970 (fall-winter sampling) and 0.9576 (spring sampling), with almost perfect 1:1 relationships (Figure 19B). **As with fall-winter egg sampling, any adjustment in detectable parasitism after egg rearing of spring sampled eggs had no impact on the original population forecast.** Again, that is not to say all parasitized eggs were identified prior to lab rearing. After correcting for egg parasitism following lab rearing, the estimated mean number of sound eggs/branch/plot declined at some plots sampled in the fall-winter plots, and at even more plots sampled in the spring. However, since parasitism was quite relatively low for spring-sampled eggs (mean of 14.6%), and especially low for the same plots sampled in the fall-winter months (mean of 4.8%), any detectable change in parasitism had no impact on the original forecast.

As already stated, **egg parasitism was *not* fully accounted for without actual lab rearing.** This is more evident by comparing ***detectable parasitism rates*** before and after lab rearing of

eggs. Since parasitism by fall-attacking parasitoids was quite low to begin with, corrected parasitism rates after lab rearing did not change substantially for the ***fall-winter egg samples*** (Figure 20). The regression lines was very strong (r^2= 0.8897) and had a virtual 1:1 relationship. However, there was a **noticeable increase in detectable parasitism following lab rearing of spring sampled eggs** (Figure 20). The regression line falls well below the slope of a 1:1 relationship, and had a significantly lower r^2 value (0.4908) than the fall sampled eggs. Most likely a lab technician's ability to identify eggs as parasitized depends on whether sufficient time has elapsed between parasitoid attack and when eggs are examined to detect any morphological changes in eggs brought on by looper and/or parasitoid embryo development. **One might reasonably conclude that *if* egg parasitism were very high under localized conditions, and eggs were *not* sampled in spring *nor reared for verification*, then the egg survey could over-predict the population forecast.** From the looper outbreak of 1989-1993, DNRE reported that egg parasitism rates were "as high as 23%…from spring-collected looper eggs incorrectly classified as viable until incubated in the lab" (Hartling *et al.,* 1999**).**

Objective 6(b) (and 1). Relationship between fall and spring egg parasitism; relationship between winter and spring egg counts: Sentinel placement to monitor for spring parasitism

Sentinel eggs were placed in four stands to replicate the DNRE experiment that first proved the existence of spring attack by the hymenoptera parasitoid *Telenomus* nr. *alsophilae* (Hartling *et al.,* 1999). **DNRE's 1994 study** exposed sentinel eggs to potential parasitoid attack in spring and established that spring parasitism does occur (Hartling *et al*., 1999). That study was at the collapse of the 1989-1993 outbreak.

In this current (and limited) experiment, no sentinel eggs appeared to be parasitized and no parasitoids emerged from any looper eggs. The previous experiment in 1994 was done at the end of an outbreak (when presumably a large parasitoid complex had had time to build). It is tempting to state that the lack of sentinel parasitism in the present experiment was because New Brunswick (it now appears) was only experiencing a temporary spike in populations, and as such the parasitoid complex was low. However, spring egg parasitism was clearly shown in another component of this project [see results under Objective 6(a)]. A more likely reason no sentinels were parasitized was that while antidotal evidence at the time of the original experiment (several plots over several years) suggested where spring parasitism might exist, there was no information available to suggest likely plots for the current study.

Objective 7. Relationship between degree-days and various life stages

In past studies during the looper outbreak of 1989-1993 DNRE confirmed five larval instars in New Brunswick (Hartling *et al*., 1991), similar to Ontario (de Gryse and Schedl, 1934) and one more instar than in Newfoundland (Carroll, 1956; West *et al.*, 1987). At that time relationships were developed between accumulated degree-days (threshold of 3^0C) and both egg hatch and larval development (Hartling *et al*., 1991; Hartling and Carter, 1991; N.B. DNRE, unpublished data). DNRE's historical curves are presented using accumulated degree-days calculated from (1) *uncorrected* maximum-minimum temperatures at the closest available weather station (Figure 21A) and (2) maximum-minimum temperatures *corrected* to appropriate latitude and elevation using the BioSIM model (Figure 21B). Clearly, both regression curves are similar with a high degree of confidence around each equation (r^2's of 0.9340 and 0.8834, respectively). While the regression lines well describe the *average* accumulated degree-days required for the

average larval index there is a significant scatter about the line. In other words, predictions at any one plot may be inaccurate. **It may be best to actually interpret the scatter around the regression line to identify when *to expect the "first" plots to have a specific instar index* rather than to predict with accuracy actual larval development for all plots.**

For the current project, as egg hatch and early larval development (L1 to L3) data were collected they were compared in *real time* to DNRE's historical "hemlock looper development-degree-day curves" depicted in Figure 21A and B. **Results consistently fell within the range of data points scattered about the historical regression curves.** However when runs of BioSIM were made at seven sites, predictions using accumulated degree-days and thresholds based on historical data ran 3-4 days later than real events (D. Lavigne, pers. comm., June 19,2001). The apparent discrepancy between the fit on the historical curves and the predictions with BioSIM may in part be a reflection of how well the historical data really fits what is actually happening (i.e. the historical data might have inherent problems from infrequent sampling at many locations). Secondly, the apparent discrepancy may simply reflect differences in data interpretation. The BioSIM model using a regression model which best describes when "on average" larval development events will unfold. This approach is "less forgiving" or more restrictive than using an actual historical regression *with all its associated scatter of data points plotted around the regression line* (i.e. $\pm$ the regression line). In either case, there may be components affecting egg hatch and larval development that are not accounted for in the regression models. A detailed analysis of BioSIM is outside the scope of this project. Further analysis will likely be conducted in-house by DNRE.

Since the *primary thrust of this objective* was to *build on existing relationships* between hemlock looper larval development and accumulated degree-days, not only were the project data analyzed, but also pooled with DNRE's historic information to enhance existing relationships. In Figure 22, the pooled data (1990-1992; 2001) were plotted using degree-days calculated from maximum-minimum temperatures at the nearest "suitable" weather stations, and corrected to appropriate latitude and elevation using the BioSIM model. The regression line can only be described as strong (r^2=0.874). As was said for the regressions using the historical data, the regression lines well describe the *average* accumulated degree-days required for the *average* larval index. Since there is a significant scatter about the line, predictions at any one plot may be inaccurate. **It may be best to actually interpret the scatter around the regression line to identify when *to expect the "first" plots to have a specific instar index* rather than to predict with accuracy actual larval development for all plots.**

Three pairs of regressions were compared, using pooled (1990-92; 2001), current (2001) and historical data (1990-92). Each pair of regressions were plotted using accumulated degree-days calculated from maximum-minimum temperatures (1) *uncorrected* from the nearest available weather station and (2) *corrected* for latitude and elevation with the BioSIM model (Figure 23)[11]. All 3 pair of regressions had a very strong positive association between larval development and accumulated degree-days (r^2=0.874 to 0.934). **For each pair of regression lines, predicted larval development events were several days earlier with the BioSIM model compared to *uncorrected* temperatures from the nearest weather station.** For example, using the

[11] For the current project, regressions derived from BioSIM model used temperature readings individually "corrected" for each site. BioSIM runs were based on projections at two weather stations from a pool of four (Houlton, Fredericton, Brockway and St. Stephen). For the regressions based on the nearest available weather station ("uncorrected" temperatures) a single weather station at Houlton, Maine was selected. Houlton was chosen because temperature readings were readily available for the entire period of April 1 to July 31 and the station was closer to the majority of monitored plots ($\leq$40 km for most; $\leq$80 km for all plots) than Fredericton and St. Stephen.

regression equations in Figure 23, a larval index of 3.0 (mean instar = 3rd) was reached 32-42 accumulated degree-days sooner with the corrected (BioSIM) heat units.

When conducting research, planning survey logistics or "biologically timing" hemlock looper spray programs in New Brunswick it is suggested the regression line of the pooled data may be *best suited* to describe the relationship between larval development and accumulated degree-days. That regression line is intermediate between the ones using only historical data or only data from this project. Furthermore, that regression represents the largest database collected over more years.

Objective 8(a). Pheromone trap catches: Relationship between pheromone trap catches and subsequent egg counts

The Province of *Québec* uses the same lure concentration, pheromone trap (Multi-Pher I ®) and suppliers as does New Brunswick[12]. Recent reporting from that jurisdiction suggested a poor relationship between moth catches/trap and egg counts on 100-cm lower-crown branch (r^2=0.2206) (Berthiaume and Hébert, 2001). Trap catches of 1200 moths resulted in a range of egg counts varying from "0-35 eggs/3000 sq. *cm* of foliage" (i.e. "0-117 eggs/sq. m of foliage").

In *New Brunswick*, similar results were found. Data were *pooled* for 41 plots originating in this project and 28 plots from the previous year's (2000) operational **DNRE** survey. The relationship between moth catches and subsequent egg density was poor. **However, the data did indicate that at plots with trap catches approximately as high as 1200 moths, subsequent looper egg populations never exceeded 15 eggs/100-cm lower-crown branch.** Furthermore, it did not exceed 5 eggs/branch in 94% of the plots evaluated (Figure 24). **For making sound management decisions, this information can help in the interpretation of when egg surveys should (or needn't) be implemented.** Whether a relationship will be revealed at higher population levels remains to be determined.

Objective 8(b). Pheromone trap catches: Effect of early trap placement on total moths captured

If there were no apparent "treatment" effects (i.e. timing of trap placement had no effect) then each trap should have captured ~33.3% of the total moths in each 3-trap configuration. When plotted as a relative frequency distribution the trend suggests counts were somewhat higher for traps placed 2 weeks in advance, followed by traps placed 1 week in advance and then those placed at the standard operational time (Figure 25). Furthermore, the mean (±SD) percent capture per 3-trap configuration was somewhat greater for traps placed 2 weeks in advance (39%±18 for the traps) compared to traps placed 1 week in advance (31%±15) or at the standard operational time (30%±15). However, a statistical analysis indicated no significant difference (ANOVA-single classification; F=1.24, P=0.29). While the difference was not statistically significant, the variability in moth captures between traps in each 3-trap configuration would suggest that further testing is warranted.

[12] The supplier of the Multi-Pher I ® pheromone trap is Bio-Controle (2600 rue Dalton, Sainte-Foy, Québec G1P 3S4). The hemlock looper pheromone lure is purchased from Phero Tech Inc. (7572 Progress Way, Delta, B.C. V4G 1E9).

Objective 8(c). Pheromone trap catches: Assessing the optimal timing of trap retrieval by evaluating the pattern of moth catches over time

In New Brunswick, the Province uses a phenological contour map as one planning tool for anticipating the expected *relative sequence* of spring phenology around the Province, and ultimately the relative development of shoot flushing and larval development. It was specifically developed as a tool for use in the biologically timing of aerial protection programs against the spruce budworm. The phenological zones were originally developed using indicators visible from the air such as the disappearance of ice from lakes and snow from the woods, first signs of leaf flushing on hardwoods and the first appearance of new shoots on balsam fir (MacDonald, 1963; Webb, 1958).

Since elevation and latitude are two variables which influence the aforementioned indicators, the phenological zones have some value when making predictions of the relative rate at which degree-days accumulate and hemlock looper development happens over the season (i.e. first in the south and later in the higher elevations of north-central New Brunswick). Conversely, these same phenological contours reflect the relative sequence of cold days, cold nights and occurrence of frost. For these reasons, hemlock looper pheromone traps were monitored throughout the moth flight period not only in the 80-plot project area of southwestern New Brunswick but across most of the phenological zones of the Province (refer back to Figure 2).

For ease of interpretation, cumulative moth captures were plotted against *calendar dates* rather than Julian dates. Several conclusions were drawn from the analysis. Firstly, 94-100% of the ultimate moth captures in phenological categories 0 to 3 had occurred by September 19-26 and 92-100% in phenological category 4 by October 4-5 (Figure 26). In other words, **>90% of the cumulative moth captures had occurred by the first week of October in all phenological categories monitored** (in a year of extended warm autumn temperatures). Secondly, despite colder day and night temperatures in September and October at the higher elevations (i.e. higher phenological zones), such conditions did not prematurely end moth captures when compared to the lower elevations. Finally, **cumulative moth capture had reached 100% by mid-October.** Standard practice by DNRE and forest industry is to leave the operational pheromone traps in the woods until mid-October, when moth activity in the woods has largely tapered off, and then begin trap pick-up before (hopefully) snowfall occurs in the areas of higher elevation. Based on this study, it would appear that virtually no moths are captured in traps past the commencement of these pick-up dates. For decision-making purposes, data of cumulative male moth capture were also plotted against *accumulated degree-days* (threshold of 3^0C) for each phenological zone (Figure 27). Cumulative capture exceeded 90% *in all traps, in all phenological zones* when the accumulated degree-days had reached 1892 (range across the phenological zones was 1678-1892). The cumulative moth capture had reached 100% *in all traps, in all phenological zones* when the accumulated degree-days had reached 2053 (range across the phenological zones 1755-2053).

4. SYNTHESIS OF PAST & CURRENT RESEARCH

The pheromone-trapping network operated by the Province of New Brunswick serves as an early warning system. Complimenting this system are egg surveys, implemented when there is a need to collect more detailed and accurate biological information for contemplating a hemlock looper control program. Sound interpretation of survey data depends on a good understanding of the relationships between moths to egg densities, eggs to larval populations, and eggs to defoliation.

DNRE had no experience during the outbreak of 1989-1993 with the then recently developed pheromone lure used in Newfoundland for outbreak detection. Although the Canadian Forest Service in New Brunswick had conducted pheromone trap research, there was no information on the relationship between male flight activity and accumulated degree-days, and little on trap catches to subsequent egg densities. At that time DNRE believed the most probable use of pheromone traps was as a tool to identify where intensive egg sampling should be conducted to delineate an outbreak and subsequent need for protection (Hartling and Carter, 1993). That belief is still held. A similar viewpoint exists in Newfoundland (Bowers and West, 1996).

What is the threshold of moth counts in pheromone traps at which egg sampling should be contemplated? In *Québec* the relationship between trap captures and egg densities was poor (Berthiaume and Hébert, 2001). B. Pendrel (CFS–Maritimes) reported at a hemlock looper workshop held in Fredericton in 1996, based on data from cooperators in Newfoundland and Nova Scotia, 750 moths/trap (200-μg lure) "may be the threshold levels where defoliation …can be expected" (West, 1997). However, once again the actual correlations of moths to defoliation were poor. Furthermore, the Newfoundland data indicated there were no positive association between moth captures and egg or larval densities (West, 1997). As reported by Berthiaume and Hébert (2001) and McNeil (1991), the number of moths captured in pheromone traps is highly variable and depends on several factors that are unknown and probably can't be controlled.

Although Pendrel suggested a threshold of 750 moths/trap (200-μg lure) (or <500 moths/trap with the lower potency 10-μg lure now used by DNRE), evidence in New Brunswick would suggest this threshold is too low. Antidotal observations from DNRE's operational surveys from 1997 to the present suggest moth counts of that magnitude do not typically represent detectable defoliation. Furthermore, as reported in this project looper populations never exceeded "15 eggs/100-cm lower-crown branch" for counts almost as high as "1200 moths/trap" (10-μg lure), and populations were "≤5 eggs/branch" at 94% of the plots evaluated. While this information is very useful for interpreting the threshold at which egg surveys should be implemented, perhaps a more important aspect of any moth-trapping program is whether it is sufficiently sensitive to identify changes (trend analysis) occurring in the population dynamics.

New Brunswick places its operational pheromone traps in the field approximately one week prior to moth eclosion. Does placing traps out several weeks in advance of moth eclosion affect the total capture? From the present study there was no evidence to suggest placing traps out 1 or 2 weeks in advance of standard practice (i.e. ~2 or 3 weeks in advance of anticipated moth flight) had a negative impact on the number of moths captured. However, research has shown that numerous factors including inter-trap differences can affect pheromone trap captures (Elkinton and Cardé, 1988; McNally and Barnes, 1981; Wall and Perry, 1980), and many factors probably can't be suitability controlled (McNeil, 1991). Any conclusions reached by interpreting moth capture data from multiple trap placements (including this study) should therefore be treated with caution.

