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EVALUATION OF THREE EGG MASS SURVEY METHODS AND TWO BIOLOGICAL CONTROL AGENTS FOR GYPSY MOTH MANAGEMENT

By

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ABSTRACT

EVALUATION OF THREE EGG MASS SURVEY METHODS AND TWO BIOLOGICAL CONTROL AGENTS FOR GYPSY MOTH MANAGEMENT

By

Lyle Jay Buss

Three gypsy moth (*Lymantria dispar* L.) egg mass survey methods were evaluated in northwestern Lower Michigan. Although counts from the fixed-radius plot and 100-tree plot methods were better associated with subsequent defoliation, the timed walks required much less time to complete. However, when common egg mass density thresholds were used with egg mass counts to evaluate the study stands for hypothetical suppression, the 100-tree plot method provided fewer overspraying errors than the other 2 methods.

A Sunfleck Ceptometer® was used to measure leaf area index in hardwood stands at the Michigan State University Kellogg Experimental Forest. The ceptometer provided relatively consistent estimates, indicating that the ceptometer could be used to quantify insect defoliation.

Two biocontrol agents, *Cotesia melanoscela* (Ratzeburg) and *Entomophaga maimaiga* Humber, Shimazu and Soper, were released at Kellogg Forest in 1994 and 1995. Their impact on the gypsy moth population was very low, possibly due to low gypsy moth densities and other factors.

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INTRODUCTION

The gypsy moth (*Lymantria dispar* L.) is one of the most serious defoliators of hardwood trees in the eastern United States. Outbreaks of this exotic insect often result in heavy defoliation over large geographic areas, sometimes in excess of five million ha annually (Butalla 1996). Defoliation can annoy recreationists, and can damage or kill trees through growth loss, decreased vigor, and increased susceptibility to other stresses (Kulman 1971, Wargo 1977, Herrick and Gansner 1987). Management of the pest can be difficult. Population densities may vary within each infested stand, and estimates of local population density therefore may not accurately predict the amount of subsequent defoliation. Dispersal of early instars and mortality caused by predators, parasitoids, and pathogens can contribute to inaccurate predictions of defoliation based on population survey methods. Other management difficulties include introduction and evaluation of biological control agents and quantification of defoliation.

This project was initiated because gypsy moth populations were expanding into southwestern Michigan. Michigan State University's W. K. Kellogg Experimental Forest in Kalamazoo County provided an opportune site to evaluate some of the gypsy moth management tools that are currently being used. Gypsy moth had been detected in this area for several years, but had not yet caused noticeable defoliation. Research was also conducted in the Huron-Manistee National Forest in northwestern Lower Michigan where gypsy moth populations had gone through the initial outbreak in the early 1990s.

Population surveys measuring egg mass density and quality are frequently used to predict defoliation. Numerous protocols have been developed, each with various advantages and disadvantages (Ravlin et al. 1987). An ideal survey would be easy to conduct, require little time, and provide accurate predictions of defoliation. In Chapter 1, we present a comparison of 3 egg mass survey methods, discuss their time requirements, and evaluate their accuracy in predicting defoliation. The first method we evaluated was the fixed-radius plot (Kolodny-Hirsch 1986), which is generally considered to provide the most accurate and precise estimate of actual egg mass density (Kolodny-Hirsch 1986, Liebhold et al. 1991). We also tested the timed walk (Eggen and Abrahamson 1983), a method used by county gypsy moth suppression personnel in Michigan until 1997. The third method was the 100-tree transect, a method developed in Michigan that may combine the accuracy and precision of the fixed-radius plots with the time efficiency of the timed walks (Chilcote et al. 1994). These methods were evaluated in stands with low to high gypsy moth populations in the Huron-Manistee National Forest area.

Gypsy moth defoliation can be quantified in many ways. Aerial surveys are useful for determining broad categories of defoliation over large areas. Stand-level defoliation may be estimated visually, but this provides only qualitative estimates that are subject to observer bias. In Chapter 2, we describe the use of a Sunfleck Ceptometer to quantify leaf area between bud break and leaf fall in 9 hardwood stands. The objective was to evaluate consistency of the measurements and determine the potential of the ceptometer for measuring changes in leaf area caused by gypsy moth defoliation. This work was conducted at the Michigan State University W. K. Kellogg Experimental Forest.

Gypsy moth management often includes the release of biological control agents to regulate gypsy moth populations without the environmental effects associated with conventional insecticides. In Chapter 3, we present results from a study designed to establish the braconid wasp *Cotesia melanoscela* (Ratzeburg) and the fungus *Entomophaga maimaiga* Humber, Shimazu and Soper at Kellogg Forest, an area only recently invaded by gypsy moth. We also identified other gypsy moth natural enemies that were already present in the area. These natural enemies include previously established biocontrol agents that had moved along with the gypsy moth, and native generalist parasitoids and predators that were already present.

Much of the gypsy moth research conducted to date and most management strategies were developed in the northeastern United States where gypsy moth has been present since 1869. However, research findings and management options have not been adequately evaluated in recently infested areas like Michigan where different climate and cover types may cause changes in gyspy moth population dynamics. The goal of this project was to provide information that will help improve gypsy moth management in Michigan and other North Central states.

CHAPTER 1

Predicting Gypsy Moth (Lepidoptera: Lymantriidae) Defoliation: A Comparison of Three Egg Mass Survey Methods

INTRODUCTION

The gypsy moth, *Lymantria dispar* L., is a major defoliator of hardwood trees in the eastern United States. It is a pest not only in forests, but also in forested rural and urban areas. Defoliation has serious impacts on trees, including growth loss, decreased vigor, increased susceptibility to other stresses, and even mortality (Kulman 1971, Wargo 1977, Herrick and Gansner 1987). Suppression of high gypsy moth populations with biological or chemical insecticides is often necessary to keep damage and annoyance at acceptable levels. Defoliation in the United States between 1980 and 1995 ranged from 293,000 to 5.2 million ha (Butalla 1996). Annual suppression costs during this time period were as high as $22.5 million (Butalla 1996).

Decisions on the need for gypsy moth suppression using pesticides are based largely on population surveys used to predict subsequent defoliation. Inaccurate population estimates can lead to overspraying and associated economic and environmental impacts. Forest managers prefer to survey overwintering egg masses because they are present longer than other life stages and because sampling can be completed in time to plan suppression programs (Fleischer et al. 1991). Egg mass density thresholds and information on population trends (increasing or decreasing) are typically used to delineate treatment areas (Onken et al. 1996).

At least 13 different protocols have been used by managers in the United States to sample gypsy moth egg masses (Ravlin et al. 1987). Two common methods are the timed walk and the fixed-radius plot. In the timed walk (Eggen & Abrahamson 1983), the observer walks in a predetermined direction for 5 min (a usual distance of 0.16 km), counting all visible egg masses on the trunks and branches of trees. Then the observer reverses direction and repeats the survey back to the starting point and averages the 2 counts. Tables derived from regression equations are used to convert counts into density estimates of egg masses per acre (Eggen & Abrahamson 1983). This method is easy to learn and use and has a relatively low cost (Ravlin et al. 1987). However, the association between timed walk counts and actual egg mass density is confounded by differences among observers and habitats (Fleischer et al. 1991, Liebhold et al. 1991).

Observers using the fixed-radius plot method count egg masses within a circular plot usually 0.01 ha (1/40th acre) in size (Kolodny-Hirsch 1986). Egg masses are counted on ground debris and standing trees within the plot. This method is more labor-intensive than the timed walk, particularly in high populations, and often requires binoculars for counting masses high in tree canopies (Liebhold et al. 1994). However, the fixed-radius plot is considered the most accurate and precise method for estimating egg mass density (Kolodny-Hirsch 1986, Liebhold et al. 1991). Researchers commonly use fixed-radius plots to determine egg mass density, but in operational projects there may not be adequate resources to thoroughly survey large areas using this method.

Chilcote et al. (1994) recently developed an alternative egg mass survey method in Michigan based on a linear transect of 100 trees. The observer counts new egg masses on the lower 2 m of tree boles that are at least 5 cm in diameter. New and old egg masses can be easily distinguished since all egg masses are within reach of the observer. Chilcote et al. (1994) developed a two-step procedure for decision-making based on a goal of preventing more than 37% defoliation (the defoliation level of concern for many managers). In step one, control is not warranted if the average number of new egg masses per tree is less than or equal to 0.2, and control is recommended if the average is 2.0 or greater. If the average number of egg masses per tree is between 0.2 and 2.0, a regression model is used to predict defoliation and make the control decision. This model requires 3 variables: the proportion of the trees that are oak (*Quercus* spp.), average egg mass length, and the ratio of the number of new to old egg masses. Although this method has not yet been integrated into an operational program, it may reduce time needed to survey large areas while retaining a high level of accuracy.

Associations between egg mass density and subsequent defoliation are often complicated by the presence of pathogens. The nuclear polyhedrosis virus (NPV) has historically been a major factor regulating gypsy moth in the United States, typically causing crashes in outbreak-level populations. Another pathogen is the fungus *Entomophaga maimaiga* Humber, Shimazu and Soper. This larval pathogen mysteriously appeared in outbreak gypsy moth populations in 7 northeastern states in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990a). In 1991, Smitley et al. (1995) introduced this pathogen at several sites in northwestern Michigan. Favorable weather and subsequent introductions have resulted in widespread distribution of *E. maimaiga* in the northeastern region and in Michigan..

Our first objective was to evaluate the accuracy and time requirements of 3 egg mass survey methods, the 100-tree plot, fixed-radius plot, and timed walk in oak forests of Michigan's lower peninsula. We also examined the associations between defoliation and various egg mass variables. Our second objective was to evaluate gypsy moth mortality caused by *Entomophaga maimaiga* and NPV, and how these pathogens differed among gypsy moth populations.

METHODS

Study site

Study sites were established in 32 stands in the western half of the Huron-Manistee National Forest. These stands are located in the northwestern part of Michigan's lower peninsula in Manistee, Mason, Lake, Newaygo, and Oceana counties. Each study stand met the following criteria: the terrain was flat to gently rolling, there was no history of *Bacillus thuringiensis* application, at least 60% of the basal area was mature oak, and each stand was at least 10 ha in size. This area had been through at least 1 gypsy moth outbreak prior to this study (Defoliation maps, Michigan Department of Natural Resources).

Gypsy moth defoliation maps from the Michigan Department of Natural Resources and the USDA Forest Service, State and Private Forestry in St. Paul, Minnesota were used to generate a list of 100 potential study stands. Stands across a range of gypsy moth population levels were randomly selected from this list and sampled after verifying that they met the selection criteria. Dominant overstory species in the study stands were white oak (*Quercus alba* L.), red oak (*Q. rubra* L.) and black oak. (*Q. velutina* Lam.). The understory plants ranged in height from 0.1 to 3.0 m, and varied in species and abundance.

Egg mass surveys

One study site was established in each stand at least 50 m from the edge of the stand or road to avoid edge effects. Surveys were conducted between 20 December 1994 and 12 May 1995 before egg hatch occurred. The 3 egg mass surveys were conducted in random order at each site. The 0.01 ha fixed-radius plot and 100-tree plot were centered at a wooden stake marking each study site, and a timed walk was conducted in a random direction from the stake.

Fixed-radius plots. We carefully examined all trees within the 0.01 ha plot for egg masses, scanning the entire tree with binoculars. Logs and branches on the ground within the plot were overturned in search of egg masses.

Timed walks. After randomly choosing a direction, we tallied egg masses with a hand counter along a transect for 5 min. We repeated the count along the route back to the starting point and then averaged the 2 counts (Abrahamson 1987).

100-tree plots. We modified the 100-tree transect method of Chilcote et al. (1994) into a circular plot of the closest 100 trees around a central point so that counts could be expressed on an area basis. We began from the tree at the center of the plot and walked in a circular path, spiraling outward from the center until we had examined 100 trees that were at least 4 cm in diameter. A hand counter was used to count the new egg masses on the lower 2 m of each tree bole, and each tree was marked with chalk to ensure that it was not examined twice. Old egg masses were tallied at the same time and used to compute the percentage of new egg masses for the site. This percentage was then used to adjust counts from the fixed-radius plots and timed walks, where all egg masses were counted. To determine the ground area of each 100-tree plot, we measured the distance between the plot center and the farthest tree examined in each cardinal compass direction. The mean of these 4 "pseudo-radii" was used to calculate the circular area of the plot. Egg mass counts were then converted into a standard unit of egg masses per ha.

At each site we measured the length along the longest axis of the first 10-15 egg masses encountered. The time needed to complete each survey was recorded as well. Time needed to travel to the plot center was the same for all sampling methods, so only the time required to complete each survey was recorded.