Pheromone traps are left in the woods until mid-October, when moth activity in the woods has largely tapered off, and retrieved before (hopefully) snowfall occurs in the areas of higher elevation. It is not clear if traps left out until mid-October can provide forest managers with a clearer picture than if retrieved at an earlier date. For example, does the lure still retain sufficient potency to attract male moths to the traps by this date, is the killing strip still sufficiently potent to kill arriving moths, or are male moths still sexually interested in the "female" commercial pheromone? Any of these variables could influence whether traps are still "active" after a particular time period. From the present study cumulative moth totals in weekly and periodically monitored pheromone traps were found to reach 100% by mid-October, the time when operational trap retrieval commences for DNRE and forest industry. Furthermore, cumulative moth capture had reached 94-100% by September 19-26 in the lower phenological zones (0-3) and 92-100% by October 4-5 in the higher phenological zones (4-5). **In New Brunswick, being able to retrieve pheromone traps from the field one or two weeks earlier than normal practice would give pest managers improved operational flexibility that should far outweigh the fact a small percentage (<10%) of the potential capture is missed.**

Québec reported a similar pattern of moth captures in a 2-year study from 1991 and 1992. Its moth flight period started in mid-August and was almost completed by late September. Males were always captured in traps (pheromone, light and pheromone-light traps) earlier in the flight period than females and all males were captured by mid to late September in both years (Delisle *et. al*, 1998). Furthermore, Delisle *et al.* (1998) reported that in Newfoundland male flight activity started later than in Québec but most males were captured by mid-October.

As already stated, while pheromone traps may be a suitable tool for flagging an increase in previously endemic looper populations, operational egg surveys are a more appropriate way to delineate the severity and distribution of feeding damage for planning a control program. Hemlock looper overwinters in the egg stage (Martineau, 1984) singly or groups of two or three eggs on twigs, needles and bark (de Gryse and Schedl, 1934), as well as on the forest floor (Otvos and Bryant, 1972). Since the tiny eggs are extremely difficult to find on branches, Otvos and Bryant (1972) developed a solvent extraction process using household bleach, to remove the eggs attached to the branch substrate. This system was in operational use by the Canadian Forest Service in Newfoundland when it was adopted by New Brunswick in 1989, and modified as required over the ensuing years.

What is the population threshold from egg sampling predicted to cause moderate to severe defoliation? *Nova Scotia and Québec* both use "5 eggs/branch" as the density threshold to predicted moderate defoliation. In *Québec*, SOPFIM reported the following population thresholds for their 1999 operational egg survey[13]:

(1) "0 eggs/branch" - no defoliation;
(2) "1-4 eggs/branch" - light defoliation;
(3) "5-9 eggs/branch" - moderate defoliation;
(4) "10-19 eggs/branch" - severe defoliation; and
(5) "20+ eggs/branch" - extreme defoliation.

[13] From map entitled "Hemlock Looper Egg Survey 1999", produced December 1, 1999 by M.R.N. and SOPFIM and from L. Hartling and D. O'Brien (pers. comm., Alain Dupont and Denise Moranville of SOPFIM, Oct. 31, 2000).

Similar thresholds are reported for *Nova Scotia*. That Province reported the following population categories in use during operational egg surveys in 1997-1998 (N.S. Natural Resources, 1998):

(1) "0 eggs/branch" - no defoliation;
(2) "0.1-4.5 eggs/branch" - light defoliation;
(3) "4.6-9.5 eggs/branch" - moderate defoliation; and
(4) ">9.5 eggs/branch" - severe defoliation.

At this time, provincial authorities in Nova Scotia do not check for spring parasitism in order to make adjustments in the population forecast when conducting operational egg surveys. Furthermore, spring parasitism has yet to be documented in that Province[14].

In Newfoundland, where the Canadian Forest Service first developed the population thresholds, a slightly lower threshold of "4 eggs/branch" was the benchmark for predicting moderate defoliation. Reports from 1989 (Clarke *et. al*, 1990) and 1990[15] (forecasts for 1990 and 1991, respectively) suggested the following thresholds:

(1) "<1 egg/branch" - minus light defoliation;
(2) "1-3 eggs/branch" - light defoliation;
(3) "4-9 eggs/branch" - moderate defoliation; and
(4) "≥10 eggs/branch" - severe defoliation.

Around the same time, published research by Dobesberger (1989) in Newfoundland suggested "≤4 eggs/branch" as light defoliation and "≥10 eggs/branch" as severe defoliation. Dobesberger subsequently revised his forecast categories and suggested "<10 eggs/branch" still represented light defoliation. He felt the Canadian Forest Service were being too conservative using a threshold of "≤ 4 eggs/branch" for light defoliation[16].

More recently (2000-2001), *provincial authorities in Newfoundland* reported using a population threshold[17] of ">3 eggs/branch" to predict moderate defoliation. A complete breakdown of their population categories is as follows:

(1) "0 egg/3 branches" - no defoliation;
(2) "1-2 eggs/3 branches" – very light (trace) defoliation;
(3) "3-9 eggs/3 branches" - light defoliation (≤20 total tree defoliation[18]);
(4) "10-29 eggs/3 branches" – moderate defoliation (21-40% total tree defoliation); and
(5) "≥30 eggs/3 branches" - severe defoliation (41%+ total tree defoliation).

At this time, provincial authorities in Newfoundland do not check for spring parasitism in order to make adjustments in the population forecast when conducting operational egg surveys[19].

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[14] L. Hartling in personal communications with E. Georgeson (Nova Scotia Department of Natural Resources) on March 18, 2002.
[15] Fax from Canadian Forest Service – Newfoundland and Labrador Region to N. Carter of Forest Pest Management Section, N.B. Natural Resources and Energy, dated October 10, 1995.
[16] L. Hartling in personal communications with E.J. Dobesberger in October, 1989.
[17] Fax from H. Crummey (Newfoundland and Labrador Department of Forest Resources and Agrifoods) to L. Hartling, dated January 10, 2001.
[18] Total tree defoliation means defoliation to all age classes of needles.
[19] L. Hartling in personal communications with H. Crummey (Newfoundland and Labrador Department of Forest Resources and Agrifoods) on March 22, 2002.

Furthermore, to the best of our knowledge, spring parasitism has not been documented in that Province.

Compared to other jurisdictions, New Brunswick has historically used higher egg density thresholds for predicting defoliation. For the hemlock looper outbreak of 1989-1993, DNRE utilized operational information and research from Newfoundland (Dobesberger, 1989; Clark *et al*., 1990). Initially DNRE adopted "10+ eggs/branch" as the population threshold expected to cause moderate defoliation on balsam fir (Hartling and Carter, 1991). Local data soon became available and population categories were "refined" and interpreted in the following way[20]:

(1) "0-4 egg/branch" - little or no defoliation expected;
(2) "5-9 eggs/branch" & "10-19 eggs/branch" - "gray areas for interpretation"; defoliation was very light to moderate in intensity, with "15+ eggs/branch" more probable of moderate levels of defoliation (where defoliation had occurred "10-19 eggs/branch" more likely to represent at least moderate defoliation); and
(3) "≥20 eggs/branch" - significant defoliation expected.

From all information to date, decision boxes can be drawn around the *egg to defoliation* data for making management decisions. For *New Brunswick*, it is suggested that egg counts up to approximately "22-27 eggs/100-cm lower-crown branch" will seldom exceed 10% needle loss of the current year's foliage and seldom exceed 25% loss of the 3 older age classes of needles.

Since hemlock looper populations tend to be highly localized in distribution (Berthiaume and Hébert, 2001; DNRE, unpublished data), **a challenge facing forest managers is determining an adequate egg sampling effort to accurately delineate the severity and distribution of an outbreak.** The appropriate sampling intensity should be significantly greater than that of *spruce budworm* egg-mass or overwintering larval surveys. Forecast surveys during the last spruce budworm outbreak in New Brunswick generally used a sampling intensity of one plot per 4 600-6 700 ha (Hartling and Carter, 1993). During the *looper outbreak* of 1989-1993, DNRE adopted a two-tier approach to sampling. An *extensive* survey was conducted by staff of Forest Pest Management, followed by a more *intensive* (i.e. higher intensity) survey in areas of highest risk. The latter, conducted by DNRE staff (Branch and Regional) and forest companies represented a sampling intensity of one plot per 250-400 ha and was designed to delimit as many pockets of infestation as possible for protection or to redirect harvesting operations.

Accurate population thresholds for predicting defoliation and an adequate sampling intensity are but two components of a reliable operational egg survey**. A 3rd requirement of a good egg survey is that egg parasitism be assessed.** In New Brunswick DNRE has always corrected its forecast to account for egg parasitism so as to prevent over-forecasting in years of high parasitism. The egg parasitoid *Trichogramma minutum* was frequently detected in looper eggs at *incidental* levels throughout New Brunswick (Hartling and Carter, 1993; Hartling *et al*., 1991). In the fall of 1991, DNRE found a second egg parasite, originally identified as *Telenomus* sp. (Hartling and Carter, 1993; Hartling *et. al*., 1991) and subsequently identified as *Telenomus* near *alsophilae* (Hartling *et al*., 1999). To the best of DNRE's knowledge, finding these two species of looper egg parasitoids was the first on record for the Province (Hartling and Carter, 1993). Furthermore, while it was an established fact that *Telenomus* sp. will attack looper eggs in late summer and fall, a 1994 DNRE experiment first proved the existence of *spring* egg parasitism by *Telenomus* nr. *alsophilae* (Hartling *et al.,* 1999**).** It was later corroborated in

[20] Based on memo from L. Hartling to Y. Moreault, W. Clowater and R. Walker dated Dec. 11, 1992.

Québec, and its impact on natural populations demonstrated in the field (Hébert *et al*., 2001).

This current study again corroborated the significance of spring egg parasitism reported by Hartling *et al.* (1999) and Hébert *et al*. (2001). Significant increases in parasitism were noted between eggs sampled in the fall-winter compared to the spring. An astounding 78% of monitored plots had higher egg parasitism levels when sampled in the spring, just prior to egg hatch. Since most parasitism was attributed to the egg parasitoid *Telenomus* nr. *alsophilae,* **any forecast derived solely from egg sampling in the fall-winter months could overestimate the number of *sound* eggs hatching in spring, and thus over-estimate the forecast.**

It is not enough that egg parasitism (and especially *spring* egg parasitism) be taken into account when conducting egg surveys. Staff must be well trained in distinguishing sound eggs from parasitized eggs. Unlike eggs parasitized by *Trichogramma minutum, Telenomus*-parasitized eggs are not black, but rather have dark shading (opaque black) or a "spot" over part of the chorion (Hartling *et. al*., 1999; Hébert *et al*., 2001). Especially for spring-attacked eggs, the ability to identify eggs as parasitized depends on the length of time that lapsed between parasitoid attack and actual lab assessment. For this reason, even well-trained staff may not actually detect all parasitized eggs without lab rearing. In this study all eggs were actually reared to verify the initial diagnosis of an egg being parasitized (or sound). **One is left to conclude that *if* egg parasitism were very high under localized conditions, and eggs were *not* sampled in spring *nor reared for verification*, then the egg survey could conceivable over-predict the population forecast.**

From the information collected to date on *eggs and larval counts*, decision boxes can be drawn around the data for making management decisions. For *New Brunswick*, it is suggested that when mean egg densities are "≤22-25 eggs/100-cm lower-crown branch", larval density does not exceed the range of "69-94 larvae/sq. m of foliage" or "21-34 larvae/100-cm branch length".

Likewise, a decision box can be drawn around the *larvae to defoliation* data to identify thresholds at which *defoliation* should not be significant. In *New Brunswick*, populations of "<69-95 larvae/sq. m foliage", or "≤27-30 larvae/100-cm branch length" cause <25-34% needle loss of the old age classes of foliage. Secondly, these larval densities caused <10-27% needle loss of the current year's age class of foliage. The existence of relationships between larval counts and defoliation may be moot; in order to have ample time for planning a protection program the critical forecast is the egg survey. Nevertheless, larval to defoliation relationships are useful when assessing program efficacy.

Although not a primary objective of this project, egg and larval sampling methodologies were evaluated, as were some defoliation assessment methods. In the case of larval sampling, assessing population density presents some challenging problems. Larvae are easily dislodged from the branches. Using a basket to catch the falling larvae can overestimate the branch population if larvae are dislodged from jarred branches as the pole prunners are raised into the tree crown. If a basket is not used then there is concern of dislodging larvae from the actual sample branch as it is cut. The branch must be gently cut and dropped to a tarpaulin without striking any other branches as it falls. It is difficult to standardize the branch sample unit. If the desired branch size is 45-cm (or 100-cm), it is difficult to cut that exact size and it can't be shortened once it is brought to the ground. That is because many larvae will be dislodged when it strikes the tarpaulin (or dislodged in the basket), so every single larvae must be counted regardless of the branch size. To try and standardize the way larval densities are compared, counts can be expressed "per length of branch" or "per unit area of foliage". Hartling and Carter

(1993) and West *et al.* (1987) reported that sampled populations can be enormously variable between branches and sampling periods, whether using branch collections or beatings. Antidotal field observations further suggest weather conditions such as rain and wind have a major impact on population density sampled, and on the numbers of larvae situated on the tree or ground (Hartling and Carter, 1993). Consequently, sampling for spray program assessment and population dynamics is very crude, lacking good statistical analysis as to its accuracy.

It appears that the mid-crown branch sample is a reasonable sampling unit for estimating looper *larval populations* in the early instars (larval index ≈1.5). The present study revealed no statistically significant difference in the distribution of larvae in the upper, mid and lower crown levels of the tree, when sampling 45-cm branches. Having said this, it would appear that a single 45-cm branch from the upper, mid or lower portion of the crown did not generally have a strong positive association with the mean larval density from 3 crown levels. This suggests that the intra-tree sampling intensity (or sampling methodology) was inadequate or at least suspect.

How to express egg or larval density is probably not an issue when conducting population surveys. Both can be expressed "per 100-cm branch" and "per sq. m of foliage". While larval density can be expressed either way, whether based on a 100-cm or 45-cm mid-crown branch it did not follow that counts from 100-cm mid-crown branches correlated with 45-cm branches. This opens the question of what is the optimal branch length?

Looper defoliation on balsam fir was evident in the lower crown, within approximately 6-7 m of the ground. Results clearly indicate the greatest proportion of damage to the current year's needles was in the lower crown. This is undoubtedly a consequence of wind and rain continuously dislodging larvae downwards in the tree crowns. This ultimately leaves a greater percentage of surviving larvae to exert feeding pressure in the lower crown. While defoliation on the older age classes of foliage was somewhat greater in the lower-crown, the difference was less pronounced.

Defoliation assessed from a mid-crown branch is a reasonable representation of the average of branches from all 3 crown levels. Furthermore, the Fettes and ocular methods are both adequate for evaluating defoliation of the *current* year's needles on individual branches. Both methods are used to evaluate defoliation by spruce budworm, hemlock looper and many other defoliators. The Fettes method is based on examining individual shoots of individual branches in hand; the ocular method gives a single rating for individual branches in hand or for the entire tree crown. All methods have their pros and con's. The more precise Fettes method rates defoliation at least one 10% class higher than the ocular method. In the final analysis, the ability and consistency of the technicians assessing the damage, and the number of branches assessed per tree is perhaps more important than the specific method selected.

Optimal timing of pre-spray larval sampling, pesticide applications and deployment of field crews is made easier when regression lines to predict insect development are a component of any foliage protection program. The present study builds on historical DNRE data for New Brunswick, and clearly shows that accumulated degree-days are good predictors of biological events. Such information can assist forest managers to maximize the efficient use of field crews and in making accurate predictions of larval development. As such, this information compliments rather than replaces the need for field staff to be on-site monitoring larval *and* shoot development. Moreover, the value in using local data was clear when comparing predictions made by BioSIM with New Brunswick historical regression lines as compared to predictions made with the same BioSIM model but using Québec data. The latter did not reflect events very well in New Brunswick.

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6. APPENDIX 1: FIGURES REFERENCED IN TEXT

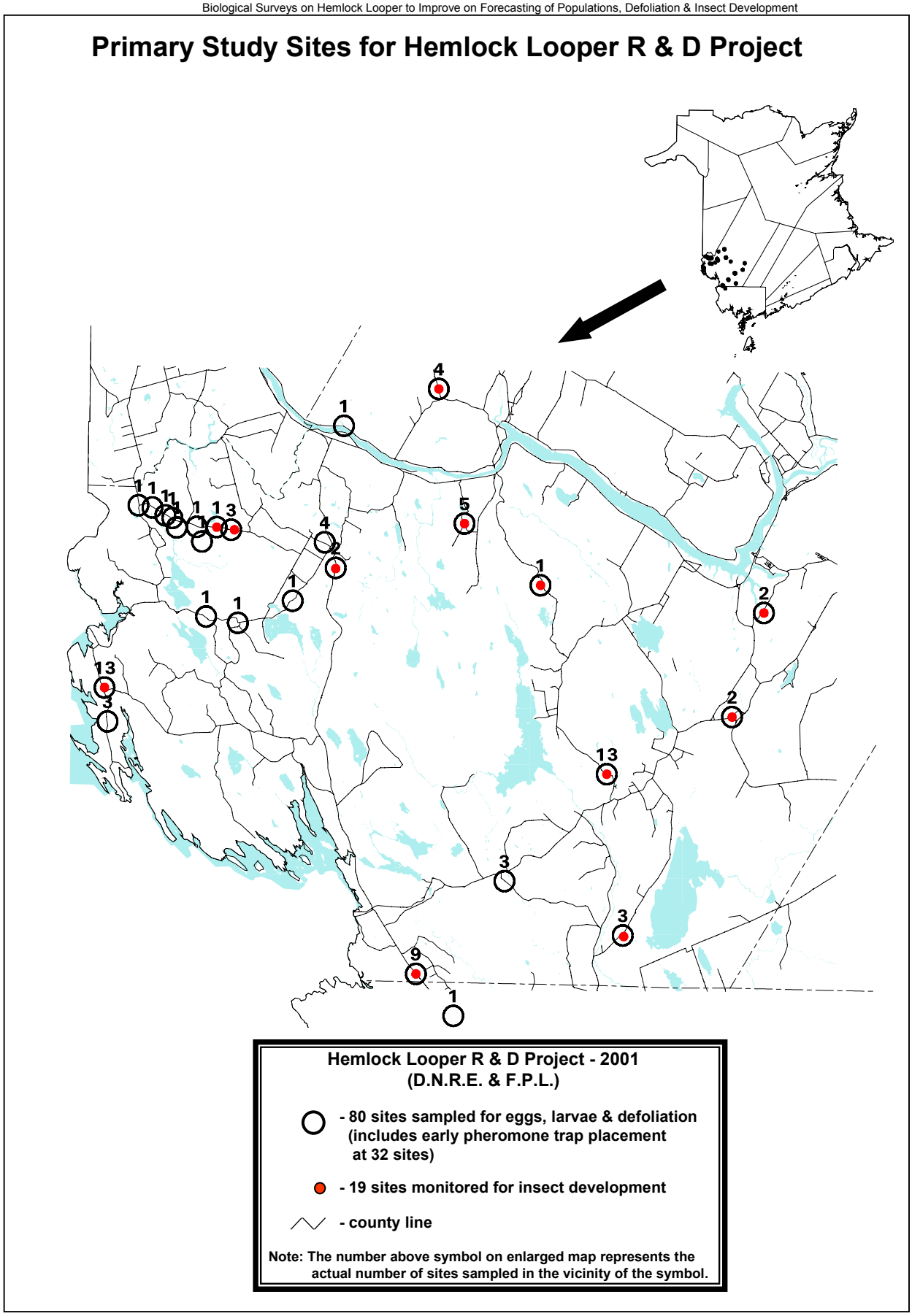


Figure 1. Map showing study sites for most components of the project [Objectives 2-5, 6(b), 7 and 8(b); parts of 1 and 6(a)]. Plot locations from 2 years of data comparing pheromone trap catches to subsequent egg densities [Objective 8(a)] are not depicted. Refer to the map on next page for the component that evaluated capture rates in hemlock looper pheromone traps over time [Objective 8(c)]

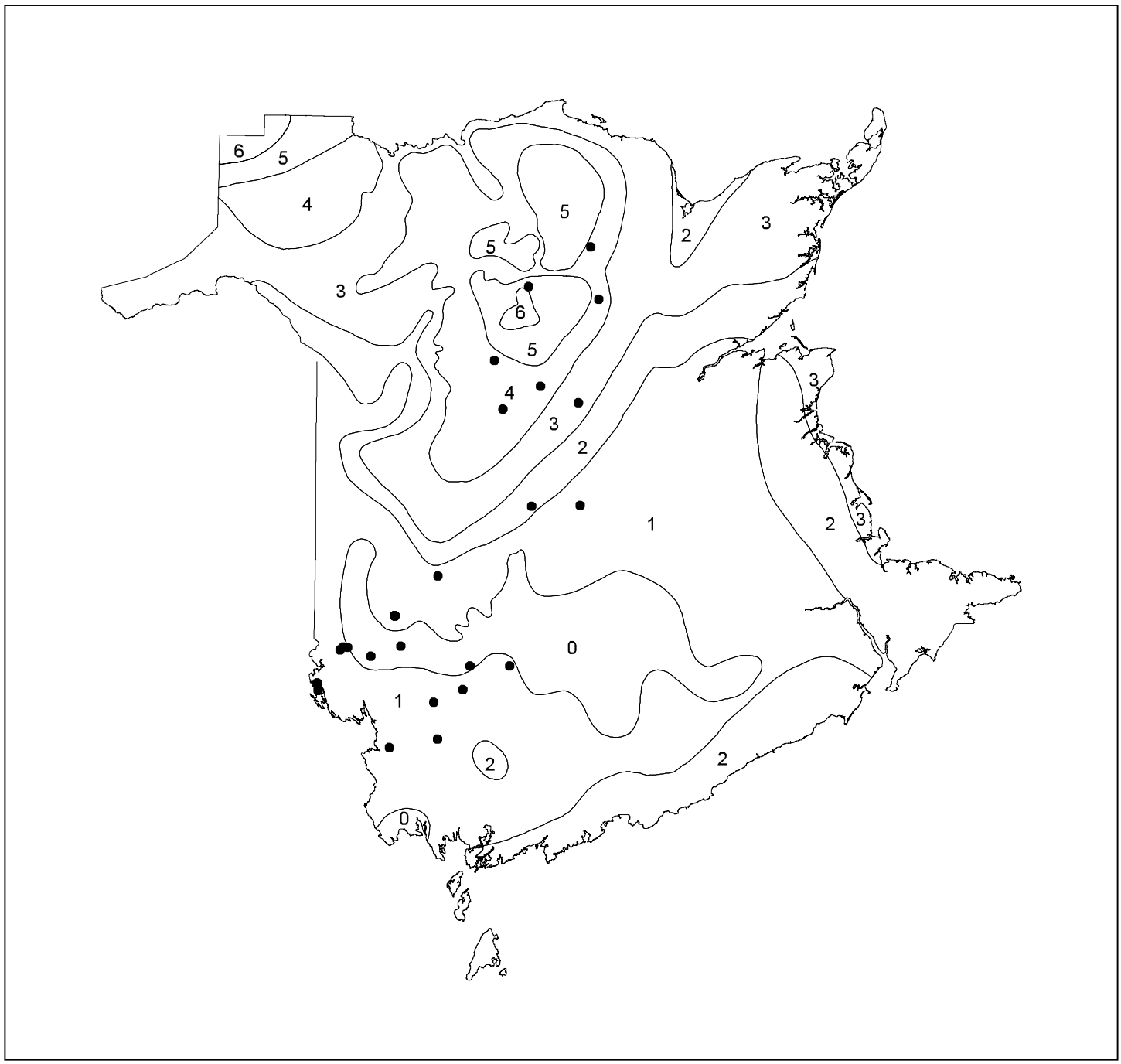
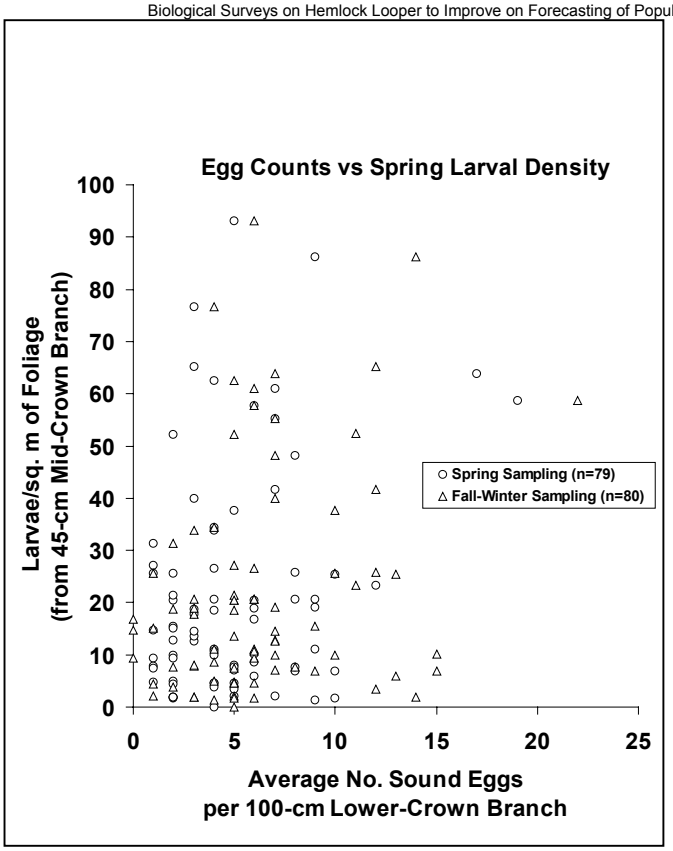


Figure 2. Map showing general locations of pheromone trap placements to evaluate capture rates in hemlock looper pheromone traps over time [Objective 8(c)]. A single trap was placed at each of 21 plots and monitored at weekly (or more frequent) intervals; 34 additional trap locations were monitored on a periodic basis for supplemental information. The phenological contours represent historical spring phenological zones developed for predicting the relative development of spring when conducting spruce budworm control programs. Refer to the map on the previous page for plot locations where the majority of the research components were conducted.



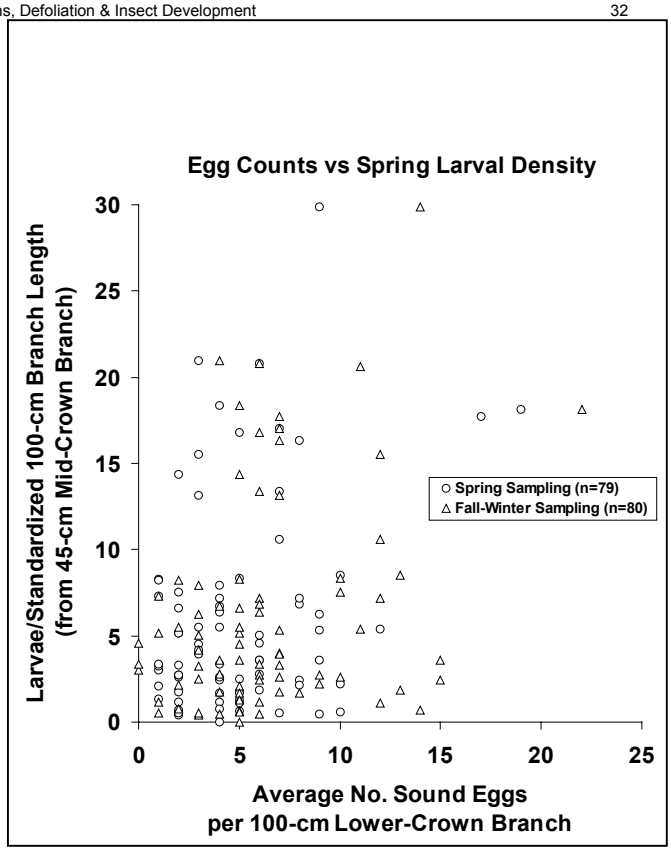
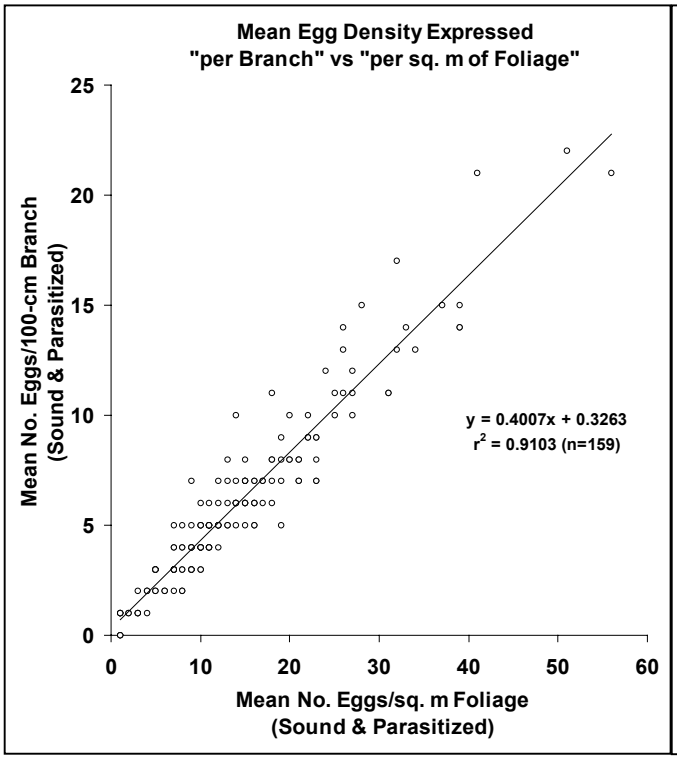


Figure 3A & B [Objective 2(a)]. Relationship between mean egg densities and mean larval density, when larval density on 45-cm branches was expressed "per sq. m foliage" (Figure A – left) and "per 100-cm branch length" (Figure B – right).



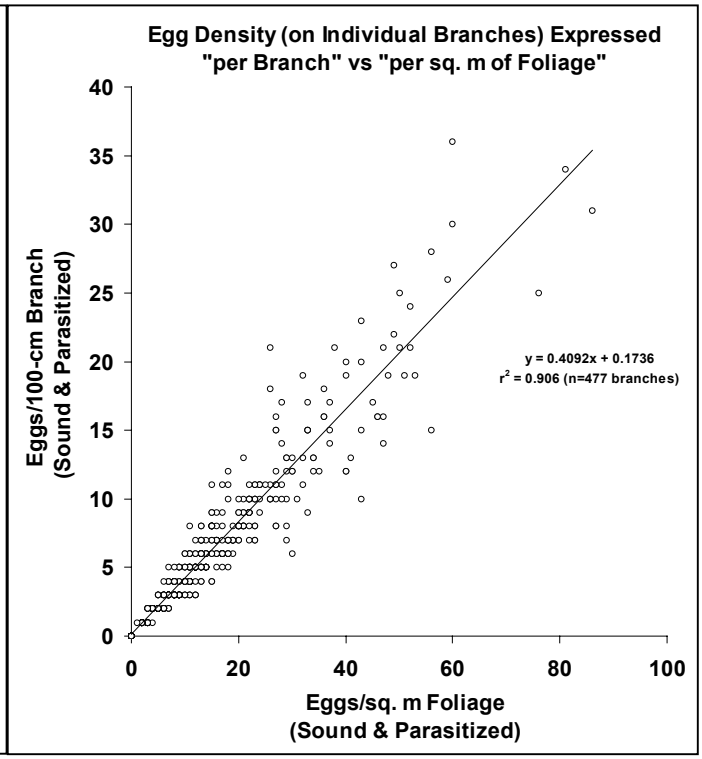


Figure 4A & B [Objective 2(b)]. Relationship between egg density expressed "per branch" and "per sq. m of foliage" from plots sampled in the fall-winter and spring. Figure A (left) represents plot means (n=159); Figure B (right) represents individual branches (n=477).