Defoliation

We assessed defoliation at each study site during peak defoliation in July 1995 after most gypsy moths had pupated. Defoliation of trees within 30 m of the plot center was visually estimated and assigned to 1 of 8 percentage classes: 0 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 75, 76 to 90, and 91 to 100%. This follows the methods of Carter et al. (1991). Estimates were made separately for overstory oaks, and for understory oaks and witch-hazel (*Hamamelis virginiana* L.) shrubs. Oaks and witch-hazel are preferred hosts for gypsy moth (Liebhold et al. 1995). The percent cover of gypsy moth-preferred hosts in the understory was visually estimated as none/sparse, common, or abundant. We also noted the presence or absence of live gypsy moth larvae and pupae while doing defoliation estimates. If live larvae were found, we returned 2 wks later to verify the original defoliation level estimates. If more defoliation had occurred, we used the estimates from the later visit.

Pathogens

We collected the first 1 to 6 gypsy moth larval cadavers that we encountered on tree boles at each site, depending on how common the cadavers were. Each cadaver was taken from a different tree whenever possible. We collected a total of 108 cadavers at 28 sites (mean = 3.9 cadavers per site); no cadavers were present in 4 sites. Cadavers were placed into individual plastic containers, returned to the laboratory, and frozen for later analysis.

A sample of internal tissue and integument from each cadaver was smeared on a microscope slide and stained with lactophenol cotton blue fungal stain (Poinar and Thomas 1978). Slides were examined at 100X for *E. maimaiga* resting spores and conidia and at 400X for NPV polyhedral inclusion bodies (Hajek and Roberts 1992). Head capsule widths of approximately half of the cadavers were measured to determine the frequency of each larval instar (Jobin et al. 1992).

Statistical analyses

Comparison of egg mass measurements. Linear regression (Sokal and Rohlf 1995) was used to examine associations among egg mass length, percentage new egg masses, egg mass densities from the 3 methods, and time needed to complete each survey. Since egg masses were counted on all substrates (ground debris and trees) within the fixed-radius plots, we considered counts from this method to be measures of absolute egg mass density (Fleischer et al. 1991). Limited portions of the total substrate are searched with the timed walks and 100-tree plots; therefore they provide measures of relative density. Estimates of relative density (timed walks and 100-tree plots) were regressed as a linear function of absolute density (fixed-radius plot) estimates to evaluate associations among the 3 methods. The tables provided by Eggen and Abrahamson (1983) for converting timed walk counts into per ha densities are not considered valid when the average count for the 2 walks is less than 20 egg masses and the area is not experiencing its initial outbreak (Abrahamson 1987). Five sites did not meet these criteria and were therefore dropped from analyses that used timed walk counts. We examined associations among egg mass variables using parametric (Pearson product-moment) and nonparametric (Spearman's rank) correlations, as appropriate (Sokal and Rohlf 1995). Data were transformed [$\log_{10}(x+1)$] when necessary to meet the assumptions of normality and homogeneous variances.

Egg mass measurements and defoliation. Spearman's rank correlation was used to examine associations among the 8 defoliation classes, egg mass measurements, and abundance of understory vegetation. Defoliation categories were combined for further analysis due to low sample sizes in some categories. Final categories used in analyses were 0 to 30% and 31 to 100% defoliation. Forest managers often use the 30% threshold as the defoliation level where control treatment becomes needed (Liebhold et al. 1994, Onken et al. 1996). A t-test was used for each survey method to determine if mean egg mass densities differed between sites that experienced low defoliation (0-30%) and sites that experienced moderate to severe defoliation (31-100%).

Pathogens. We calculated the percentage of larval cadavers collected that were killed by each of the 2 major pathogens in each site. We then examined the associations between this variable and both defoliation level and egg mass density using Spearman's rank correlation. We used the nonparametric Wilcoxon two-sample test (Sokal and Rohlf 1995) to test for significant differences in pathogen infection rates within and between sites with low defoliation and sites with moderate to severe defoliation. Contingency table analysis (Sokal and Rohlf 1995) was used to determine if pathogen infection rates varied among instars and between sites where pupae and live larvae were present or absent. Significance of these models were tested with the likelihood ratio Chi-square (χ^2) test (Sokal and Rohlf 1995). All significance tests were evaluated with alpha = 0.05. Analyses were conducted using JMP statistical software (SAS Institute 1995).

RESULTS

Comparison of egg mass measurements

The 100-tree plot egg mass densities explained more of the variation in fixed-radius plot density estimates (R^2=0.58) (Figure 1) than did the timed walks (R^2=0.44) (Figure 2). Counts from the timed walks and 100-tree plots were also significantly associated with each other (R^2=0.32, Figure 3). Egg mass length varied inversely with egg mass density estimated with each survey method (Table 1). The percentage of new egg masses was not significantly associated with egg mass density or with length (Table 1).

Egg mass counts and defoliation

Defoliation maps from the Michigan Department of Natural Resources and the U.S. Forest Service indicated that defoliation was less than 75% in our 32 study sites in 1993, and was between 50 and 100% in 22 of our sites in 1994. During 1995, the year of this study, 26 sites had less than 50% defoliation. All of the sites with over 50% defoliation in 1995 also had over 50% defoliation in 1994, according to defoliation maps. Defoliation of susceptible host species in the understory (oaks and witch-hazel) was below 30% in 24 sites, between 31 and 75% in 6 sites, and over 75% in the remaining 2 sites in 1995. Initial statistical analyses were conducted using the original 8 categories of defoliation.

Overstory defoliation was significantly correlated with egg mass counts from the fixed-radius and 100-tree plots but not with timed walk counts (Table 2). Understory defoliation followed the same pattern; it was significantly correlated with the fixed-radius and 100-tree plot counts but not with the timed walk counts (Table 2). The amount of cover of susceptible host species in the understory (none/sparse, common, or abundant) was not significantly associated with the amount of defoliation in either the understory or overstory. Defoliation levels in the understory and overstory were positively correlated with each other (r_s=0.627, p<0.001).

We conducted further analyses to determine how well each method could be used to distinguish between low (0 to 30%) and moderate to heavy (31 to 100%) defoliation. A t-test was used to evaluate differences between mean egg mass densities in the 2 groups. Differences between means were significant for estimates from the fixed-radius (t=3.60; df=30; p<0.002) and 100-tree (t=3.54; df=30; p<0.002) plots, but not for the timed walks (t=1.45; df=25; p=0.16). Although there was overlap in egg mass densities between the 2 defoliation levels for all 3 survey methods, the overlap was greatest in the timed walk counts.

Percentage of new egg masses in a plot was correlated with overstory defoliation but not with understory defoliation (Table 2). Egg mass lengths were not significantly correlated with overstory or understory defoliation. Mean egg mass length ± SEM was 25.2 mm ± 1.2 and 23.7 ± 1.8 for lightly defoliated stands (0 to 30%) and moderate to severely defoliated stands (31 to 100%), respectively. Mean egg mass length was not significantly different between the 2 defoliation levels (t=-0.65, df=29, p=0.52).

Figures 4 - 6 show various combinations of 3 potential defoliation predictor variables, percentage new egg masses, egg mass length, and egg mass density from fixed-radius plots. The high degree of overlap of data in these graphs makes it difficult to select a set of variables that best predicts defoliation. Percentage new egg masses and egg mass density appear to be the best combination of predictors (Figure 4). All stands with defoliation over 30% had at least 84% new egg masses and 6563 egg masses/ha. Use of egg mass length added little benefit over using 1 of the other variables alone (Figures 5 and 6).

We used common egg mass density thresholds along with counts from the 3 egg mass survey methods to evaluate the 32 stands for gypsy moth suppression (Table 3), simulating the decision-making process of a manager. The timed walks and fixed-radius plots gave similar results, correctly recommending treatment for all the stands where defoliation actually exceeded 30%. However, these methods recommended treatment for 17 to 20 stands where defoliation was less than 30% and treatment would be unnecessary. We used the protocol in Chilcote et al. (1994) and Chilcote et al. (unpublished data) to make control decisions based on our 100-tree plot data. Since tree species were not recorded, we estimated that 60% of the trees in each stand were oak. Using the model, control decisions were made with the first step in 14 of the 32 sites, with 1 incorrect decision. The other 18 decisions were made in the second step using the regression model, with 10 incorrect decisions. All incorrect decisions erred on the side of recommending treatment when it would not have been necessary.

Time

Timed walk surveys took the least amount of time, always requiring 10 min. Fixed-radius plots took an average of 18.1 min to complete while 100-tree plots required the most time, averaging 30.0 min each. On average, 3.5 min (12%) of the total time required for the 100-tree plots were used to measure the pseudo-radii. Counting old egg masses during the 100-tree surveys did not increase the time required to any significant degree. As expected, regressions showed that egg mass density influenced the amount of time needed for the fixed-radius plots (R^2=0.38, F=18.4, df=31, p<0.001) and 100-tree plots (R^2=0.40, F=19.9, df=31, p<0.001) (Figure 7). The difference in time requirements between the 2 methods increased with increasing egg mass density (Table 4).

***Entomophaga maimaiga* and NPV**

We found gypsy moth cadavers in 28 of the 32 stands we surveyed. *E. maimaiga* was the primary mortality agent for 65 of the 109 cadavers collected. NPV killed 35 larvae, and 9 larvae died of unknown causes. The unknown deaths may have been caused by parasitoids, predators, stress, pathogens at undetectable levels, or unknown factors. *E. maimaiga* was found in 21 of the 28 sites where cadavers were collected and NPV was found in 16 of the 28 sites. We determined the instar of 69 of the 109 larvae. Most were sixth instars (74%), 22% were fifth instar, and 4% were fourth instars. Contingency table analysis indicated that the primary cause of mortality did not vary depending on instar (χ^2=0.333, p=0.85).

Percentage mortality caused by each pathogen was significantly associated with defoliation and preseason egg mass density (Table 5, Figure 8). As expected, NPV was primarily associated with high gypsy moth populations. We found NPV in all sites that had over 30% defoliation, but in only 8 of 20 sites with low defoliation. *E. maimaiga* had a negative association with defoliation. It was found in 17 of the 20 stands with low defoliation where we found cadavers, and in half of the high defoliation stands. Amount of mortality caused by either pathogen was not significantly correlated with egg mass length or the percentage of egg masses that were new ($p>0.27$).

We observed live gypsy moth larvae in 22 of the 28 stands where we collected cadavers. Stands where no live larvae were observed had significantly higher infection rates of *E. maimaiga* than NPV ($\chi^2=7.022, p=0.008$). The 6 stands where we did not find live larvae all had *E. maimaiga* infections rates of 75 to 100%. It follows that sites where live larvae were observed tended to have higher levels of NPV than stands with no live larvae ($\chi^2=3.969, p=0.046$). Contingency table analysis also indicated that defoliation category and the presence/absence of live larvae were independent ($\chi^2=0.575, p=0.45$).

We observed gypsy moth pupae in 11 of the 28 stands where we collected cadavers. As expected, pupae were more commonly found in stands with high defoliation ($\chi^2=11.476, p<0.001$). High infection rates of *E. maimaiga* tended to occur more frequently in stands where no pupae were found ($\chi^2=4.550, p=0.033$). NPV infection rates did not vary between stands where we did or did not observe pupae ($\chi^2=1.226$, $p=0.27$). Also, there was no significant association between the presence/absence of pupae and live larvae ($\chi^2=1.797, p=0.18$).

Percentage mortality attributed to *E. maimaiga* and NPV are measurements of the relative abundance of these 2 pathogens in each stand, but do not reflect the percentage of all larvae in the stands that were killed by these pathogens. These results should be interpreted cautiously since the abundance of pupae and live larvae were not quantified in each stand, although our observations are based on large amounts of time spent in the stands.

DISCUSSION

Egg mass densities determined by the 3 egg mass survey methods are not directly comparable because different areas are searched with each method. An observer using the fixed-radius plot method searches for egg masses in the canopies and under logs and rocks. The 100-tree plots include counts from only the first 2 m of tree boles. Egg masses counted during timed walks are primarily on the boles and undersides of major branches. However, the tables for converting timed walk counts to egg masses per acre were developed by regressing timed walk counts on counts from fixed- and variable-radius plots (Wilson and Fontaine 1978), which include counts taken on trees and the ground (Eggen and Abrahamson 1983). Egg mass density estimates from the timed walks underestimated those from the fixed-radius plots by an average of 16% in this study. Therefore, thresholds used for predicting defoliation will vary depending on the survey method used.

Liebhold et al. (1991, 1994) concluded that the timed walk should not be used for estimating gypsy moth egg mass densities because of low precision and high susceptibility to observer bias. Kolodny-Hirsch (1987) and Liebhold et al. (1991) considered the fixed-radius plot method to be the most cost-efficient and precise sampling method for determining egg mass densities in forest stands. In our study, egg mass density estimates from the timed walks and 100-tree plots were both significantly associated with fixed-radius plot counts (R^2=0.44 and 0.58, respectively).

The next question we addressed concerned relative accuracy of the 3 methods in predicting defoliation. Gypsy moth managers must be able to accurately delineate areas where defoliation will be high so that appropriate actions can be taken. Suppression programs are very expensive; over $64 million was spent between 1991 and 1995 under the USDA Forest Service Cooperative Suppression Program (Butalla 1996). Although egg mass surveys are the preferred method for predicting defoliation, results can be extremely variable. Gypsy moth managers tend to be risk-averse when determining treatment thresholds to minimize potential conflicts with homeowners and forest recreationists.