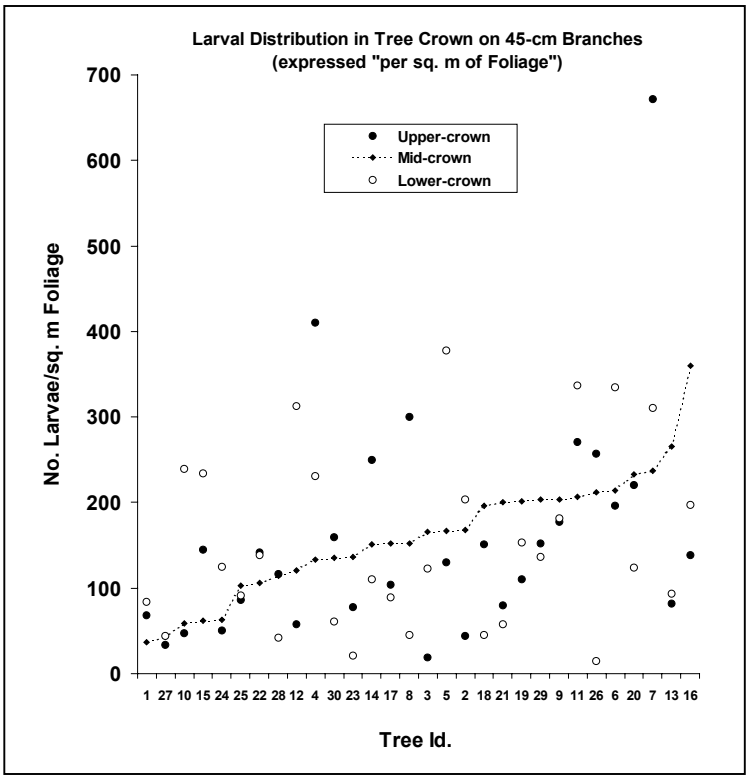


Figure 5 [Objective 2(c)]. Larval distribution in the tree crown on 45-cm branches, sorted from lowest to highest density on the mid-crown branches. Density is expressed in a standardized format as “Larvae per sq. m of foliage”.

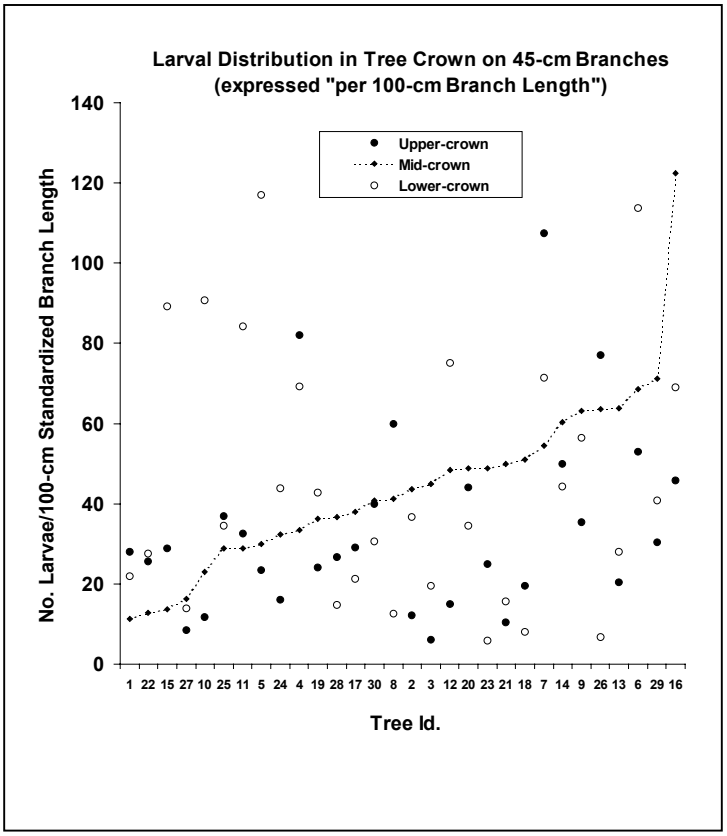
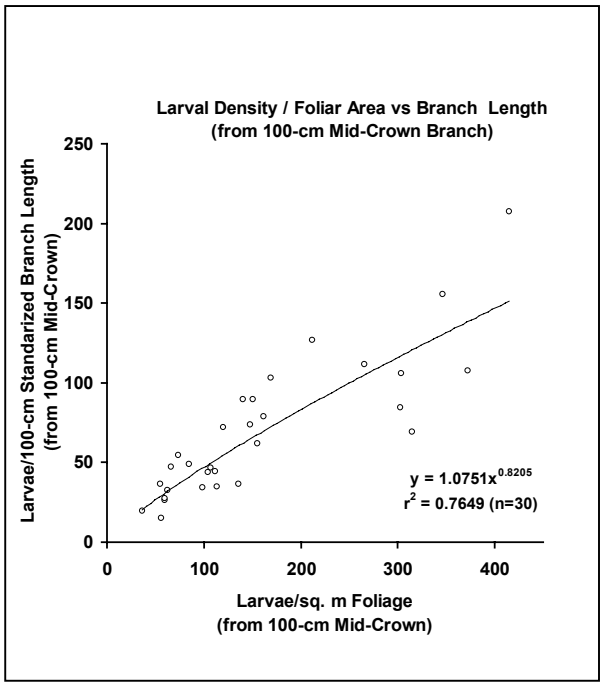


Figure 6 [Objective 2(c)]. Larval distribution in the tree crown on 45-cm branches, sorted from lowest to highest density on the mid-crown branches. Density is expressed in a standardized format as “Larvae per 100-cm branch length**”.**



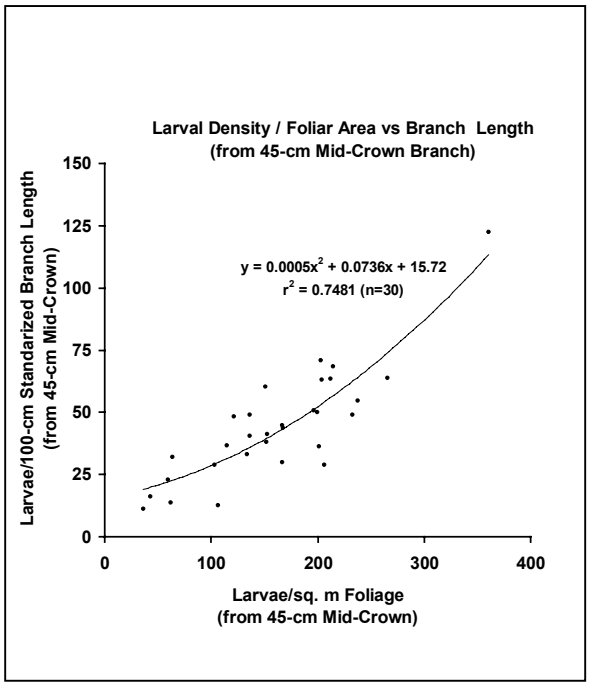


Figure 7A & B [Objective 2(c)]. Relationship between larval density expressed per standardized unit of “per 100-cm branch” and “per sq. m foliage”, using a 100-cm mid-crown branch (Figure A – left) or a 45-cm mid-crown branch (Figure B – right) as the sample unit.

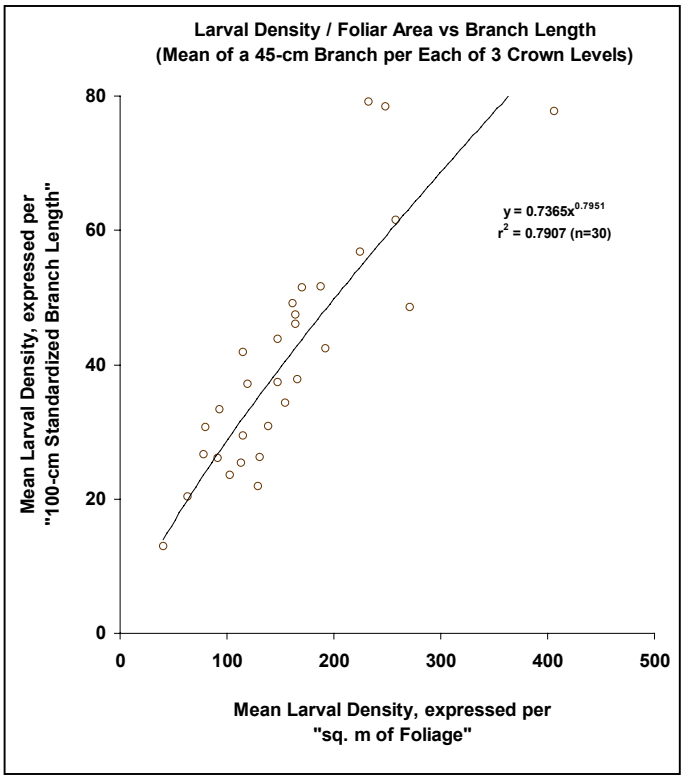
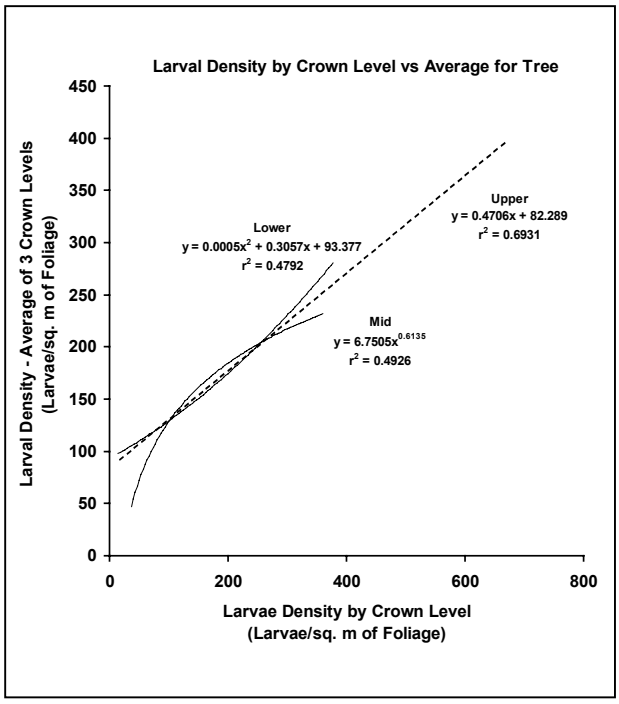


Figure 8 [Objective 2(c)]. Relationship between larval density expressed by a standardized unit of “per 100-cm branch” versus “per sq. m foliage”, when sampling 45-cm branch tips from all 3 crown levels to derive a tree average.



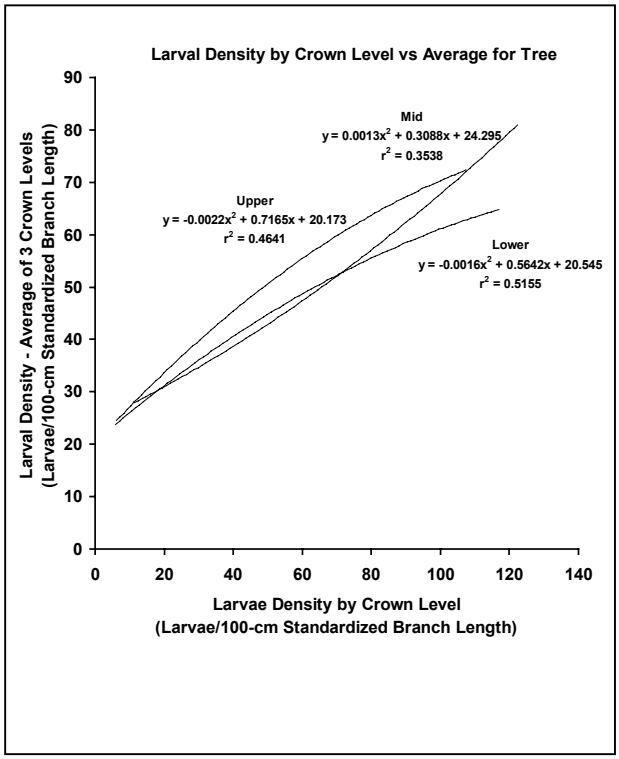


Figure 9A & B [Objective 2(c)]. Comparison of larval density on a single 45-cm branch from the upper, mid or lower portion of the crown versus the branch average from the 3 crown levels. Larval density is expressed “per sq. m foliage” (Figure A – top) and “per 100-cm branch” (Figure B – bottom).

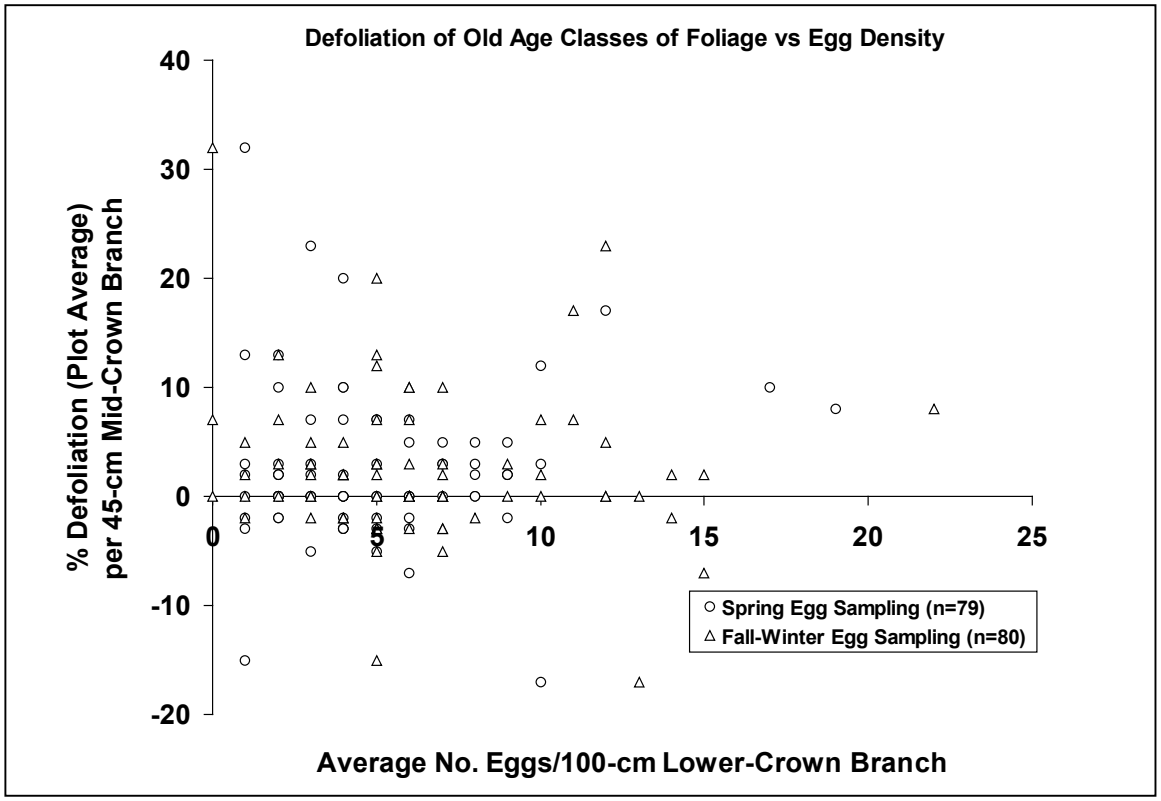


Figure 10 (Objective 4). Relationship between eggs/branch and defoliation of the older age classes of foliage.

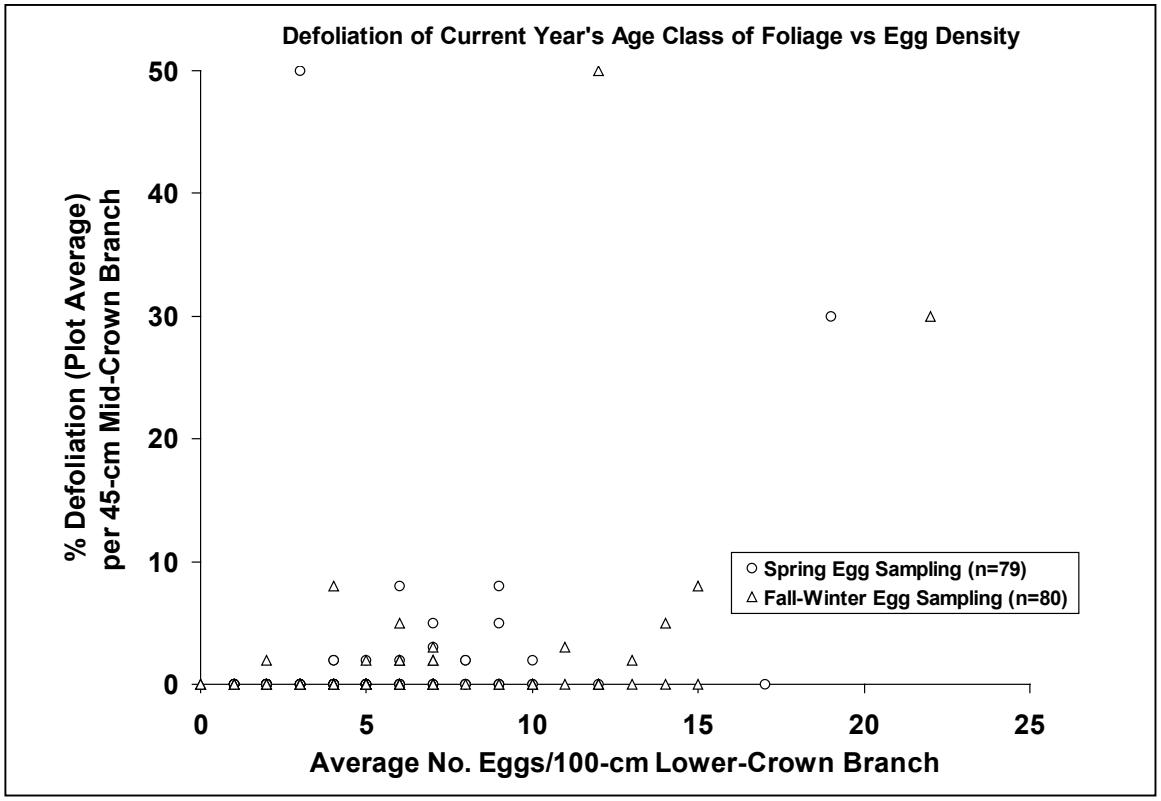
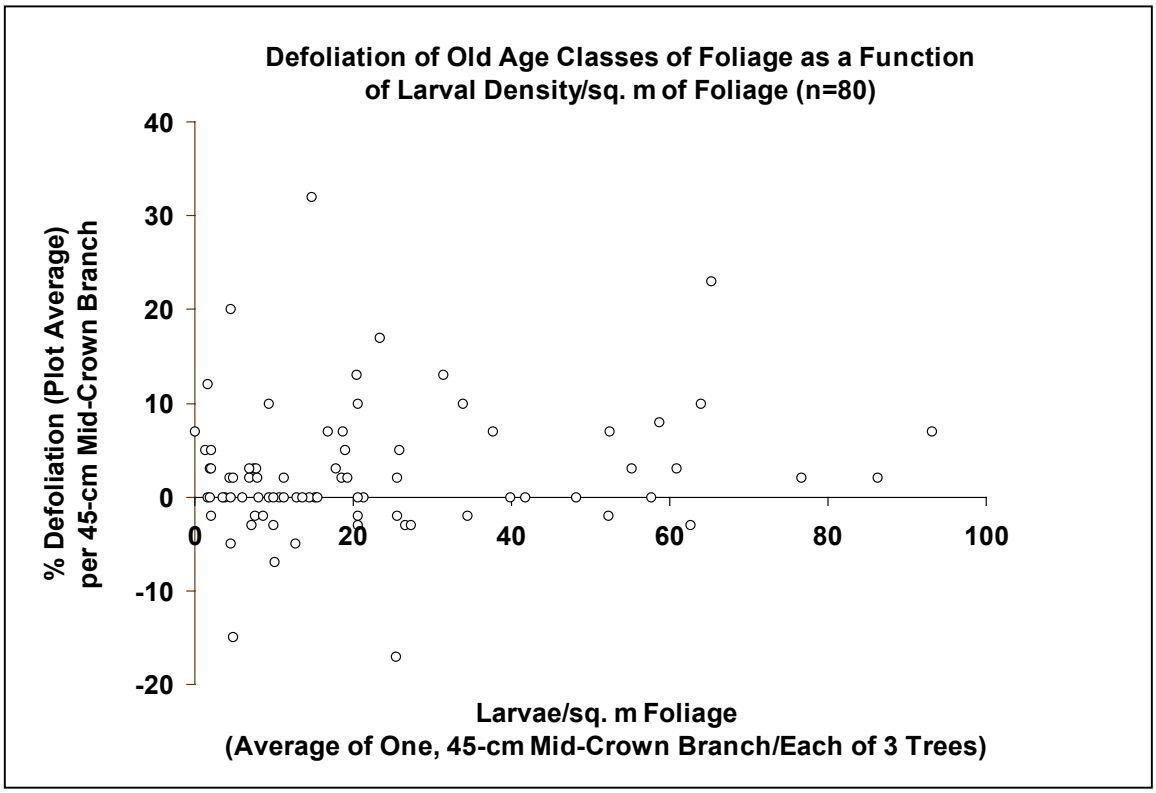


Figure 11 (Objective 4). Relationship held between eggs/branch and defoliation of the current year's age class of foliage.



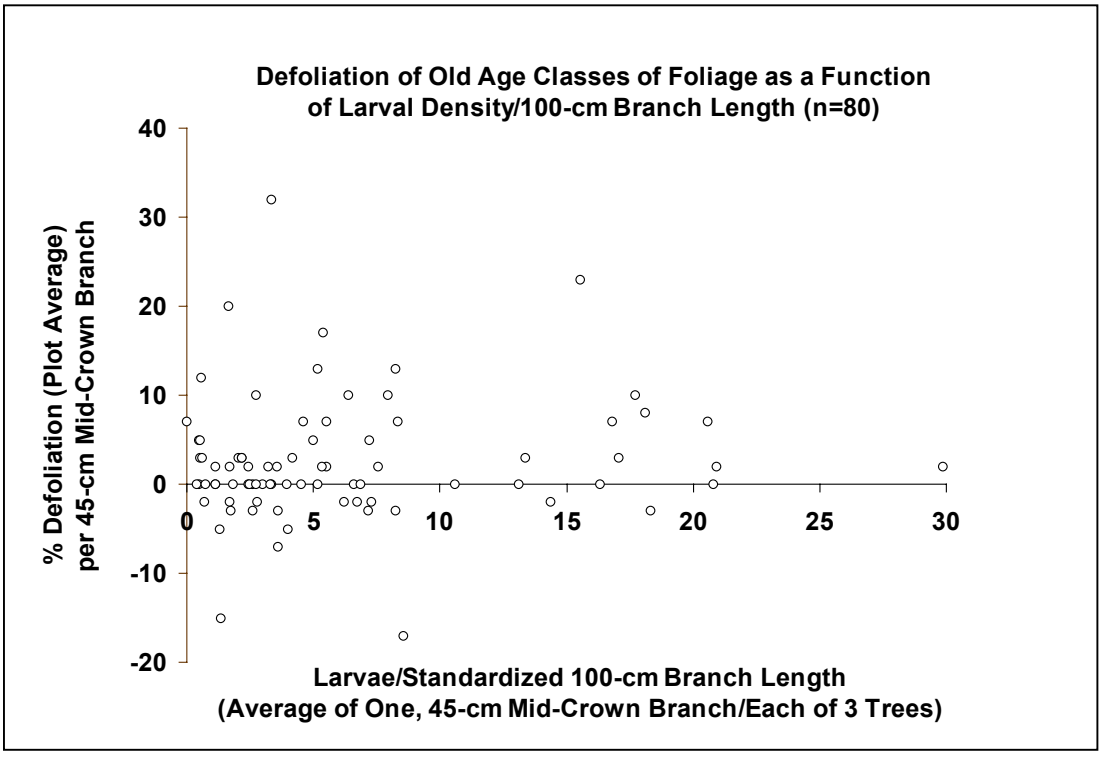
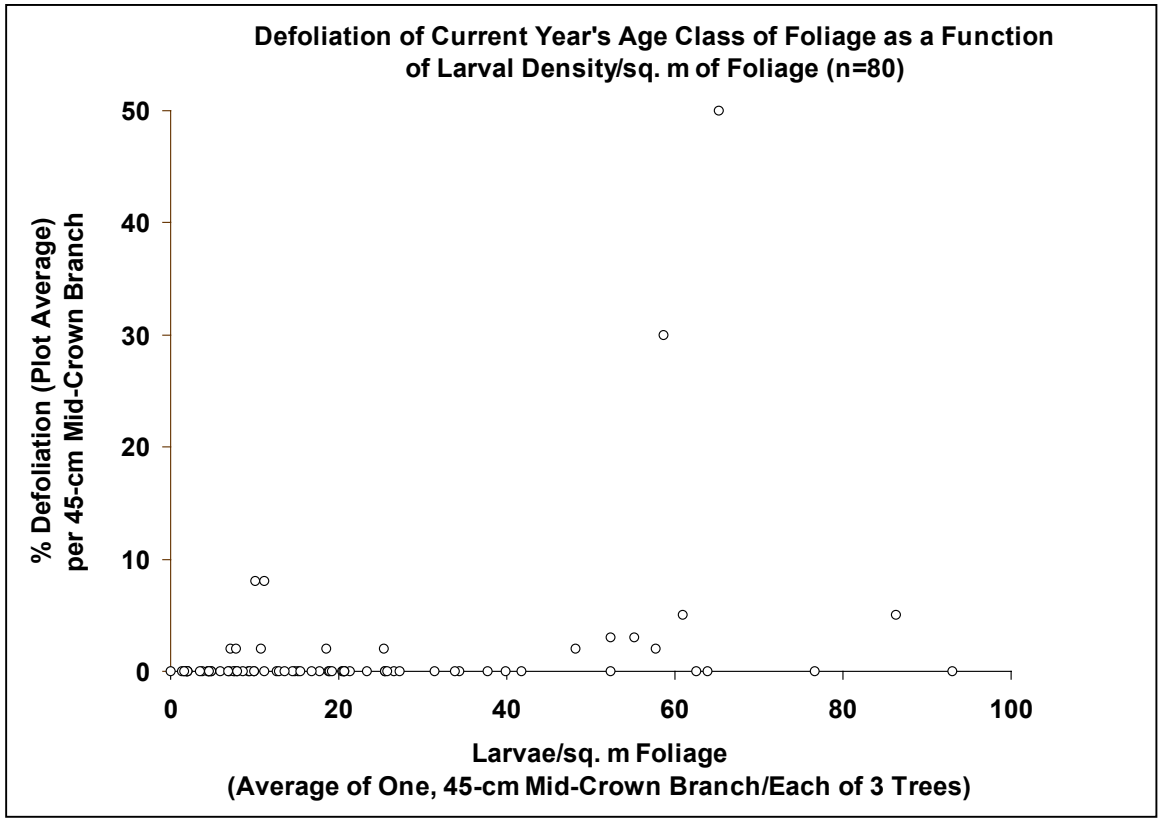


Figure 12A & B [Objective 5(a)]. Relationship between larval density and defoliation on the older age classes of foliage. Larval density is expressed “per sq. m foliage” (Figure A – top) and expressed “per 100-cm branch length” (Figure B – bottom).



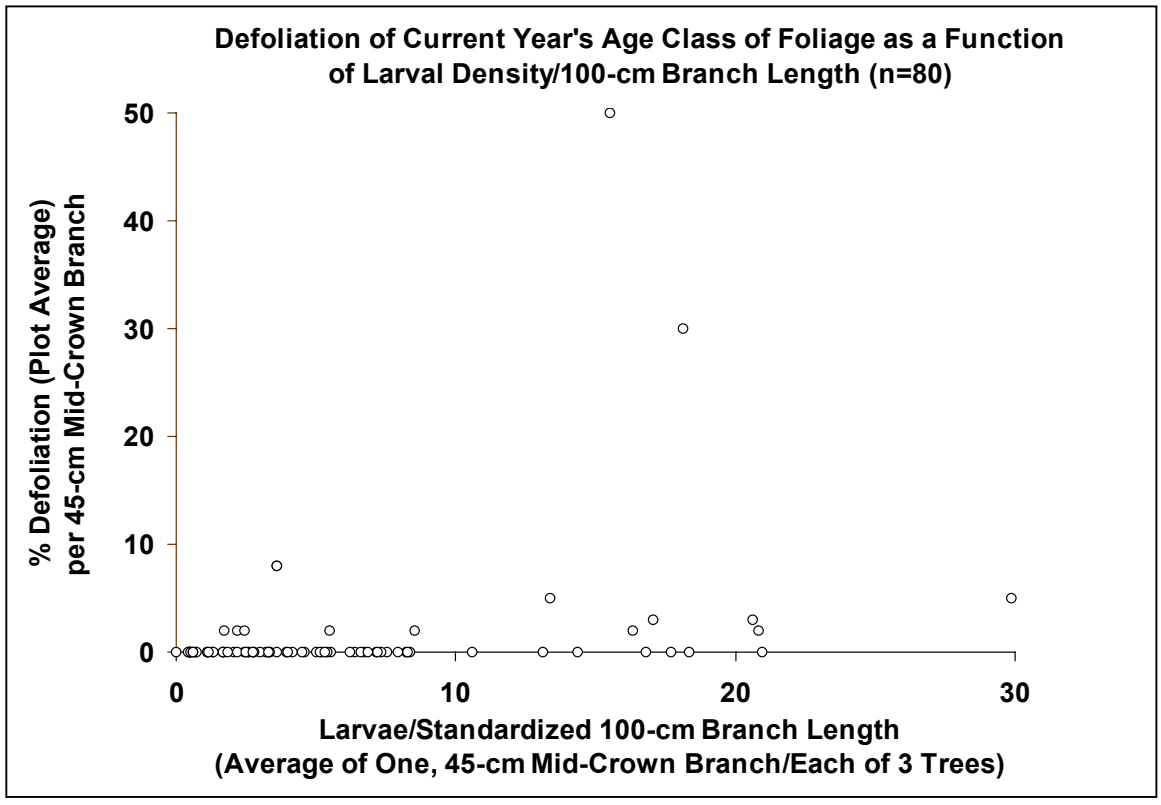
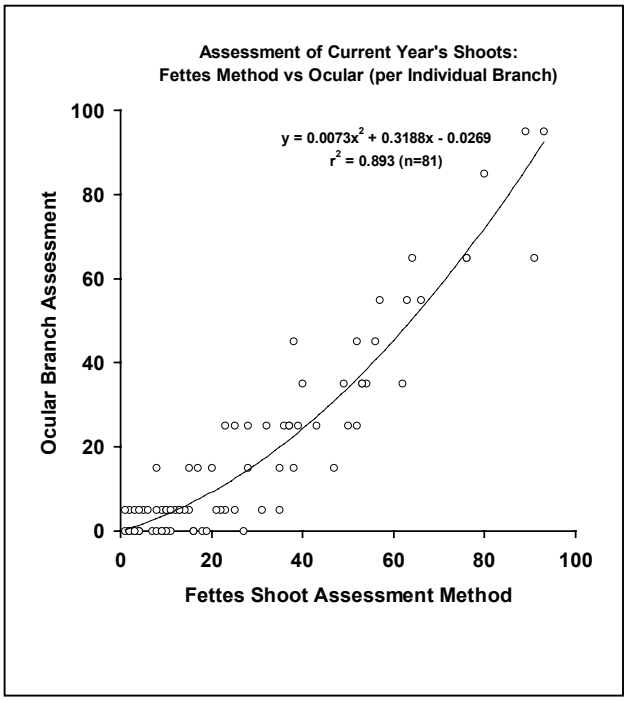


Figure 13A & B [Objective 5(a)]. Relationship between larval density and defoliation of the current year's age class of foliage. Larval density is expressed "per sq. m foliage" (Figure A – top) and "per 100-cm branch length" (Figure B – bottom).