Correlations and t-tests showed that fixed-radius plot counts were significantly associated with defoliation, while timed walk counts were not. But when counts from these 2 methods were used along with egg mass density thresholds to evaluate stands for suppression, results were very similar. If our results from fixed-radius plots or timed walks had been used to evaluate the study stands for treatment, much unnecessary spraying would have occurred. The 100-tree plot method would have provided better results in planning a suppression program for our stands than would the timed walks or fixed-radius plots, using commonly accepted thresholds. Treatment thresholds were inappropriate, partly due to the declining gypsy moth populations. Maximum defoliation typically occurs just before peak egg mass density (Williams et al. 1991). Therefore, egg mass counts may indicate that the population is still growing, even though defoliation may have just peaked. If surveys are conducted at peak egg mass density, then treatments applied the following spring will probably exceed what is actually needed will be after peak defoliation has already occurred. Also, all early research has been conducted before *E. maimaiga* became a significant factor in gypsy moth population dynamics.

Many gypsy moth managers like the timed walk survey because it is quick, easy for observers to learn, and each walk covers a lot of area. However, counts can only be converted into estimates of egg masses per ha using the tables developed in New York (Eggen and Abrahamson 1983). We also observed several problems with the timed walk in the field. Egg masses were more apparent on certain tree species. For example, they were very obvious on smooth-barked species like bigtooth aspen (*Populus grandidentata* Michx.) and dark-barked tree species like black oak, but difficult to see on trees like white oak, which have flaky bark similar in color to the egg masses. The timed walk method was also more strongly influenced by the position of the sun. It was difficult to see egg masses when our transect faced the sun during a timed walk, whereas the other methods enabled us to search the trees from a slightly different angle. The timed walk was also more hazardous for the observer, who had to continually search tree crowns while walking over stumps and logs and through brush.

While the fixed-radius plot method also is easy to learn, it does require more time than a timed walk. In areas of low egg mass densities it required an average of only 5 min more time than the timed walks. The difference increased in high populations, where the fixed-radius plot took over 13 min longer than the timed walk (Table 4).

Another problem with the fixed-radius plot method was that the egg mass density estimate was biased upward when a plot had an individual tree with an extraordinary number of egg masses. This is, of course, why mean egg mass density for a stand should be determined by sampling multiple plots. The 100-tree plot was less affected by this problem because using 100 boles diluted the effects of individual trees.

Egg mass density estimates from the 100-tree plots and the fixed-radius plots were comparable in their association with defoliation, but the 100-tree plot method took nearly 12 min longer to complete. The major drawback to the 100-tree plot method was the time factor, requiring an average of 30 min per survey. This is not acceptable for forest managers, who have limited time and resources for conducting surveys over large areas. Chilcote et al. (1994) and Chilcote et al. (unpub. data) found that accuracy in predicting defoliation was as good using 25 trees (89.8%) as it was using 100 trees (86.2%). Using 25 trees may make this method more feasible for use in gypsy moth management programs. The 100-tree method also eliminates the need to determine the percentage of the egg masses that are new, since the observer can closely examine all masses and ignore the old ones. The fixed-radius plot and timed walk methods require this extra survey when both types of egg masses are present. This time savings can be substantial; surveying 100 egg masses to determine the percent of new and old took 4 to 16 min, depending on the population density.

The average area encompassed by the 100-tree plots was 0.09 ± 0.006 ha (0.23 ± 0.014 acres, mean ± SE), 9 times larger than the fixed-radius plots. Raw counts of new egg masses per 100 trees were highly associated with the counts expressed in units of egg masses per ha (R^2=0.93, F=430.1, df=31, p<0.0001). This suggests that measuring the area of each 100-tree plot may be unnecessary, which would save an average of 3.5 min per plot. Counts of new egg masses per 100 trees were as highly correlated with defoliation and other egg mass variables as were the counts after conversion to egg masses per ha. Using the transect version of the method (Chilcote et al. 1994) may better represent the stand because it covers a longer linear area, but may be more prone to observer bias if the observer tends to orient toward big trees or trees with many egg masses. Since only tree boles are searched in the 100-tree plot method, it may also underestimate egg mass density in low populations, since a higher proportion of egg masses in low populations tend to be laid in concealed locations on the ground (Eggen and Abrahamson 1983, Skaller 1985). Whether this error is enough to result in unexpected defoliation is unknown.

Predicting defoliation from egg mass counts is confounded by a variety of factors that affect gypsy moth eggs and larvae, including pathogens, parasitoids, weather, and the presence and distribution of host trees and shrubs. Fourteen study stands had estimates of over 2471 egg masses per ha (1000 masses/acre) but received less than 30% defoliation. Cadavers killed by *E. maimaiga* and NPV were found in nearly all of these stands, with the majority of the cadavers infected by the fungus. These pathogens likely had a big impact on larval populations and subsequent defoliation.

Diagnosing *E. maimaiga* and NPV in cadavers from the study sites provided an indication of relative infection rates among sites. As expected, pathogen infection rates were associated with population levels as measured by egg mass density and defoliation. Larval mortality due to NPV was positively correlated with defoliation and egg mass density. This is typical of the density-dependent nature of the virus, which typically is more prevalent when larvae are at high density (Wallner and McManus 1981). Mortality rates caused by *E. maimaiga* were negatively correlated with defoliation and egg mass density. Since NPV is not usually prevalent in low density populations, it was not suprising that *E. maimaiga* killed a higher percentage of the larvae found in these stands. Also, the fungus is not as dependent on high gypsy moth larval density as the virus, and high infection rates have been noted in low density gypsy moth populations (Hajek et al. 1996b). All of the stands where no live larvae were found had high (at least 75%) cadaver infection rates of the fungus relative to NPV, and most of these stands also had low defoliation. Stands where pupae and live larvae were not observed usually had greater than 50% infection of cadavers by *E. maimaiga*. *E. maimaiga* may have caused so much mortality in low-density populations that live larvae could not be easily found.

We initially considered that the association of *E. maimaiga* with defoliation and egg mass density was related to the spatial distribution and spread of the fungus since its original introduction. But *E. maimaiga* was found throughout the 5400 square km study area. All sites where we did not find *E. maimaiga* were within 5.2 km of a site where it was found. Although NPV was likely present in all study stands, we detected it in fewer stands than *E. maimaiga*.

Since this study was conducted while most gypsy moth populations in the area were declining, one must be careful in extrapolating results to healthy, growing populations. More research is needed to determine how egg mass variables can be used most effectively to predict defoliation across varying population levels. Also, a better understanding of how pathogens subsequently affect population density and defoliation is needed. This will become more important as *E. maimaiga* spreads across the range of gypsy moth.

Table 1. Coefficients of Pearson's product-moment correlation (r) and Spearman's rank correlation (r_s) among gypsy moth egg mass (EM) density, EM length, and the percentage of new EMs at 32 sites in the Huron-Manistee National Forest in Michigan. Significance levels: * = p<0.05; ** = p<0.01; NS = not significant.

	Correlation of EM density with:	
Survey Methods	EM length (r)	Percentage new EMs (r_s)
Fixed-radius plots	-0.544*	NS
100-tree plots	-0.391*	NS
Timed walks	-0.394*	NS

Table 2. Coefficients of Spearman's rank correlation for comparisons between gypsy moth egg mass (EM) variables and defoliation at 32 sites in the Huron-Manistee National Forest in Michigan. Egg mass density was measured in each plot using three survey methods. Significance levels: * = p<0.05; ** = p<0.01; NS = not significant.

	Correlation of EM variables with:	
Egg mass variables	Overstory defoliation	Understory defoliation
EM density (Fixed-radius plots)	0.564**	0.472**
EM density (100-tree plots)	0.701**	0.640**
EM density (Timed walks)	NS	NS
EM length	NS	NS
Percentage new EMs	0.366*	NS

Table 3. Potential control decisions for 32 study stands based on gypsy moth egg mass (EM) density, and the actual defoliation that occurred. These thresholds are commonly used by gypsy moth managers to determine if control is needed to prevent defoliation from exceeding 30%. The protocol for the 100-tree plot method is described in the text of this paper.

		No Control Recommended		Control Recommended	
	Treatment threshold[1]	Actual defoliation		Actual defoliation	
Survey method	EM/ha (EM/ac)	0-30% No. of stands)	31-100%[2] No. of stands)	0-30%[3] No. of stands)	31-100% No. of stands)
Timed walks	741 (300)	5	0	19	8
	1236 (500)	7	0	17	8
Fixed-radius plots	1236 (500)	4	0	20	8
	2471 (1000)	6	0	18	8
100-tree plots		14	0	10	8

[1] For treatment thresholds using timed walks and fixed-radius plots see Onken et al. (1996). For 100-tree plots see Chilcote et al. (1994).
[2] This column represents stands where control measures would not have been recommended and would therefore have had defoliation exceeding 30%.
[3] This column represents stands that would have been treated unnecessarily.

Table 4. Average time needed to conduct gypsy moth egg mass (EM) surveys using fixed-radius and 100-tree plots at various population levels. Egg mass density categories for the two methods are not directly comparable because the methods sample different areas.

Survey method	EM density range EM/ha (EM/ac)	Time range (minutes) Min.	Max.	Mean time ± SE minutes
Fixed-radius	0 - 1,236 (0 - 500)	8.0	25.5	15.1 ± 2.7
	1,237 - 2,471 (501 - 1,000)	11.3	20.0	15.5 ± 1.5
	2,472 - 24,710 (1,001 - 10,000)	14.0	23.5	17.6 ± 1.0
	>24,710 (>10,000)	15.8	31.8	23.5 ± 1.9
100-tree	0 - 124 (0 - 50)	17.0	31.5	22.9 ± 2.5
	125 - 494 (51 - 200)	19.3	32.8	24.9 ± 1.4
	495 - 1,236 (201 - 500)	17.5	50.0	31.5 ± 3.7
	>1,236 (>500)	26.3	52.5	37.0 ± 2.7

Table 5. Spearman's nonparametric rank correlation coefficients among the percent of gypsy moth larvae killed by the pathogens *E. maimaiga* and NPV, egg mass (EM) density counts from three survey methods, and subsequent defoliation. Egg mass surveys were conducted in the spring of 1995, and pathogens and defoliation were evaluated in the summer of 1995. n=28 stands. Significance levels: * = $p<0.05$; ** = $p<0.01$; NS = not significant.

	Pre-season EM density determined by:			**Defoliation**	
% mortality caused by:	Fixed-radius plots	100-tree plots	Timed walks	Overstory	Understory
E. maimaiga	-0.454*	-0.617**	NS	-0.648**	-0.462*
NPV	0.436*	0.509**	NS	0.693**	0.439*

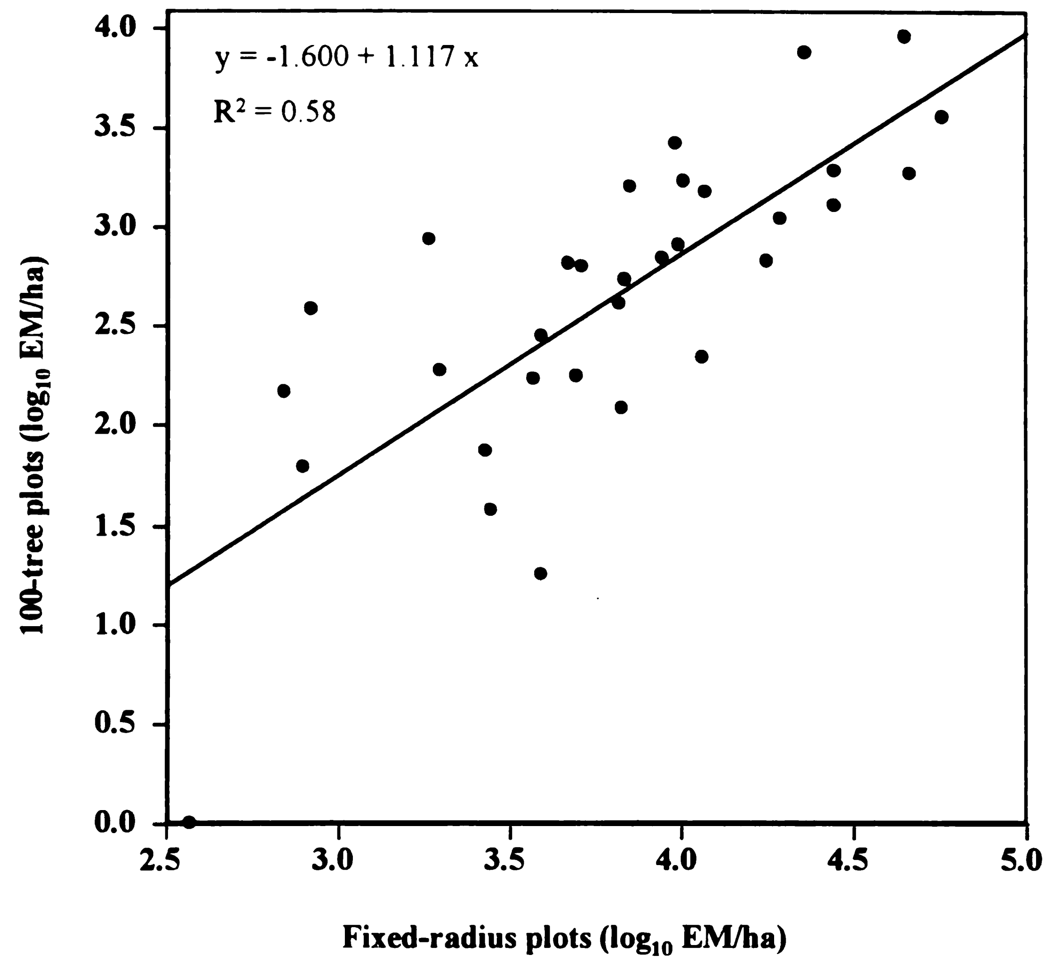


Figure 1. Linear regression of gypsy moth egg mass (EM) densities based on fixed-radius and 100-tree plot surveys of 32 sample plots in the Huron-Manistee National Forest, MI. The slope and intercept were significantly different from 0 ($F=42.1$, $p<0.0001$, $SE=0.661$; $t=-2.42$, $p=0.022$, $SE=0.172$, respectively).