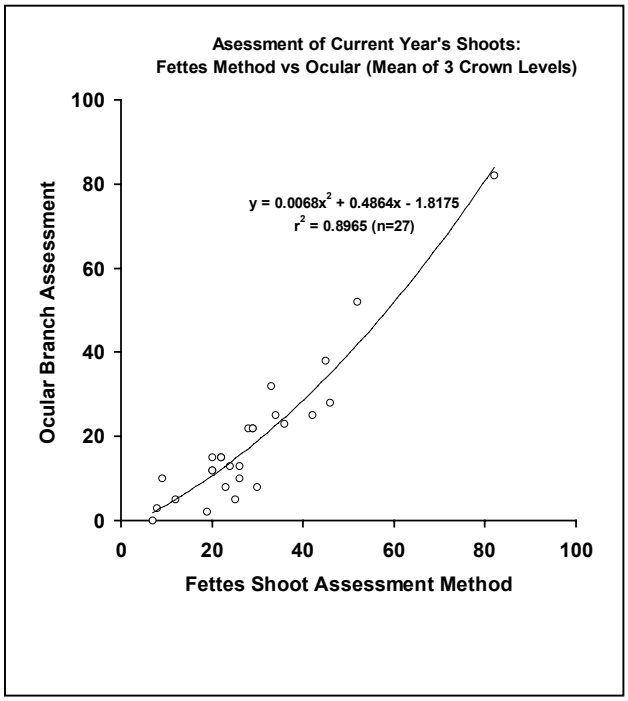
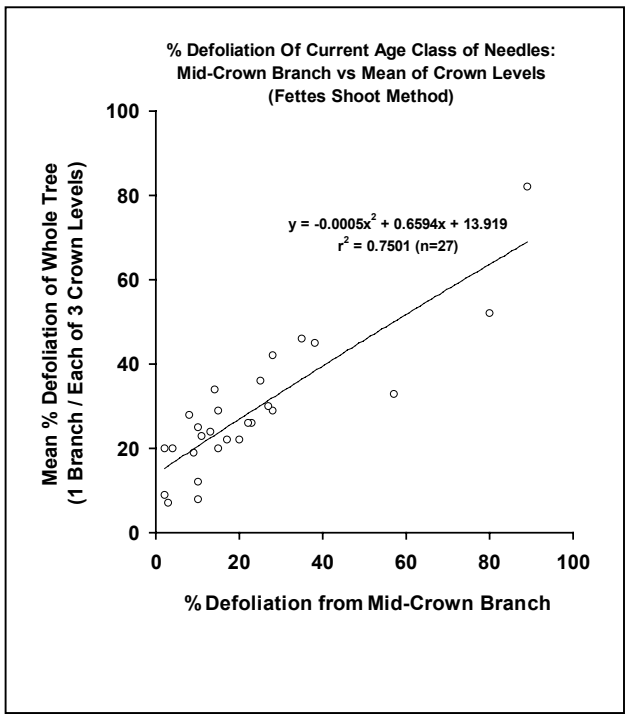


Figure 14 [Objective 5(b)]. A comparison of two methods of assessing defoliation of the current year's shoots, using the ocular branch method and the Fettes shoot assessment method. Data based on individual branches assessed from each of 3 crown levels from 27 trees, and plotted both by individual branch (Figure A – left) and by an average for the whole tree (Figure B – right).



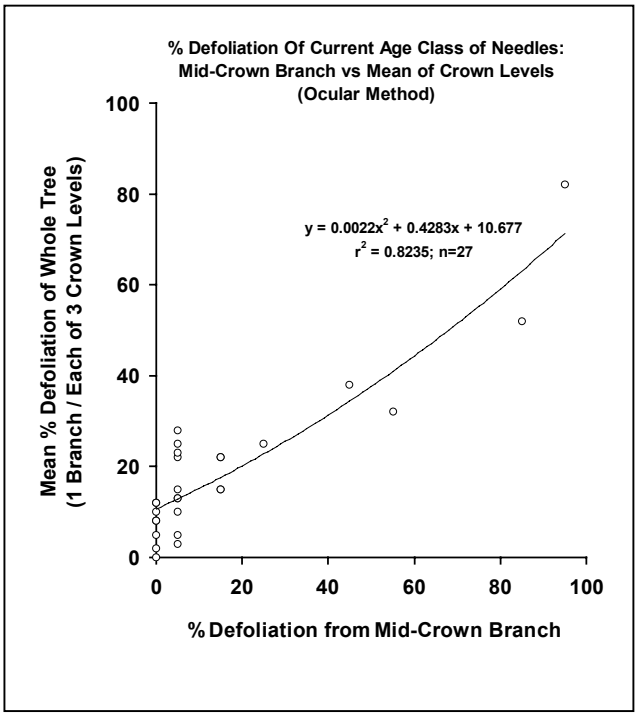


Figure 15 [Objective 5(b)]. Relationship between defoliation of the current year's shoots on a mid-crown branch versus the tree mean of branches from 3 crown levels. Assessment based on Fettes shoot method (Figure A – left) and ocular method (Figure B – right).

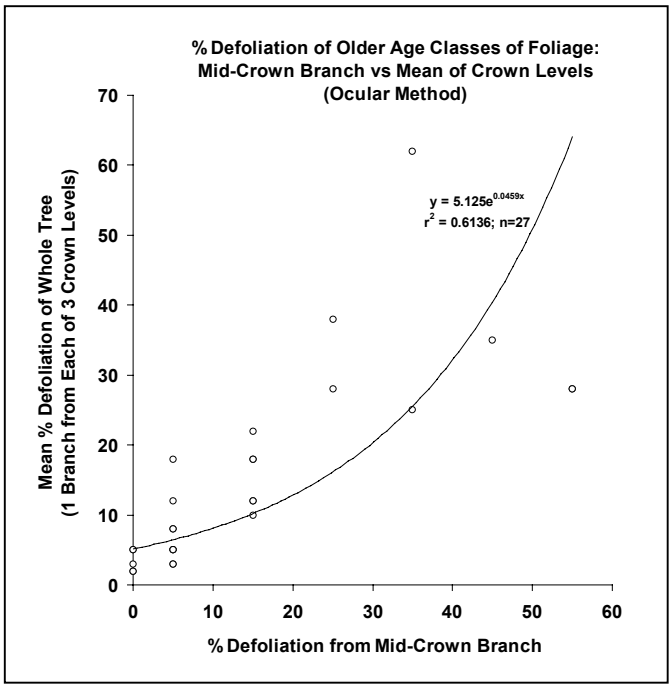
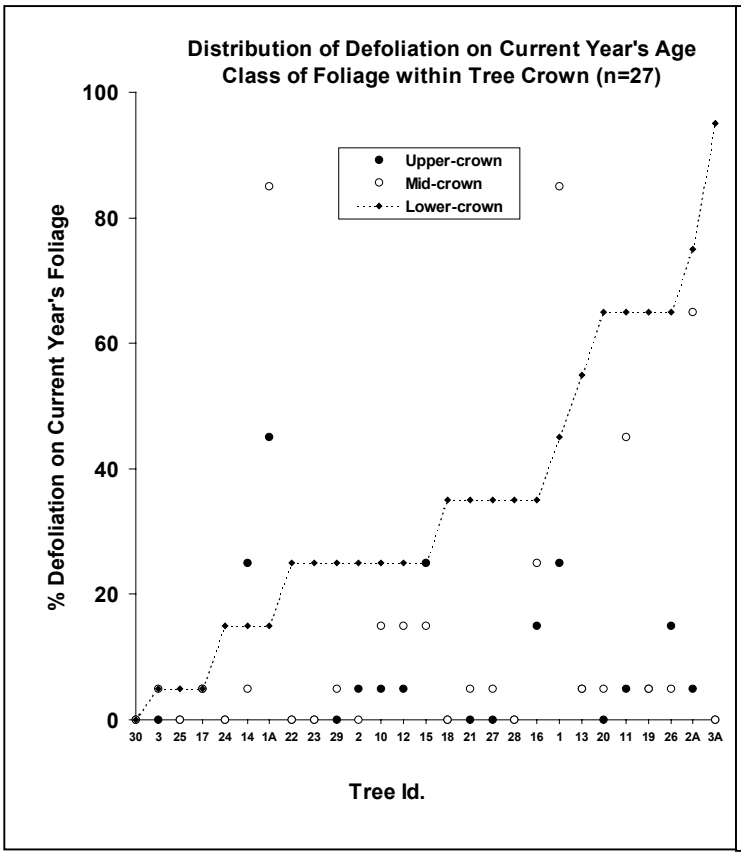


Figure 16 [Objective 5(b)]. Relationship between defoliation of the older age classes of foliage on a mid-crown branch versus the tree mean of branches from 3 crown levels. Assessment based on an ocular assessment.



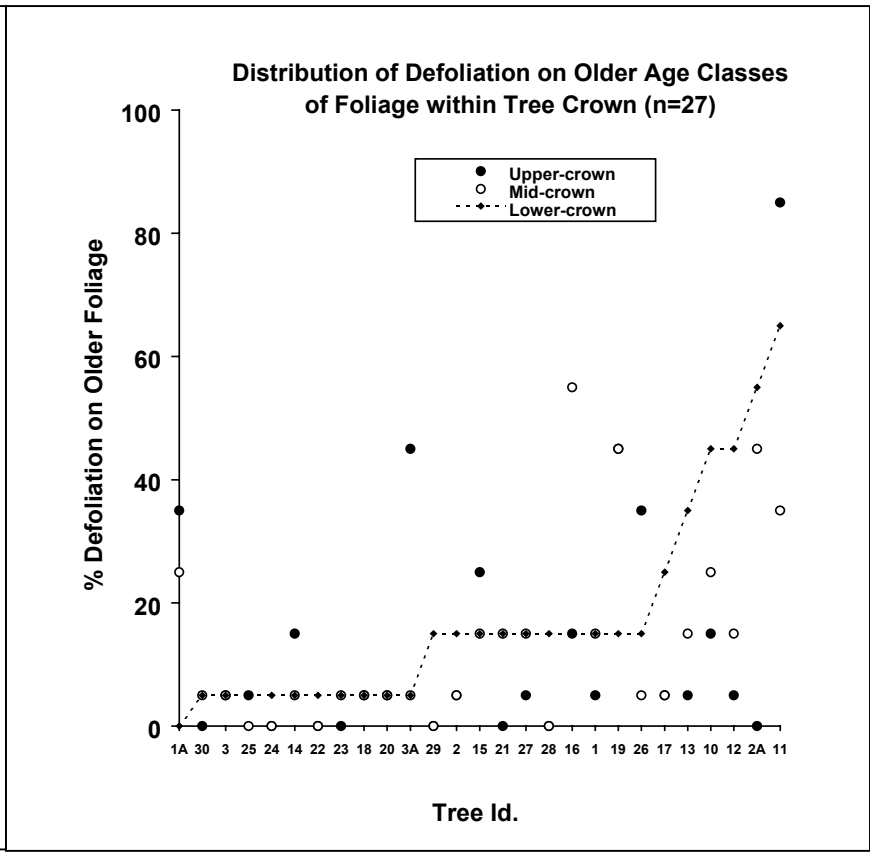


Figure 17 [Objective 5(b)]. Distribution of defoliation on the current and older age classes of foliage within 3 levels of the tree crown on 27 trees selected for their higher hemlock looper populations. Data are sorted from lowest to highest defoliation on the lower crown of each tree. Figure A (left) represents the distribution of defoliation on the current age class of foliage. Figure B (right) represents the distribution of defoliation on the older age classes of foliage.

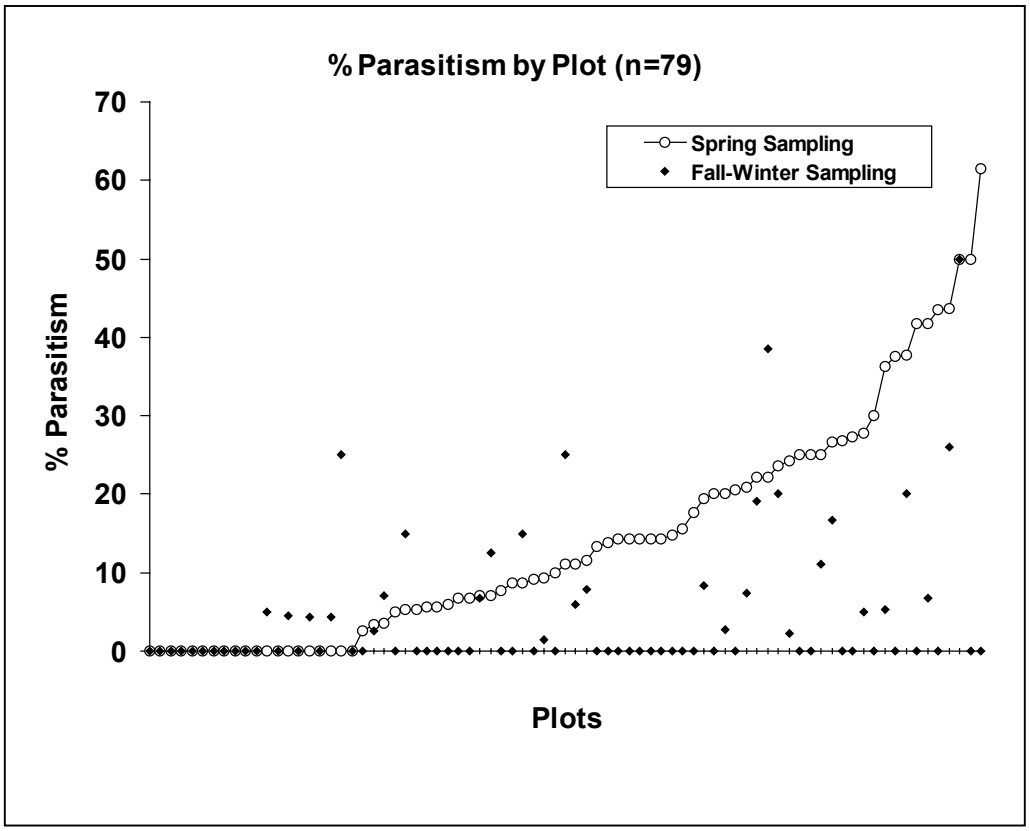
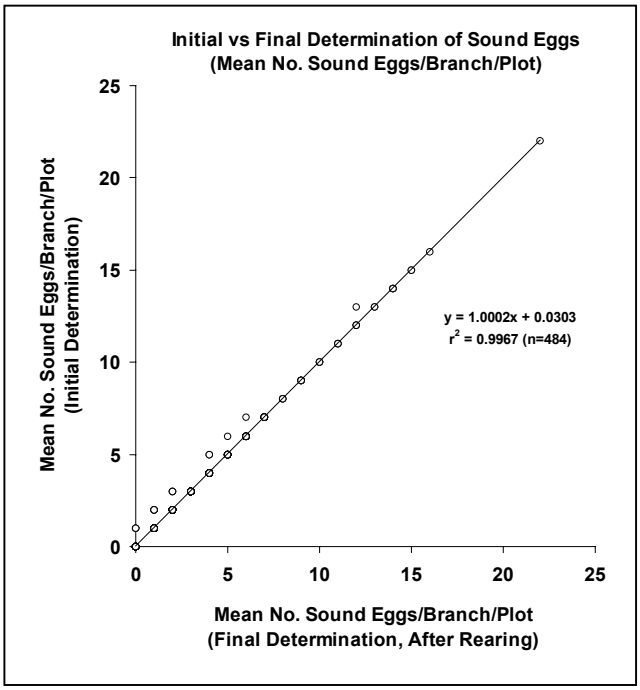


Figure 18 [Objective 6(a) & 1]. A comparison of the change in detectable egg parasitism levels from the fall to the spring after attack by spring egg parasitoids. The 79 plots are sorted along the X-axis from lowest (0%) to highest (62%) levels of detectable egg parasitism at time of spring sampling.