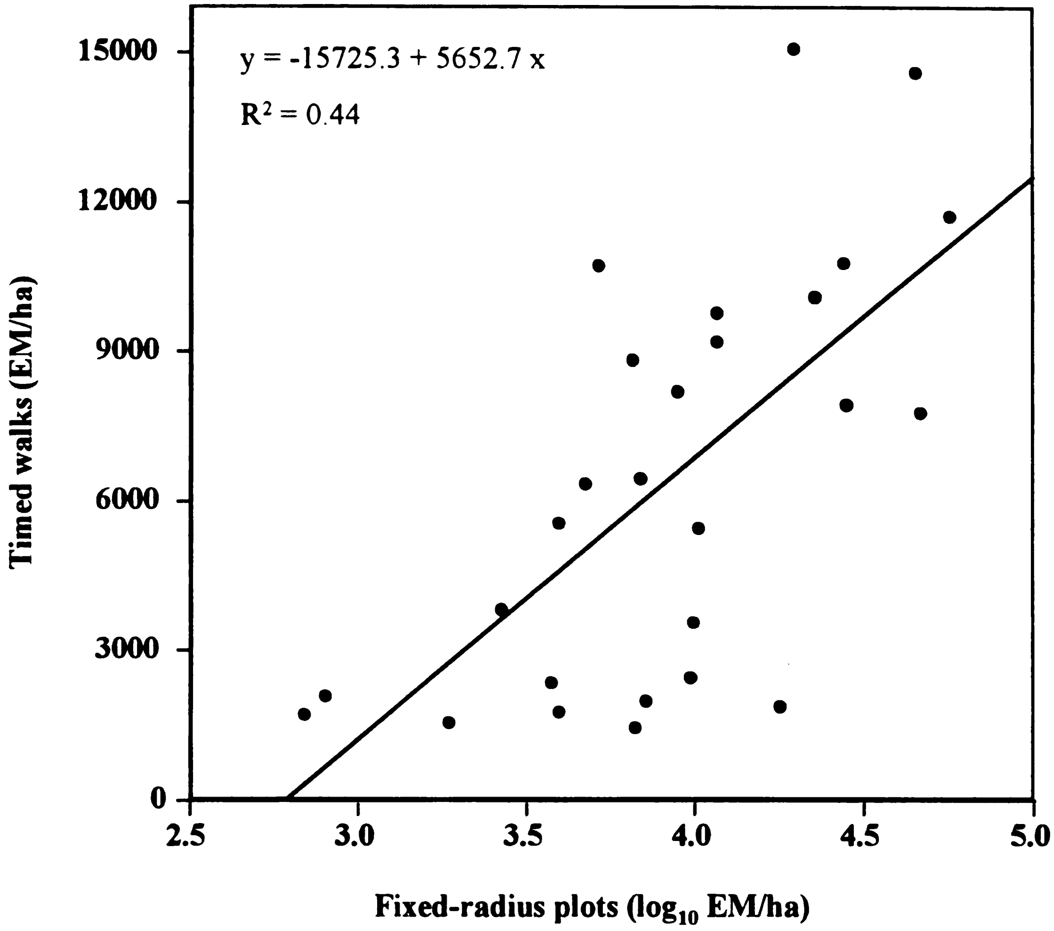


Figure 2. Linear regression of gypsy moth egg mass (EM) densities based on fixed-radius plot and timed walk surveys of 27 sample plots in the Huron-Manistee National Forest, MI. The slope and intercept are significantly different from 0 ($F = 19.3$, $p = 0.0002$, SE=1285.2; $t = -3.10$, $p < 0.005$, SE=5074.0, respectively).

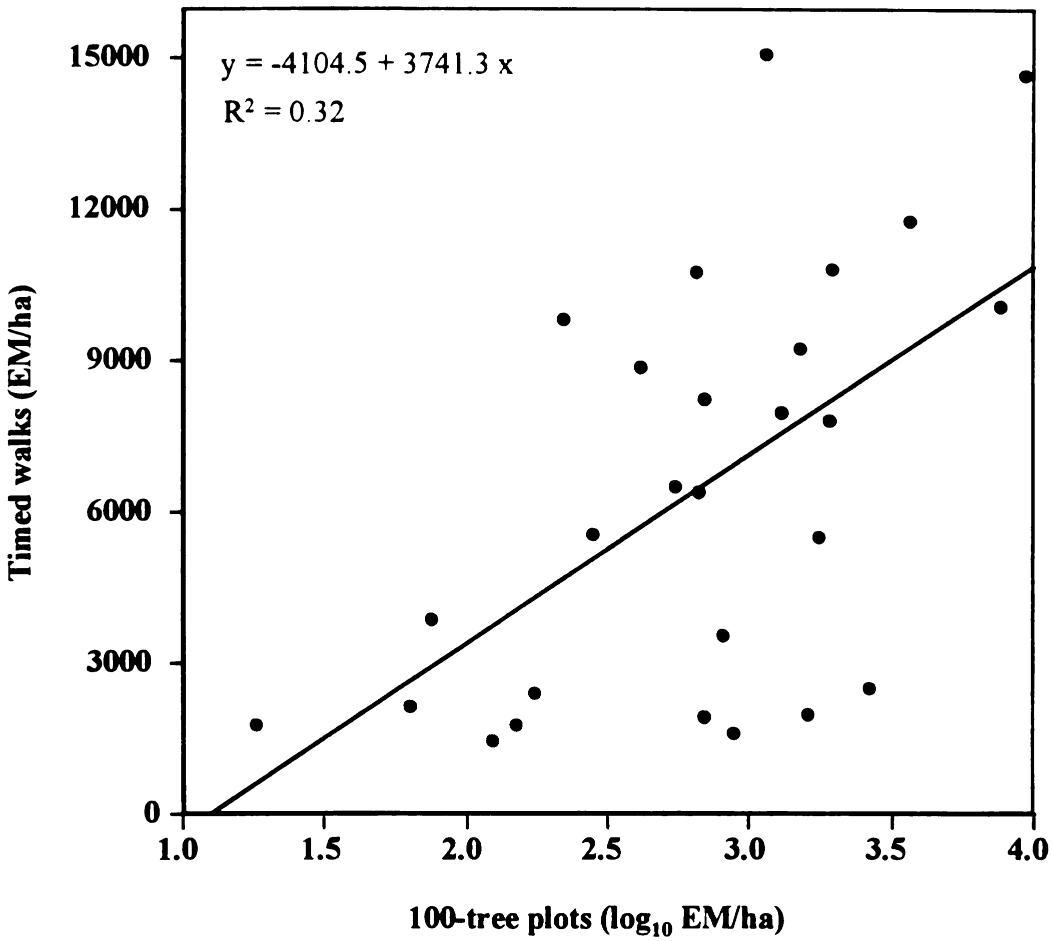


Figure 3. Linear regression of gypsy moth egg mass (EM) densities based on 100-tree plot and timed walk surveys of 27 sample plots in the Huron-Manistee National Forest, MI. The slope is significantly different from 0 (F = 12.0, $p < 0.002$, SE=3110.6), but the intercept is not (SE=1078.8).

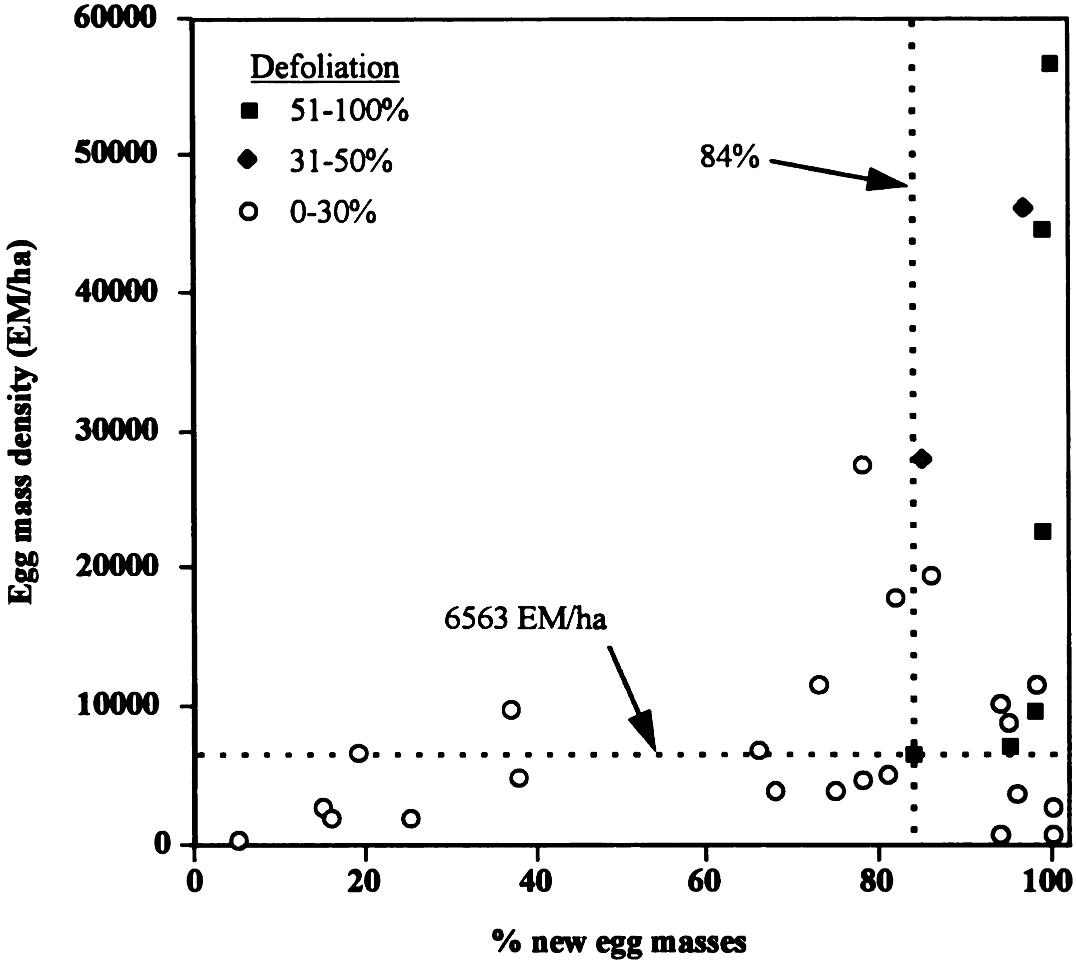


Figure 4. Percentage of new gypsy moth egg masses versus egg mass density from the fixed-radius plots. Defoliation was grouped into three categories. All stands with over 30% defoliation had at least 84% new EM and 6563 EM/ha, as indicated by the dotted lines. n=32 sites.