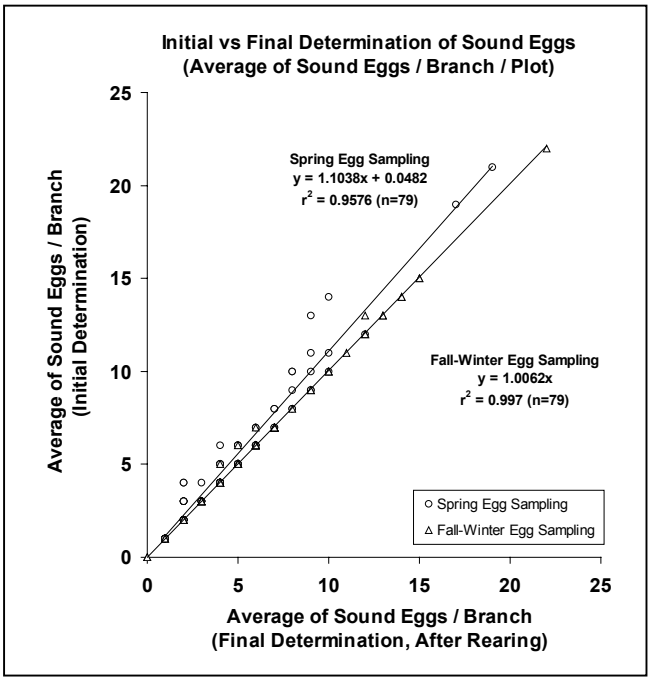


Figure 19A & B [Objective 6(a) & 1]. A comparison of mean egg densities (sound eggs/branch) before and after adjustments to account for final egg parasitism levels by rearing the eggs in the laboratory. Figure A (left) represents all plots from DNRE's operational egg sampling and supplemental project sampling in the fall-winter months of 2000-2001 *where eggs were actually detected* (n=484). Figure B (right) represents a subset of 79 plots sampled in the fall-winter months of 2000-2001 and resampled in the spring (2001), just prior to egg hatch.

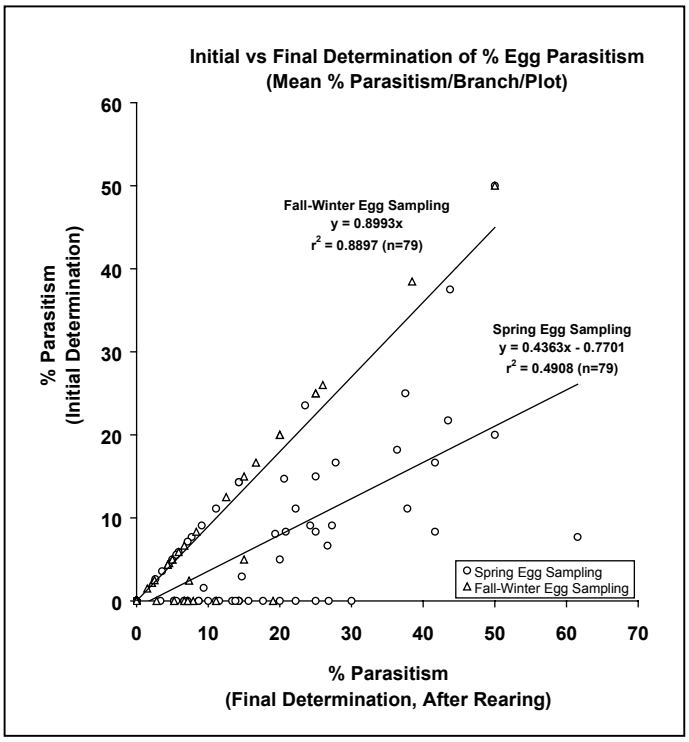


Figure 20 [Objective 6(a) & 1]. A comparison of % ***detectable*** egg parasitism before and after rearing the looper eggs in the laboratory. All 79 plots were sampled in the fall-winter months of 2000-2001 and resampled in the spring (2001), just prior to egg hatch.

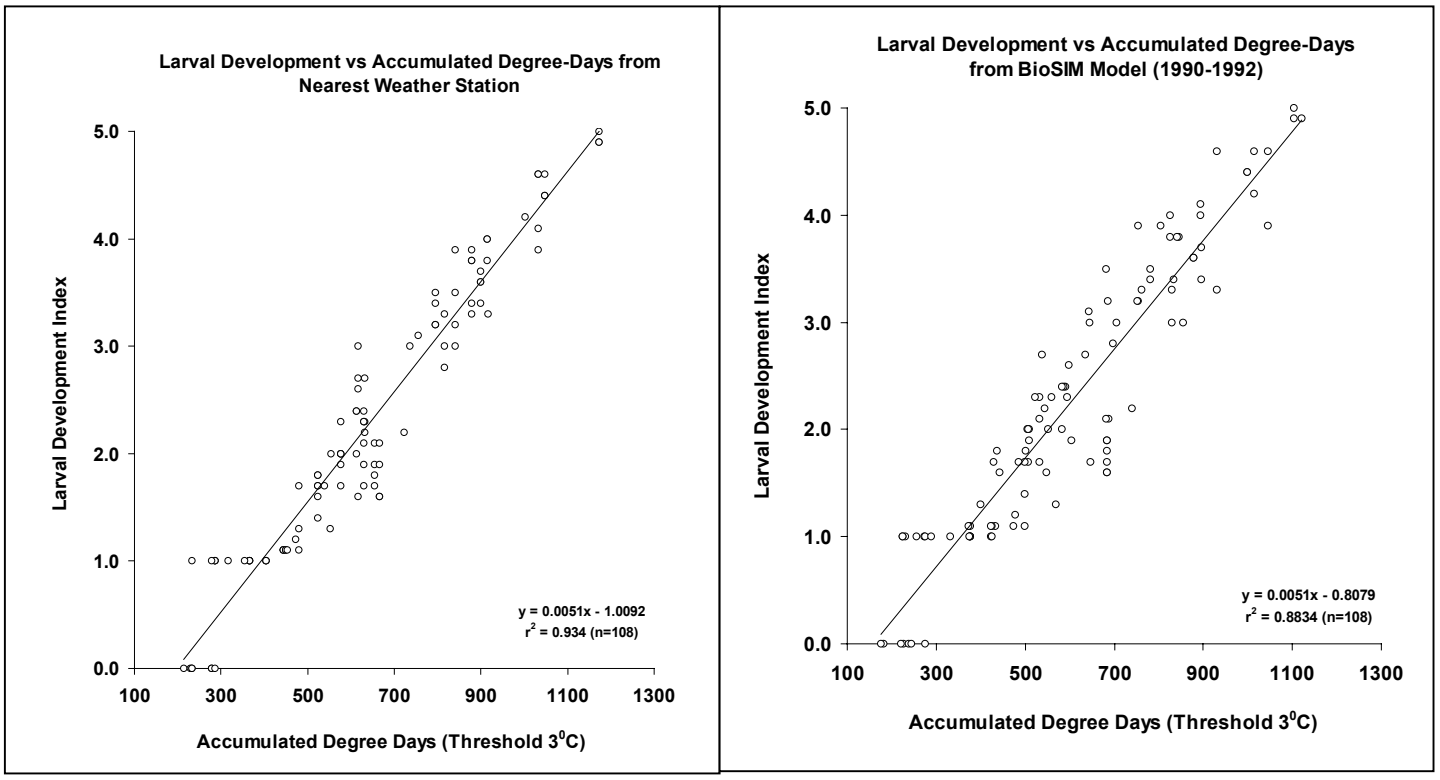


Figure 21 (Objective 7). Hemlock looper larval development versus accumulated degree-days (threshold of 3^0C) from historical DNRE data collected in southwestern New Brunswick from 1990-1992. Figure A (left) represents degree-days calculated from maximum-minimum temperature readings at the nearest available weather station. Figure B (right) represents degree-days calculated from maximum-minimum temperature readings at the nearest available weather stations, and ***corrected*** to appropriate latitude and elevation using the BioSIM model.

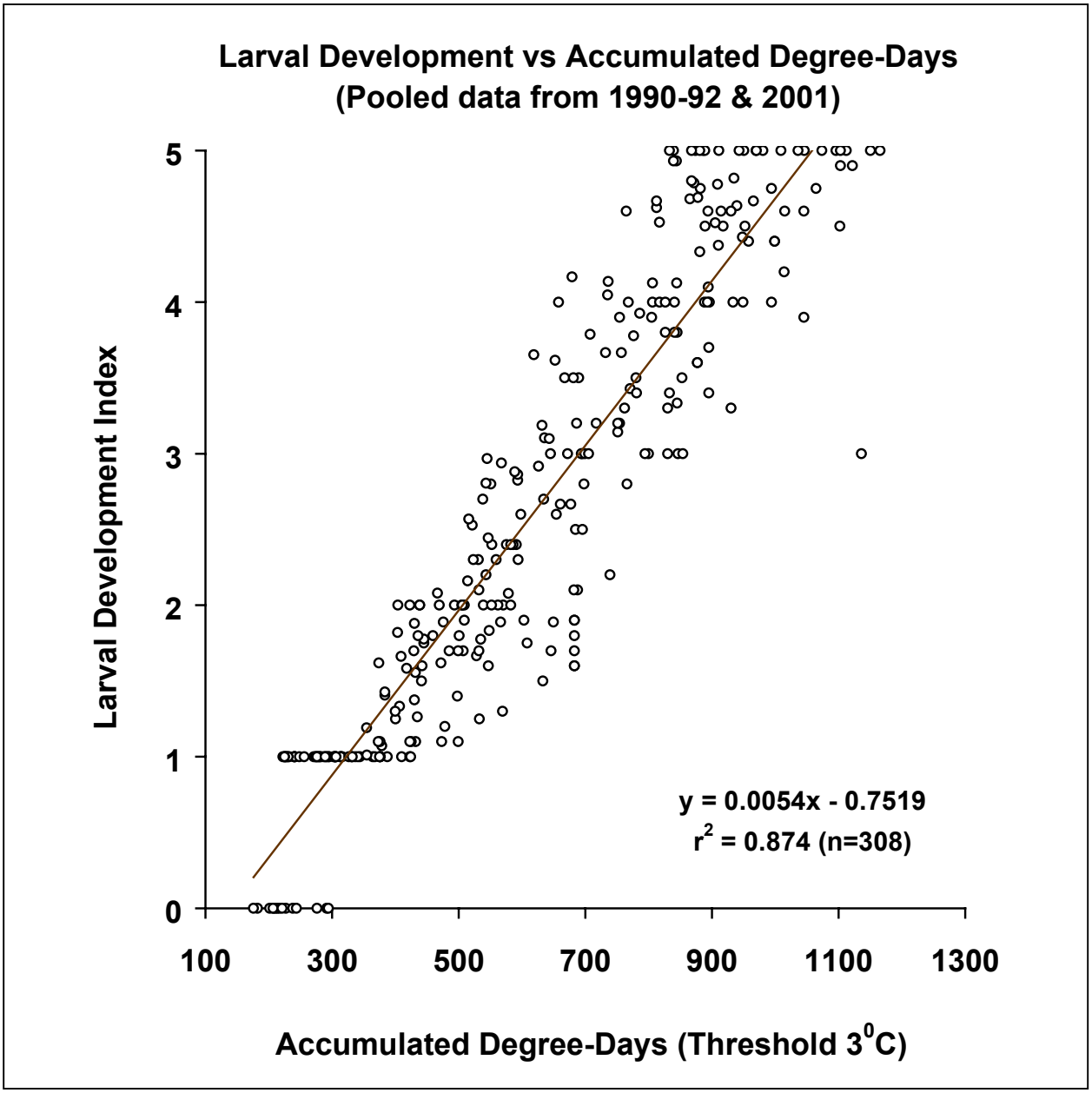


Figure 22 (Objective 7). Regression line of hemlock looper larval development versus accumulated degree-days for *pooled data* from the current study (2001) and historical DNRE data from southwestern New Brunswick in 1990-1992. Degree-days (threshold of 3^0C) were calculated from maximum-minimum temperatures at the nearest available weather stations, and *corrected* to appropriate latitude and elevation using the BioSIM model.

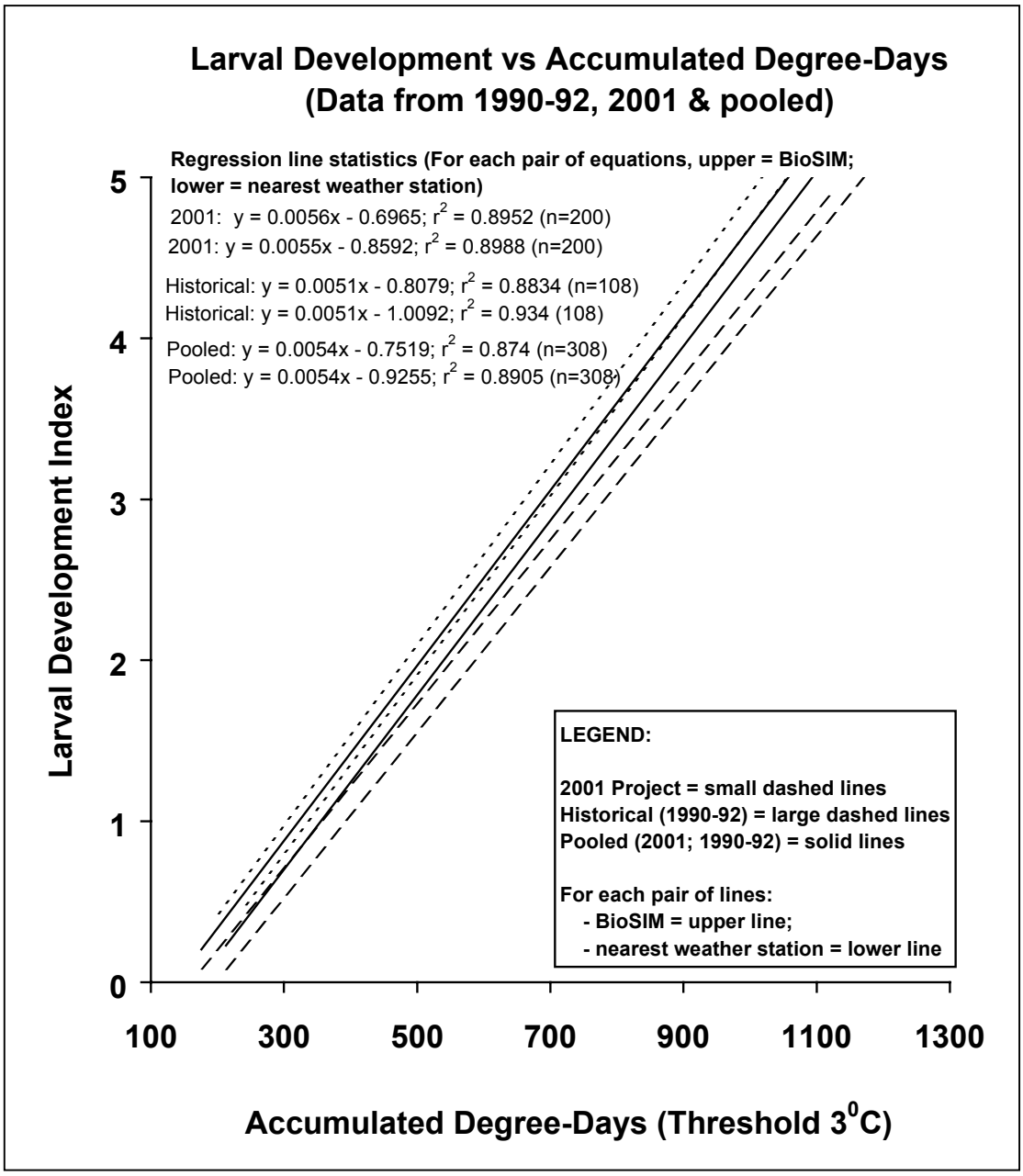


Figure 23 (Objective 7). Regression lines of hemlock looper larval development versus accumulated degree-days. There are 3 pairs of regression lines representing data from (1) the current study (2001), (2) historical data from 1990-92 and (3) pooled data from both sources. Each pair of regression lines represents degree-days (threshold of 3^0C) calculated from maximum-minimum temperatures from (1) the nearest available weather station and (2) ***corrected*** to appropriate latitude and elevation using the BioSIM model.