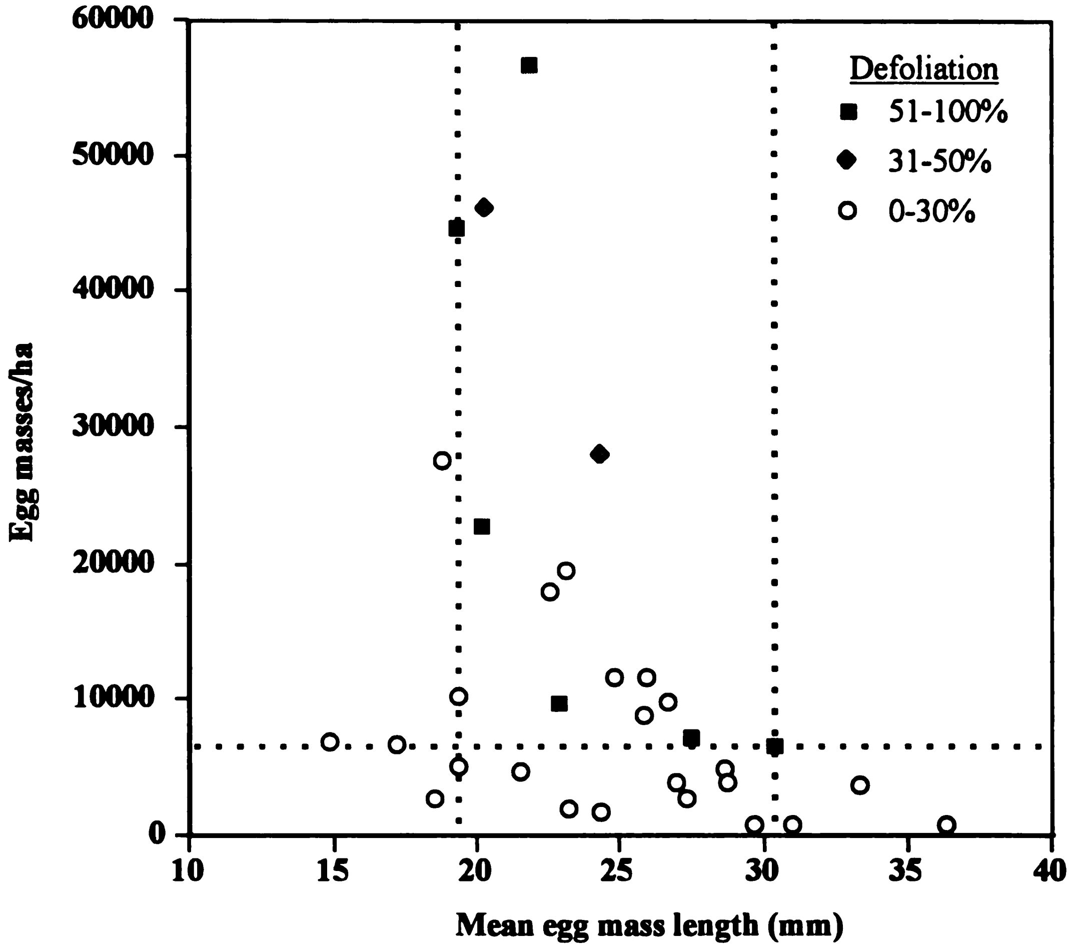


Figure 5. Gypsy moth egg mass (EM) length versus EM density in the fixed-radius plots. Defoliation was grouped into three categories. All stands with over 30% defoliation had mean EM lengths between 19.3 and 30.4 mm, and at least 6563 EM/ha, as indicated by the dotted lines. n=31 sites.

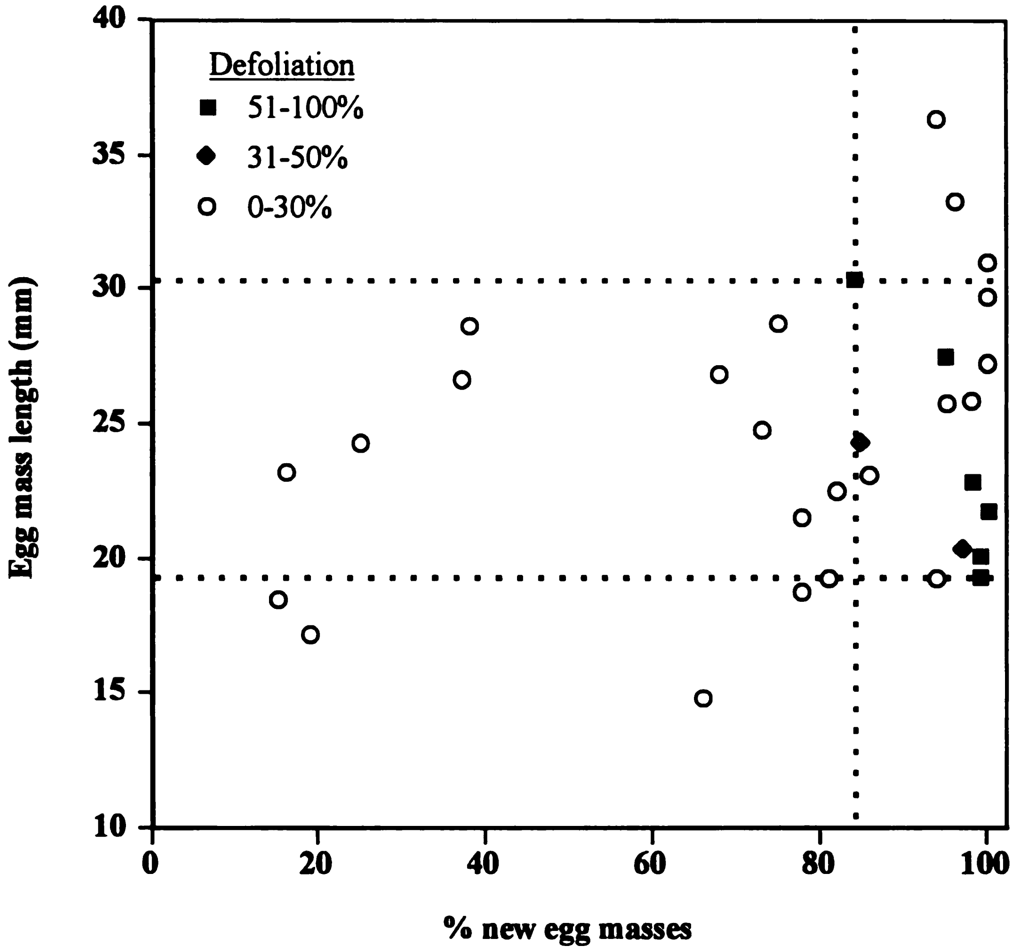
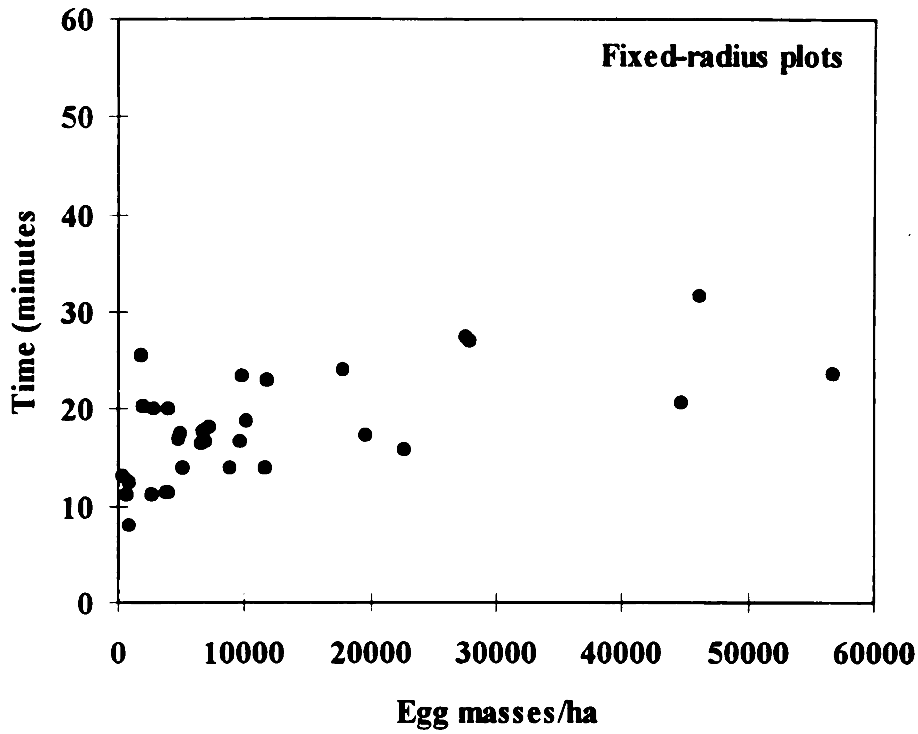


Figure 6. Percentage of new egg masses versus egg mass length. Defoliation was grouped into three categories. All stands with over 30% defoliation had at least 84% new EM and mean EM lengths between 19.3 and 30.4 mm, as indicated by the dotted lines. n=31 sites.



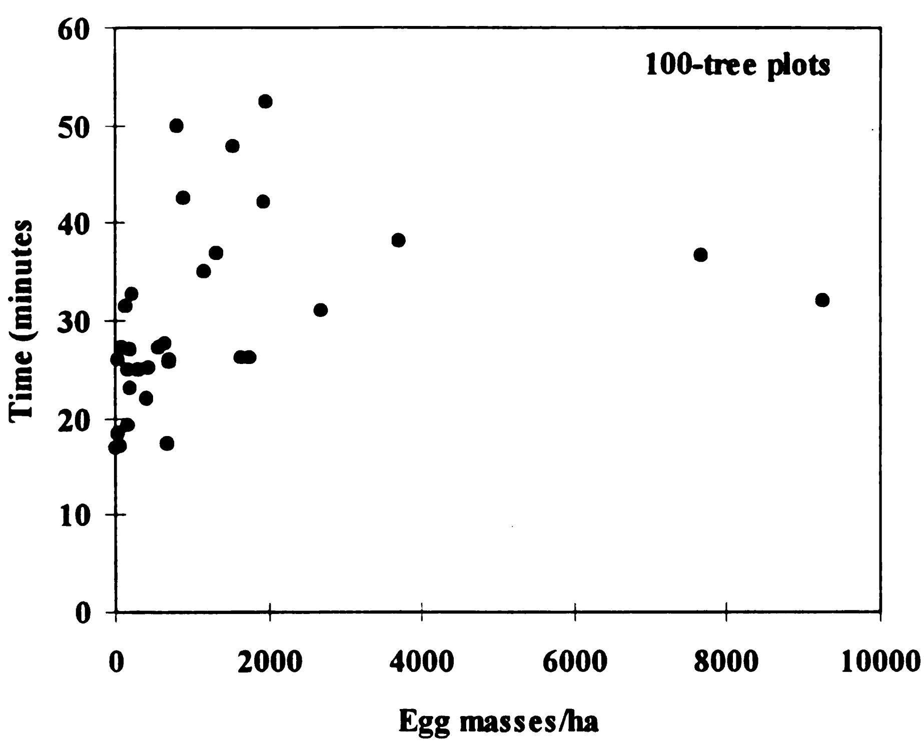


Figure 7. Time required to conduct gypsy moth egg mass surveys using fixed-radius plots and 100-tree plots by egg mass density. n=32 sites.

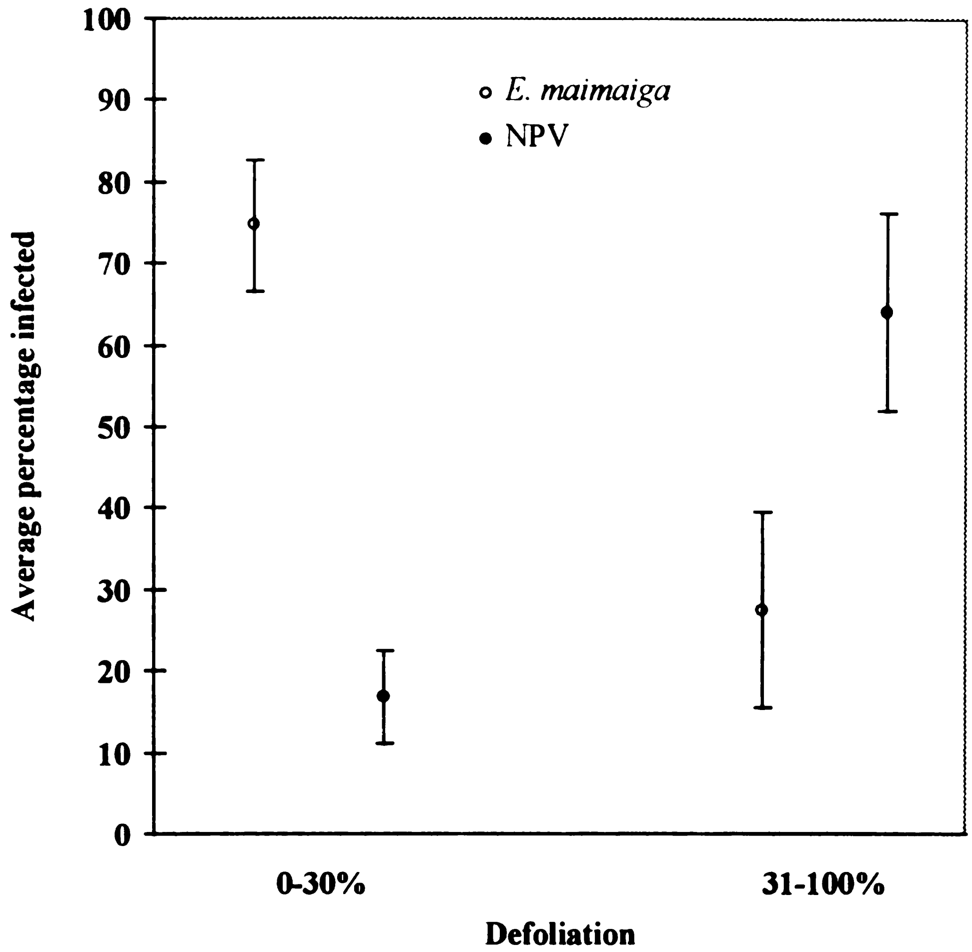


Figure 8. Average percentage mortality caused by *Entomophaga maimaiga* and NPV in gypsy moth cadavers collected at 28 study sites in mid-July, 1995 in the Huron-Manistee National Forest, MI. Error bars indicate SEM. Average percentage of mortality varied between the 2 defoliation levels for *E. maimaiga* (p=0.004) and NPV (p=0.001). Percentage mortality varied between the 2 pathogens for both low (p<0.001) and high (p=0.033) defoliation. Tests were conducted with the nonparametric Wilcoxon two-sample test.

CHAPTER 2

Estimation of Seasonal Leaf Area Index with a Sunfleck Ceptometer

INTRODUCTION

Leaf area is frequently measured in forest stands because of its close association with photosynthesis, transpiration, and forest productivity (McLeod and Running 1988). Leaf area index (LAI) is a measurement of the one-sided leaf area per unit ground area (m^2m^{-2}). Direct estimation of LAI using litterfall analysis or destructive sampling is time-consuming and labor intensive (Bolstad and Gower 1990, Chason et al. 1991, Huston and Isebrands 1995, Vose et al. 1995). Allometric equations relating LAI to sapwood basal area have been developed for many tree species. However, these equations require destructive sampling and are often specific to the geographic area and year of development (Gazarini et al. 1990, Burton et al. 1991, Huston and Isebrands 1995, Vose et al. 1995). The amount of light transmitted through the canopy can be converted to LAI based on the Beer-Lambert Law. This is an accurate and cost-efficient method of indirectly quantifying LAI, especially when many stands must be sampled (Bolstad and Gower 1990).

Many studies have evaluated the Sunfleck Ceptometer® (Decagon Devices, Pullman, WA) for use in measuring canopy transmittance (e.g. Pierce and Running 1988, Bolstad and Gower 1990, Vose and Swank 1990, Vose et al. 1995). This device measures photosynthetically active radiation (PAR) in the wavelength range of 400-700 nanometers with a linear band of 80 sensors spaced at 1 centimeter intervals. It calculates the arithmetic mean of values from each of the 80 sensors. Several readings can be taken and then automatically averaged and stored in an internal data storage unit.

A potential use of the Sunfleck Ceptometer may be to quantify defoliation, such as that resulting from herbivorous insect feeding. Theoretically, the amount of leaf area lost to a defoliator could be quantified by measuring the increase in light transmittance through a canopy. One of the major defoliating insect pests of hardwood forests in the northeastern United States is the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). Outbreak populations of this insect can defoliate millions of hectares of forest in a single year (Butalla 1996). Defoliation is typically estimated visually and assigned to qualitative classes such as low, moderate, and high (Talerico 1981). Observer bias is frequently a problem, however, and it may be difficult to consistently compare qualitative estimates of defoliation (Jobidon 1992).

We used a ceptometer to measure LAI periodically from initial leaf expansion to autumn leaf drop in several hardwood stands representing a range of ages and species. The objectives of this study were to examine seasonal within-stand variation in LAI and to evaluate the potential use of a ceptometer to estimate gypsy moth defoliation. We also examined potential associations between LAI and stand variables including basal area, tree density, and average trunk diameter.

METHODS

Study site

This study was conducted at the Michigan State University W. K. Kellogg Experimental Forest in Kalamazoo Co., Michigan (latitude, 42°21'29.0" to 42°22'22.8"; longitude, 85°20'50.1" to 85°22'22.4"). The 240-ha forest consists of a variety of genetic plantations and naturally regenerated hardwood stands. Nine hardwood stands that were part of a related study on gypsy moth biocontrol (see Chap. 3) were chosen for this study (Table 1). An experimental plot approximately 0.4 ha in size was established in each stand in May 1994 and the plot center marked with a stake. Wire flags were placed 3 m apart along transects radiating outward 30 m from the stake in the 4 cardinal directions (40 flags per plot, Figure 1). The 9 stands were grouped in blocks of 3 based on their spatial distribution within Kellogg Forest. Stand sampling order was randomized on each sampling date, first by block and then by stand within block.

PAR readings were taken with a Sunfleck Ceptometer at approximately 1 - 2 wk intervals from 11 June to 22 October 1994, and 29 April to 13 October 1995. Sampling was done on clear (cloud-free) days, primarily between the hours of 1100 and 1430 EDST, although sometimes sampling had to start as early as 1000 and end as late as 1615. Graphical inspection of the data indicated that time of day did not introduce a significant bias to the LAI estimates. Each stand was sampled 4 - 7 times in 1994 and 9 - 10 times in 1995. Readings were taken at each flagged point with the ceptometer held horizontally 4 feet above the ground. The observer slowly rotated in a circle, taking a reading every 45 degrees. The average of the 8 values at each flag was stored in the ceptometer's memory, resulting in a total of 25,600 individual PAR readings per plot on each sampling date. When clouds were present around the sun, sampling was halted until they had passed. Data was downloaded to a computer at the end of each day.

A measure of above-canopy (or total incoming) PAR was taken in a nearby clearing immediately before and after taking readings within each stand. A diffuse PAR reading, measuring reflected and scattered light, was obtained in a clearing by using the ceptometer's single sensor mode and shading the tip with an index card held approximately 1 m above the sensor (Decagon Devices 1987).

Stand variables were measured in four 0.01-ha fixed-radius plots, 1 along each of the 4 transects within each experimental plot, with each centered 10 m from the plot center (Figure 1). Basal area (cross-sectional area of tree trunks at breast height, or 1.37 m above ground), tree diameter at breast height (DBH), density, and height were measured for all trees at least 2.5 cm DBH, and means were calculated for each stand (Table 1).

Data analysis

No leaves were present on the trees during the initial sampling period in 1995 (29 April). These readings were used to estimate the woody area index (WAI), the tree bole and branch portion of the total LAI in each stand. LAI was calculated using equations based on the Beer-Lambert Law, where LAI is directly proportional to the logarithm of the canopy transmittance (Q_1/Q_0) (Decagon Devices, 1987). Q_1 is the PAR reading under the canopy, and Q_0 is the above-canopy PAR reading. Consecutive sets of above-canopy PAR and diffuse PAR readings were averaged and used in the LAI equations with beneath-canopy PAR readings recorded during that time frame. WAI was subtracted from the LAI estimates of each stand to yield LAIs representing foliage only. Final readings each year were taken after leaf abscission had begun.

Ceptometer transects in 5 of the 9 stands extended slightly beyond the edge of the stand either into a clearing or a different stand. Some individual sampling points at the end of transects were located outside the main stand because some plot centers were too close to the stand edges. We dropped 1 - 10 points from the calculation of LAI in 5 stands because they fell outside of the intended stand. This was done consistently for all sampling dates during both years of the study.

Associations among stand variables, WAI and LAI were evaluated using Pearson's product-moment correlation (r) (Sokal and Rohlf 1995). We computed the coefficient of variation (CV) of LAI estimates for each plot for sampling periods between 11 June and 4 September for each year to examine within-season variability of the LAI estimates. This was the period after full leaf expansion and before leaf fall, where LAI was expected to remain relatively consistent. A t-test was used to determine if average stand LAIs varied significantly between 1994 and 1995 for samples taken between these same dates. All significance tests were conduted with α=0.05, using JMP Statistical Software (SAS Institute 1995).

RESULTS

There were only 3 significant correlations among stand variables: basal area and stand age (r=0.872, p=0.002), tree density and average DBH (r=-0.782, p=0.013), and average height and average DBH (r=0.965, p<0.0001). All 3 associations were expected. Woody area index (WAI) was correlated with stand basal area (r=0.841, p=0.005) but not with age, average DBH, density, or height. Neither maximum LAI nor mean summer LAI were significantly associated with stand variables or WAI consistently in both years. Maximum stand LAIs in 1994 and 1995 were significantly correlated with each other (r=0.741, p=0.022), as were average summer LAIs (r=0.773, p=0.015).

As expected, measured LAI increased quickly until early-June, remained relatively stable during the summer and then began declining in early September (Figure 2). The highest average summer LAIs (between 11 June and 4 Sept.) were in stands 40, 42 and 53 (Table 2). These were older stands (50 to 75 years old) with closed canopies dominated by oak. The range of LAI estimates in this study (3.8 to 7.5) correspond well with those reported in other hardwood stands (Bolstad and Gower 1990, Chason et al. 1991, Vose et al. 1995).

Average summer LAIs differed between 1994 and 1995 in stands 15 (t= -2.46, df=6, p=0.049), 16 (t= -3.36, df=7, p=0.012) and 40 (t= 4.85, df=9, p=0.0009). There were no significant differences in LAI between years for the other 6 stands. The 2 English oak stands (15 and 16) had the lowest LAIs in 1994 (Tables 2 and 3). These stands also had the greatest increase in LAI over the 2 yrs. Trees in these plantations averaged 17 ft in height, and grew vigorously, probably accounting for the significant increase in LAI from 1994 to 1995. LAI values were quite similar for the 2 stands, as expected for stands of similar age, height and stocking density. The large decrease in LAI in stand 40 may have occurred after several trees blew down during a windstorm.

Coefficients of variation (CV) for stand LAI values during full leaf expansion ranged from 6.9 to 23.5% (Table 2). Low CV indicated that LAI values estimated with the ceptometer were consistent. CV were consistently low in stands 40 and 53 but there was no consistent association among CV and stand variables over the 2 yrs.

The ceptometer provided a relatively quick way to measure PAR transmittance in forested stands. Seven to 35 min were required to take the 40 transmittance readings in each stand, with an average of 14 min (SEM=0.461, n=141) and a mode of 12 min (Figure 3). Average time decreased from 17 min in 1994 to 13 in 1995, showing that observer experience speeded up the sampling. Many of the high time values occurred when the observer waited for clouds to move away from the sun. Clouds were common in this area due to the effects of nearby Lake Michigan.

DISCUSSION

Results from other studies that have evaluated the association of LAI with stand attributes such as basal area, stand density, average diameter, and tree height have varied. Liebhold et al. (1988) found that oak LAI measured from canopy photographs was significantly correlated with basal area measurements. Gresham (1982) and Dalla-Tea and Jokela (1991) noted an association between litterfall and basal area in pine stands. But Vose et al. (1995) found no clear associations between litterfall and several stand variables in hardwood stands, possibly because of the small range of the variables in their study. The primary reason for the lack of significant associations in our study may be that species composition varied considerably among sites. Associations among LAI and other stand attributes vary with canopy structure. Our stands were composed of both pure and mixed hardwoods species across a wide range of age, diameter, density, and height.

Accuracy of LAI estimates acquired using the Sunfleck Ceptometer have been validated by more direct methods of LAI estimation like litterfall collection (Maass et al. 1995) and allometric equations developed from destructive sampling (Pierce and Running 1988, Bolstad and Gower 1990, Vose and Swank 1990). Clinton (1995) used a ceptometer to find significant differences in PAR levels in hardwood forests with and without *Rhododendron* understories. Jobidon (1992) successfully used a ceptometer to measure PAR that was available to young spruce as a means of measuring efficacy of herbicides used to control competing vegetation. The nationwide Forest Health Monitoring (FHM) program, a joint Environmental Protection Agency/U.S. Forest Service project, currently uses ceptometers to estimate forest LAI as a means of evaluating the long-term health of the country's forests (Tallent-Halsell 1994, Huston and Isebrands 1995).

We found that the Sunfleck Ceptometer was a cost-efficient method of determining LAI. Since it measures transmittance with 80 sensors at once, it was possible to collect 25,600 point samples in each plot in an average of only 14 min. This compares well with Fassnacht et al. (1994), who estimated LAI using 3 light-measuring instruments. They found that the LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE) took only 4 min per plot, the Sunfleck Ceptometer required 22 min, and the DEMON (CSIRO, Canberra, Australia) required 4 hrs.

Leaf area index in a forest stand can vary from year to year due to local site conditions, weather variability between years, tree death, and plant resource allocation patterns such as periodic production of large seed crops (Burton et al. 1991, Huston and Isebrands 1995). Burton et al. (1991) observed a 20 to 30% reduction in LAI during the year of a prolific seed crop in a northern hardwood stand dominated by sugar maple (*Acer saccharum* Marsh.). We observed significant between-year differences in average summer LAI in 3 of our 9 stands. Two stands were young and vigorously growing plantations that had not yet reached their maximum LAI potential. The loss of several trees that blew down in a windstorm likely caused the decreased LAI in the other stand.