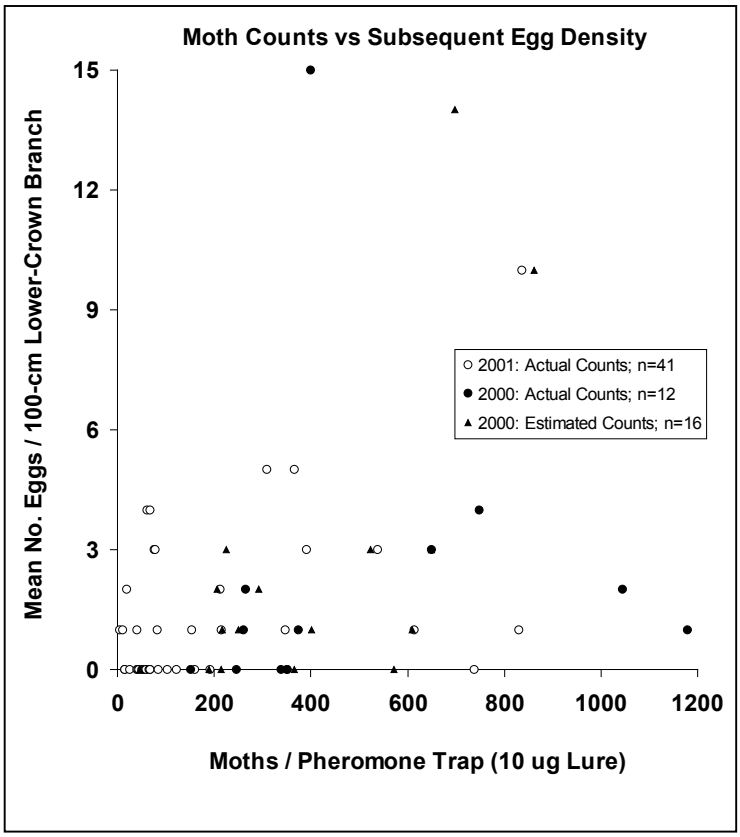
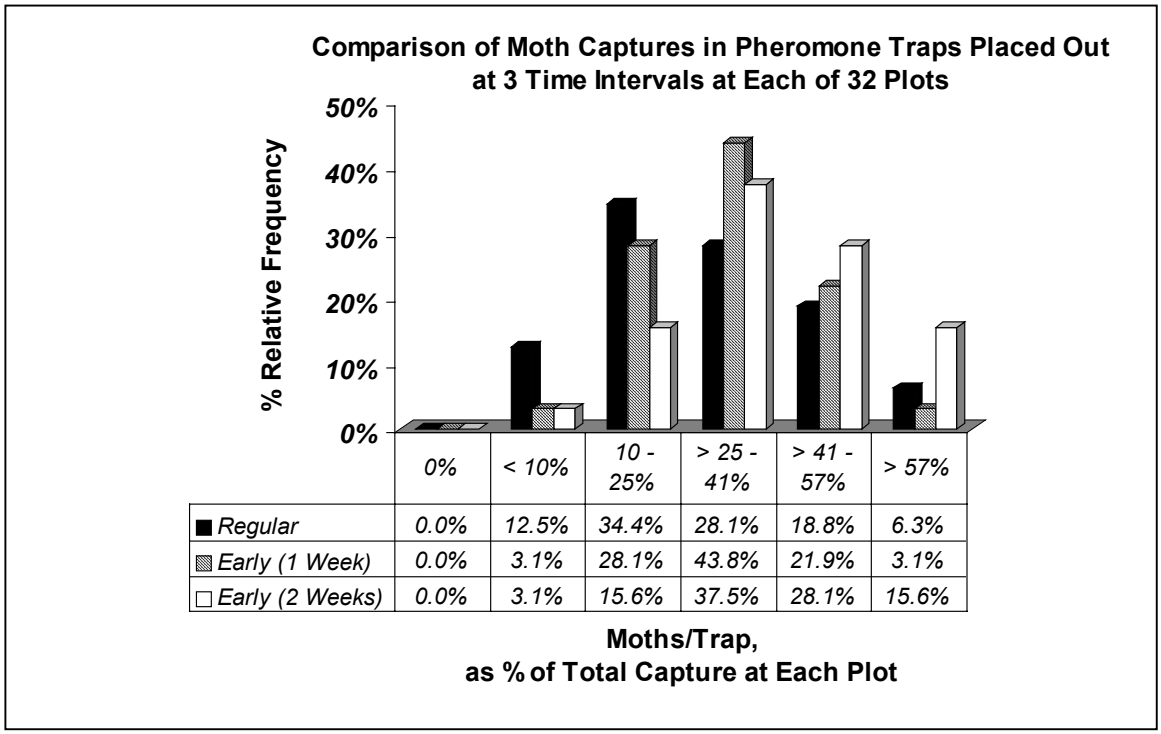


Figure 24 [Objective 8(a)]. Relationship between moth counts in pheromone traps with a 10-µg lure concentration and subsequent egg densities at the same plots. Graph represents *pooled* data from 41 plots originating from this project and 28 plots from the previous year's (2000) operational DNRE survey.



	0%	< 10%	10 - 25%	> 25 - 41%	> 41 - 57%	> 57%
Regular	0.0%	12.5%	34.4%	28.1%	18.8%	6.3%
Early (1 Week)	0.0%	3.1%	28.1%	43.8%	21.9%	3.1%
Early (2 Weeks)	0.0%	3.1%	15.6%	37.5%	28.1%	15.6%

Figure 25 [Objective 8(b)] . A comparison of moth captures in hemlock looper pheromone traps placed at 3 time intervals, at each of 32 plots. In theory, each of the 3 traps should average 33% of the total moths captured at each plot, provided there is no effect due to time of trap placement. In this graph that average represents the interval 25+ to 41% (33% $\pm$ 8% of total moths/plot).

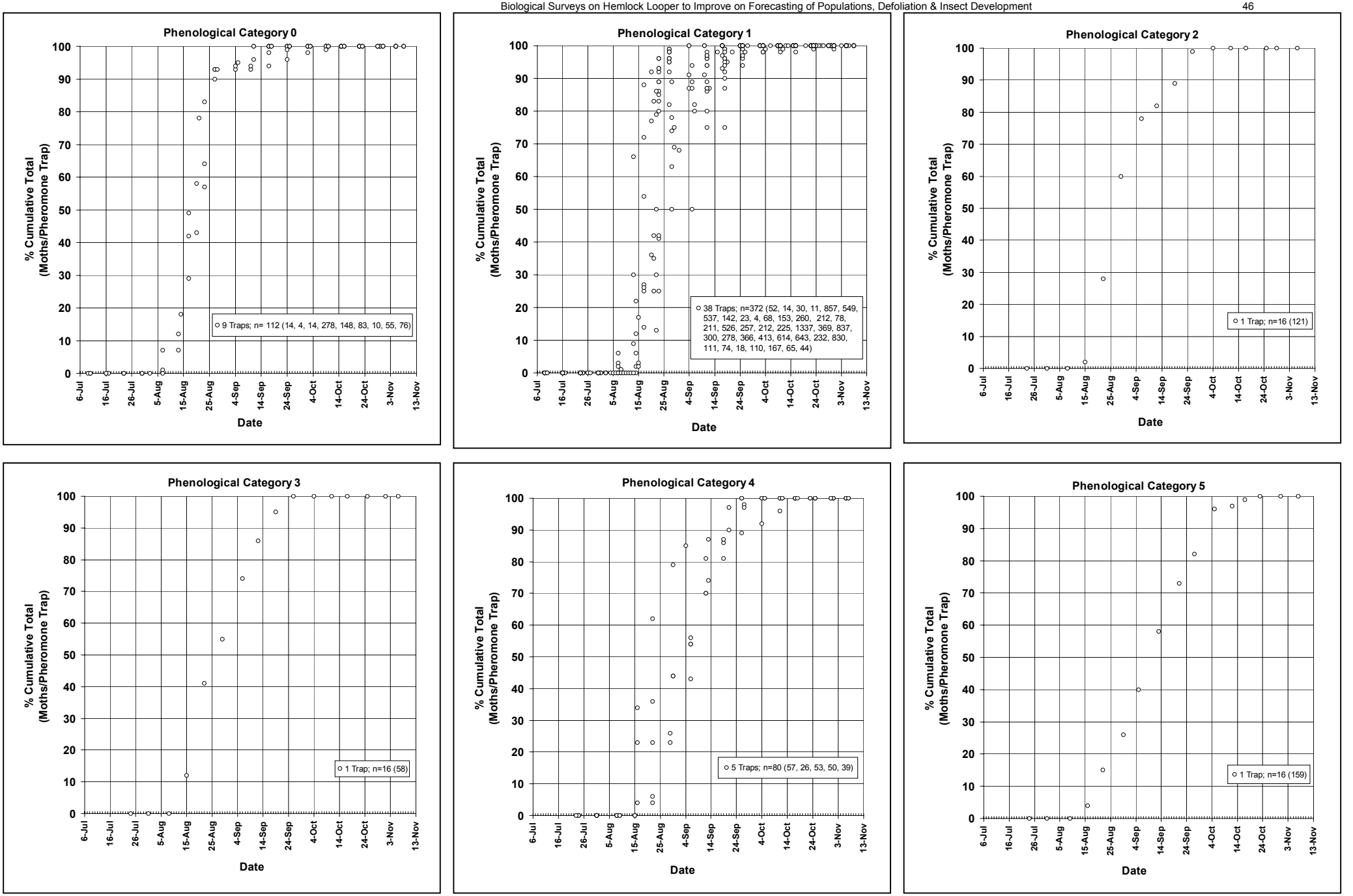


Figure 26 [Objective 8(c)]. Moth catches in monitored pheromone traps as a function of calendar date and distribution across the various spring phenological contours in New Brunswick. The legend for each graph depicts the number of traps monitored, total number of individual trap visits where moth captures were recorded (n) and the ultimate number of moths caught per trap (in brackets).

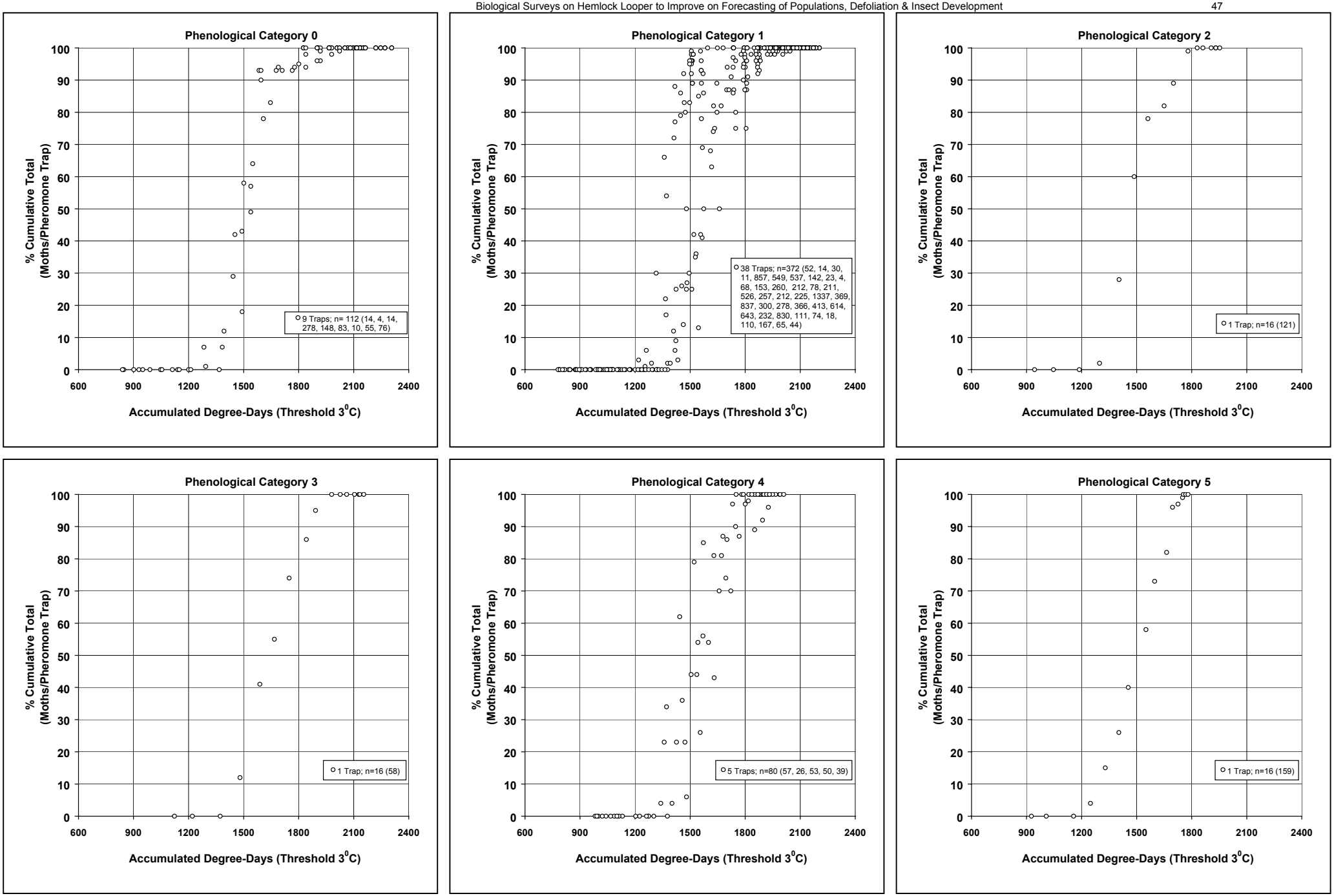


Figure 27 [Objective 8(c)]. Moth catches in monitored pheromone traps as a function of accumulated degree-days (threshold of 3^{0}C) and distribution across the various spring phenological contours in New Brunswick. The legend for each graph depicts the number of traps monitored, total number of individual trap visits where moth captures were recorded (n) and the ultimate number of moths caught per trap (in brackets). At each pheromone trap location accumulated degree-days was estimated using the BioSIM model.