Ceptometers have been found to be sensitive to within-season changes in PAR and LAI (Vose and Swank 1990, Maass et al. 1995) and provide consistent results (Bolstad and Gower 1990). In our study, the method was sensitive to foliage production for measurements taken during the spring of 1995 (Figure 2). LAI estimates during the summers of 1994 and 1995 were relatively consistent, with an average coefficient of variation of 11.5. Temporal variation within the summer may have been the result of natural variation in the method or from weather conditions. Movement of leaves and branches by the wind can cause variation in the LAI estimated by a ceptometer (Péch 1986). Variation in summer LAI was greatest in the yellow birch stand (#23, Figure 2, Table 2) in 1995. This stand was thinned in October 1994, so the trees may have been adjusting to the increased amount of sunlight and space. Another possible explanation is unnoticed growth and shedding of reproductive catkins on the yellow birch trees.

We believe that the ceptometer could be a cost-efficient method of estimating defoliation. Liebhold et al. (1988) estimated gypsy moth defoliation in oak by evaluating the change in LAI estimated from canopy photographs. Burton et al. (1991) detected significant differences in LAI between northern hardwood sites using a ceptometer. In that study, reductions in LAI were only 20 to 30%, caused in one instance by a defoliating insect, and in another instance by a prolific seed crop.

Due to the inherent variability of the method, a single ceptometer plot per stand, as in our study, may only allow the user to classify defoliation into 3 or 4 broad categories. But the ceptometer will provide a method of measuring defoliation that is repeatable, quantitative, and free from observer bias (Jobidon 1992). Low levels of defoliation may be difficult to detect with a ceptometer. Although using multiple plots may increase the accuracy and precision of the estimates, it may counterbalance the advantage of its low time requirement. It may also be difficult to account for the spatial variation of defoliation, especially in large forest tracts. Sampling along a transect through the stand may give a more representative estimate of defoliation than clumped samples within small plots.

Many studies estimating forest LAI have used several light sensors simultaneously (Chason et al. 1991, Tallent-Halsell 1994). A remote unit measures total incoming PAR in a clearing, while another unit, like a ceptometer, is operated manually below the canopy. Readings are then paired according to the times they were recorded. If resources permit, use of multiple sensors simultaneously should improve accuracy and precision of LAI measurements.

In the case of gypsy moth and late-season defoliators, which feed primarily after full leaf expansion, the before- and after-defoliation readings could be taken in the same year. Additional measurements could be taken to estimate refoliation after major defoliation events. Timing of measurements depend on local tree and defoliator phenology. Liebhold et al. (1988) found that oak LAI reached a plateau by 1 June in Massachusetts; leaf expansion at our site in southern Michigan was finished by the first week of June. For early-season defoliators that cause significant leaf damage before full leaf expansion, an initial LAI estimate should be taken during the year prior to defoliation. This may not work in stands where LAI may vary greatly between the 2 yrs, like young rapidly-growing stands or stands with significant tree mortality or windthrow. Insect population monitoring with pheromone traps, egg mass surveys, etc. may be needed to predict where defoliation will occur. Using ceptometers to provide estimates of leaf area lost to defoliators could help in predicting impacts on nutrient movement in forest ecosystems (Mattson and Addy 1975, Swank et al. 1981, Grace 1986). Measuring the increased amount of light coming through a defoliated canopy may also be useful in examining changes in understory regeneration or impacts on ground-dwelling arthropods and other wildlife sensitive to such changes.

Table 1. Summary of stand characteristics for the nine sample stands at the W. K. Kellogg Forest in Kalamazoo Co., MI. Basal area, average DBH, density, and height were measured in 1993 for all trees ≥ 2.5 cm DBH.

Stand no.	Dominant overstory species	Plantation or natural stand	Stand origin	Basal area (m^2/ha)	Avg. DBH (cm)	Density (Stems/ha)	Height (m)
15	English oak	Plantation	1984	5.3	6.1	1557	5.2
16	English oak	Plantation	1985	5.3	6.1	1705	5.2
21	Black walnut, black cherry	Plantation	1942	22.0	13.0	1112	11.0
22	Bigtooth aspen, red maple, sassafras	Natural	1951	32.8	11.4	1952	13.7
23	Yellow birch	Plantation	1968	21.4	13.2	1317	15.5
25	Red oak	Plantation	1962	20.9	19.6	667	21.3
40	Red oak, basswood, white pine	Plantation	1932	37.4	12.2	1334	12.5
42	Red oak, red maple	Natural	1946	27.8	10.7	1458	11.0
53	Black oak, black cherry, hackberry	Natural	1921	31.2	10.4	1705	11.0

Table 2. Summary of average leaf area index (LAI) and coefficient of variation (CV) at full leaf expansion for the nine sample stands at the W. K. Kellogg Forest in Kalamazoo Co., MI. Average LAI and CV were computed using stand LAIs for sampling periods between 11 June and 4 Sept. each year. LAI includes foliage only.

		Mean LAI		Change in mean LAI	CV	
Stand no.	Dominant overstory species	summer 1994 (m^2m^{-2})	summer 1995 (m^2m^{-2})	1994 to 1995 (m^2m^{-2})	1994 (%)	1995 (%)
15	English oak	4.06	5.32	+1.26	14.1	12.6
16	English oak	3.78	5.18	+1.40	11.4	14.1
21	Black walnut, black cherry	4.20	4.48	+0.28	8.4	14.8
22	Bigtooth aspen, red maple, sassafras	6.26	5.62	-0.63	8.1	16.2
23	Yellow birch	6.00	4.91	-1.10	12.8	23.5
25	Red oak	4.48	4.27	-0.22	8.4	10.4
40	Red oak, white pine, basswood	7.53	6.10	-1.43	7.1	6.9
42	Red maple, red oak	7.10	6.48	-0.62	11.6	8.3
53	Black oak, black cherry, sassafras	6.74	6.26	-0.48	9.0	10.1

Table 3. Summary of woody area index (WAI) and maximum leaf area index (LAI) from the nine sample stands at the W. K. Kellogg Forest in Kalamazoo Co., MI. Maximum LAI includes leaves only.

		WAI	Maximum LAI		Change in max. LAI
			1994	1995	1994 to 1995
Stand no.	Dominant overstory species	($m^2 m^{-2}$)	($m^2 m^{-2}$)	($m^2 m^{-2}$)	($m^2 m^{-2}$)
15	English oak	0.30	4.43	6.28	+1.85
16	English oak	0.48	4.36	6.21	+1.85
21	Black walnut, black cherry	0.65	4.67	5.26	+0.59
22	Bigtooth aspen, red maple, sassafras	1.18	6.95	6.45	-0.50
23	Yellow birch	0.91	6.91	6.69	-0.22
25	Red oak	0.78	4.72	4.94	+0.22
40	Red oak, white pine, basswood	0.83	8.01	6.59	-1.42
42	Red maple, red oak	0.77	8.15	7.02	-1.13
53	Black oak, black cherry, sassafras	1.04	7.34	6.73	-0.61

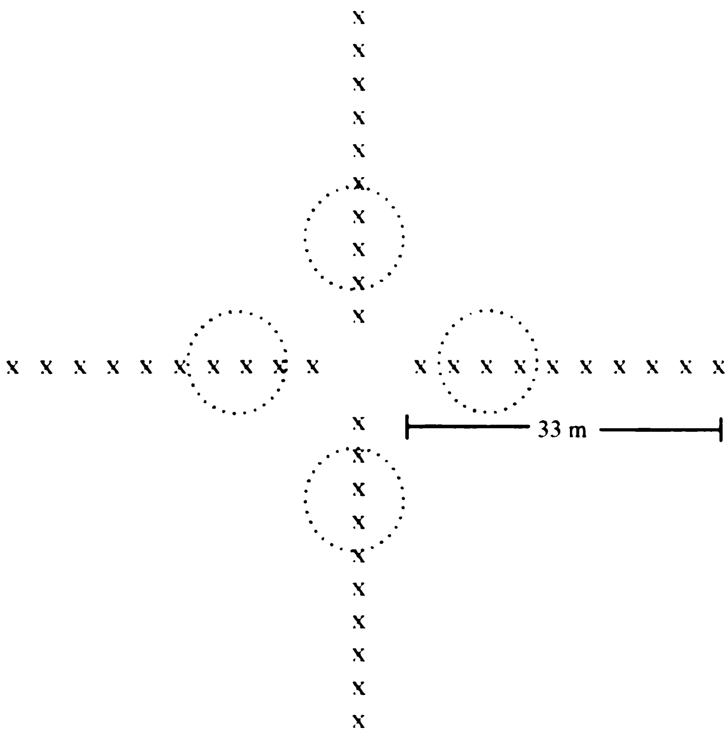


Figure 1. Plot layout for ceptometer study. Each 'x' marks a sampling point. Stand attributes (basal area, diameter, height, and density) were measured in four 0.01 ha plots indicated by the circles.

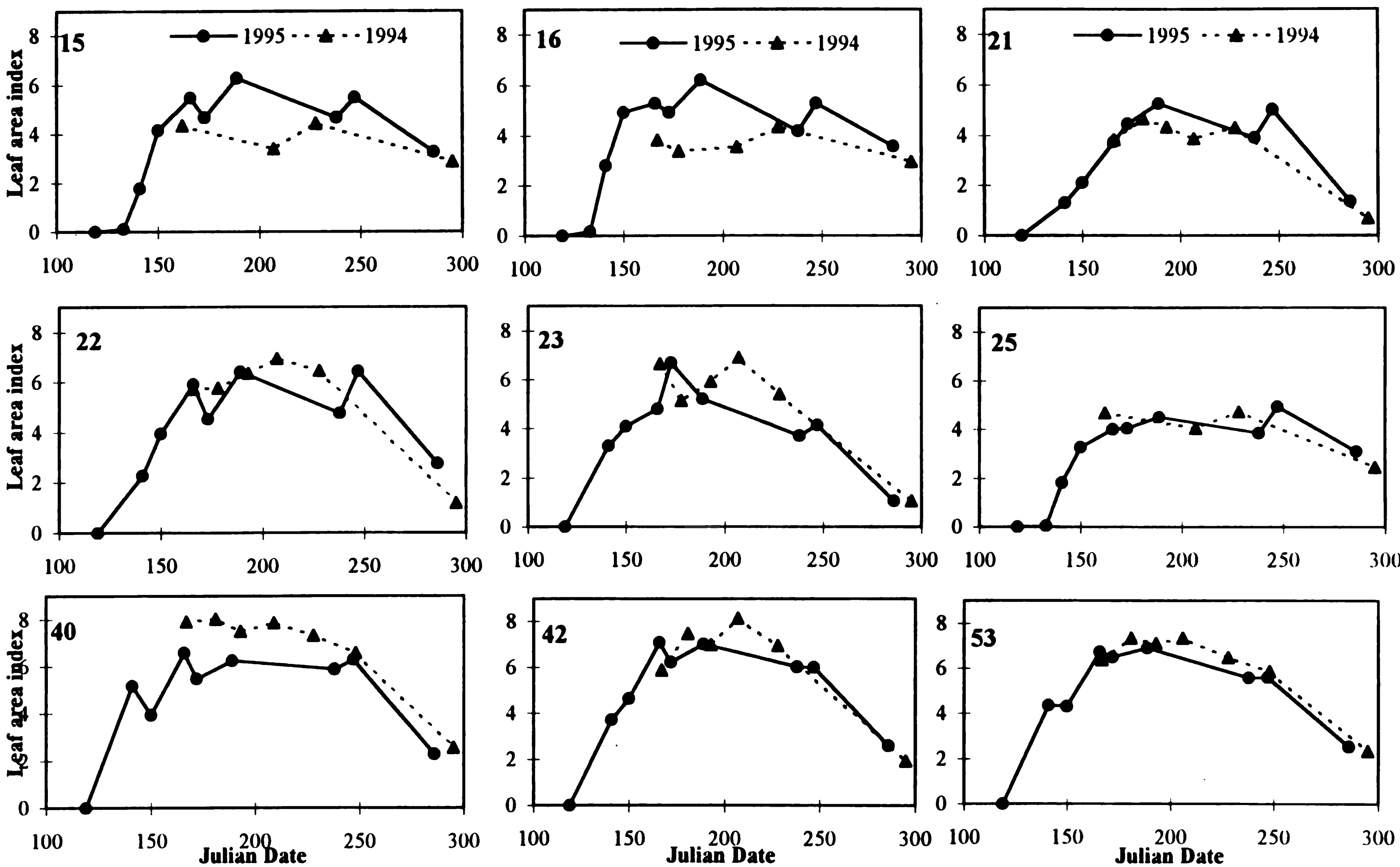


Figure 2. Leaf area index (foliage only) during 1994 and 1995 by individual stand. Stand identification numbers are in the upper left corner of each graph.

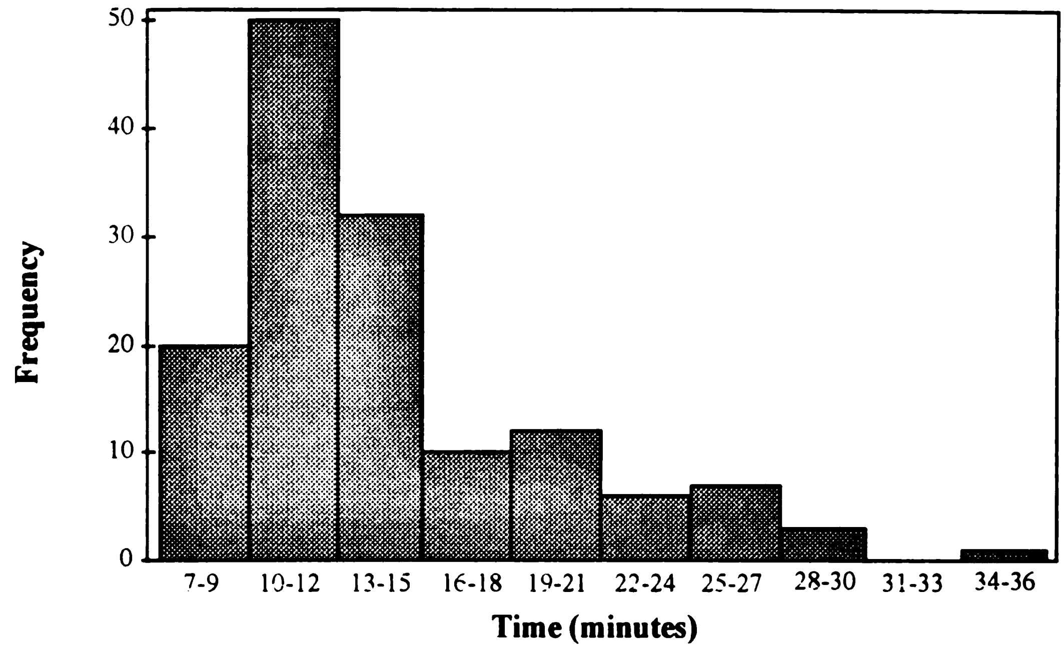


Figure 3. Frequency distribution of time required for field sampling of one plot (40 ceptometer readings) in 1994 and 1995 (n=141).

CHAPTER 3

Evaluation of Gypsy Moth Biological Control at the W. K. Kellogg Experimental Forest, Michigan, Including Introductions of Two Natural Enemies

INTRODUCTION

Introductions of exotic parasitoids, predators, and pathogens of the gypsy moth have taken place in the United States intermittently since 1905 (Hoy 1976). By 1980, at least 11 exotic natural enemies were well-established in the U.S. (Barbosa et al. 1975, Leonard 1981). Two exotic natural enemies that have become established are the braconid wasp, *Cotesia melanoscela* (Ratzeburg), and the fungal pathogen, *Entomophaga maimaiga* Humber, Shimazu and Soper. *Cotesia melanoscela* was first introduced in the eastern U.S. in 1911 (Weseloh 1981). This bivoltine wasp parasitizes young gypsy moth larvae, preferring first and second instars (Weseloh 1976). *C. melanoscela* is easily reared in the laboratory, and can be obtained from commercial suppliers for release against gypsy moth (D. McCullough, pers. comm.).

The fungus *E. maimaiga* was first brought to the U.S. from its native Japan in 1910 and released in Massachusetts. No evidence of establishment was found and the project was deemed a failure (Hajek et al. 1995). It mysteriously appeared in 1989 when an epizootic of the fungus swept through gypsy moth populations across 7 Northeastern

States (Andreadis and Weseloh 1990, Hajek et al. 1990a). The fungus is dependent on weather, needing wet, humid conditions for spore germination and gypsy moth infection (Hajek et al. 1990b, 1993). *Entomophaga maimaiga* is not commercially available at this time.

The objective of this study was to introduce *C. melanoscela* and *E. maimaiga* in a low-level gypsy moth population along the leading edge of the gypsy moth advance, and observe whether these natural enemies became established or affected the gypsy moth population in any measurable way. Our second objective was to survey for other natural enemies that were present in this gypsy moth population to determine if exotics had moved into the area with gypsy moth. Our third objective was to determine what native natural enemies were parasitizing or preying on gypsy moth larvae. This baseline information will be useful for gypsy moth managers who must evaluate releases of biological control agents to avoid overlap of ecological niches between biocontrol releases and natural enemies already present.

METHODS

This study was conducted at the Michigan State University W. K. Kellogg Experimental Forest in Kalamazoo County in southwestern Michigan. The 240-ha forest consists of a variety of genetic plantations and naturally regenerated stands of many hardwood and conifer tree species. This area was selected because low-level gypsy moth populations had been present for several years, but no significant defoliation had yet occurred.

A total of 68 gypsy moth pheromone traps were placed along a grid at a density of 28 traps per km^2 throughout Kellogg Forest between 1993 and 1996. Egg mass surveys were conducted each year after leaf fall around the 33 trap locations that were within hardwood stands. Four 0.01 ha plots, with plot centers located 10 m from the pheromone trap location in the cardinal directions, were used to determine mean egg mass density (Kolodny-Hirsch 1986).

The 9 stands with the highest egg mass counts in 1993 were chosen as release sites for *E. maimaiga* and *C. melanoscela* in 1994 and *E. maimaiga* only in 1995 (Table 2). The 9 stands were grouped in blocks of 3 based on their spatial distribution within the forest. The 3 treatments of *E. maimaiga*, *C. melanoscela*, and control were randomly assigned to the 3 stands in each block (Table 1). Basal area (cross-sectional area of tree trunks at breast height, or 1.37 m above ground), tree diameter at breast height (DBH), density, and height were measured for all trees with at least 2.5 cm DBH within the 0.01 ha plots used for egg mass surveys (Table 1). Egg masses within plots were monitored to determine hatching date and to monitor larval development.

Biological control introductions

Permits for shipment and release of the biological control agents were obtained from appropriate state and federal agencies. *C. melanoscela* were purchased from National Gypsy Moth Management Group, Inc., Landisburg, PA, and wasps were shipped as cocoons in cardboard cartons on 10 May 1994. We placed approximately 5000 *Cotesia* cocoons at the center of each of 3 release plots on 12 May (see Table 1). Paper cartons containing 500 cocoons each were stapled to tree boles facing away from the sun. *Cotesia* adult emergence and dispersal was completed within 4 d. Gypsy moths were first and second instars during the release period. The cartons of cocoons were collected from the field after adult emergence was complete so that we could determine emergence rate. A total of 100 cocoons from 8 randomly selected cartons were examined. Also, a carton of 500 cocoons was retained so we could determine the ratio of males to females that emerged (adults were allowed to eclose but were not released). No *C. melanoscela* were released in 1995 so that we could continue to evaluate the 1994 introduction.

We introduced *E. maimaiga* to the W. K. Kellogg Forest in 1994 and 1995 via soil containing resting spores. Soil for the 1994 introduction was collected by Frank Sapio (Division of Forestry, Michigan Department of Natural Resources) from Lake County in northwest Lower Michigan, and was tested for plant parasitic nematodes before introduction. This soil was acquired from one of the original release sites where Smitley et al. (1995) first introduced the pathogen into the state in 1991. An *E. maimaiga* epizootic occurred at this site in 1993 (Smitley et al. 1995). Soil for the 1995 introduction was collected by Dr. Joseph Elkinton (Department of Entomology, University of Massachusetts) at Holyoke Range State Park near Amherst, Massachusetts, where the gypsy moth population had an epizootic of *E. maimaiga* in 1994. Soil for both releases was collected from the bases of oak trees. Samples of the soil from the 2 introductions were sent to Dr. Ann Hajek (Department of Entomology, Cornell University) for quantification of resting spores concentration. Additional soil samples were saved for later use in bioassays with gypsy moth larvae.

Six trees within 11 m of the plot center were chosen in each of the 3 *E. maimaiga* release plots at Kellogg Forest (Table 1). Preference was given to large trees and to species preferred by the gypsy moth. Leaf litter was scraped away from the base of release trees, exposing bare soil. One liter of soil was spread around the base of each release tree on 12 May 1994 and 22 May 1995, and leaf litter was replaced. The soil around the release trees was watered once or twice weekly (depending on rainfall) from the introduction dates until 18 June 1994 and 23 June 1995 to provide moisture for resting spore germination (Hajek and Roberts 1991). Two to 3 liters of water were used around each release tree.

***Entomophaga maimaiga* bioassay - 1995**

We started a bioassay on 23 May 1995 to determine whether the soil obtained from Massachusetts had adequate viable resting spores to cause infection in gypsy moth larvae. A thin layer of soil was spread over the bottom of a single clear plastic container (25 x 19 x 9 cm). Gypsy moth egg masses were obtained from the rearing facilities at the USDA APHIS rearing facility at the Otis Air Force Base in Massachusetts. Larvae were reared on artificial diet (Bell et al. 1981) until they were third instars. Thirty third-instars were placed on the soil along with cubes of artificial diet. The closed container was placed in a growth chamber at 18 to 20°C and a photoperiod of 15:9 (L:D). Soil was kept moist but not wet. Fifteen larvae were removed from the container on 27 May and placed in individual cups with artificial diet but no soil. The remaining 15 larvae were left with the soil until 29 May, when they were removed and placed into individual diet cups. Cadavers were examined to determine if pathogens were present.

***Entomophaga maimaiga* bioassay - 1996**

We conducted a second bioassay for *E. maimaiga* in 1996 using soil samples from the W. K. Kellogg Forest, the Huron-Manistee National Forest, and leftover soil from the

1994 and 1995 releases. Soil from around 6 trees was collected and pooled for each one-half liter sample from the W. K. Kellogg Forest and the Huron-Manistee National Forest. Soil was scraped to a depth of 1.5 cm within 25 cm of the base of each tree. Sixteen soil samples were collected at the W. K. Kellogg Forest on 3 May 1996 from the 9 treatment plots and from 4 additional non-release sites scattered throughout the forest. One sample was collected in each control and *Cotesia* release plot. Two samples were collected within each *E. maimaiga* release plot, one from around the trees where the fungus was released in 1994 and 1995, and one from around non-release trees within 10 m of release trees. This was done because resting spores from the actual releases were likely present in the soil around release trees, whereas any spores present around non-release trees would likely be from infected larvae in the plots.

On 7 May 1996 we collected soil from 6 sites within the Huron-Manistee National Forest in northwestern Michigan. These samples were intended to be positive controls for the bioassay, since cadavers infected with *E. maimaiga* were found at these sites after peak 1995 defoliation. Spring 1995 egg mass densities ranged from 790 to 8814 egg masses per ha, and defoliation was low (0 to 20%) in these sites. We also tested soil that had been saved from the 2 releases at Kellogg Forest (2 samples from each). Approximately 50 g of soil (wet weight) from each of the 26 samples were placed in large petri dishes (25 x 150 mm). Ten third-instar gypsy moth larvae (eggs obtained from Otis Air Force Base) were put on each soil sample on 14 and 15 May 1996. The petri dishes were maintained at room temperature (20 - 25°C) and the soil was kept moist. Larvae were transferred from the soil to individual diet cups (without soil) on 21 and 22 May. Cadavers were examined for the presence of pathogens.

Subsamples of 9 soil samples collected at Kellogg Forest for the 1996 bioassay were also sent to Dr. Ann Hajek for quantification of resting spore density (Hajek and Wheeler 1994). These included soil samples from the 3 control plots and the 3 *E. maimaiga* release plots (release trees and non-release trees). Each of the 9 subsamples was analyzed separately.

Larval sampling

Since gypsy moth populations were low, we used burlap bands to sample larvae in 1994 to 1996 (Reardon 1976). Bands were wrapped around trees along transects in the 4 cardinal directions to a distance of 30 m from the plot center. We banded 18 trees in each plot (2 near the center, and 4 along each transect) at a height of 1.3 m above ground.

Bands were checked every 1 to 2 wk during the period from early instar to pupation. In 1994 and 1995, healthy-looking larvae and pupae were counted (Figures 1 - 3), while those that appeared dead or unhealthy were collected in individual containers. All larvae under bands were collected in 1996. We also collected potential predators of the gypsy moth under the burlap and noted interactions between gypsy moths and predators on trees without burlap bands.

Mortality diagnosis

Most of the gypsy moth larvae collected from burlap bands were dead when collected. Live larvae were reared on artificial diet until they pupated, died, or a parasitoid emerged. These cadavers and those from the bioassays were stored at room temperature for 2 d after death to give any internal pathogens time to develop to make diagnosis easier. Cadavers were then frozen until they could be examined. For pathogen diagnosis, a microscope slide was made of tissue from the cadaver. A drop of lactophenol cotton blue fungal stain was added to the slide (Poinar and Thomas 1978, Smitley et al. 1995). Diagnosis was made with a phase-contrast compound microscope, using 100X for *E. maimaiga* conidia and resting spores, and 400X for NPV polyhedral inclusion bodies (PIBs) (Andreadis and Weseloh 1990, Smitley et al. 1995).

Statistical analyses

Our data included annual gypsy moth pheromone trap counts (1993 to 1996, up to 68 locations), annual egg mass counts (1993 to 1995, 33 locations), and annual larval counts (1994 to 1996 from the 9 treatment plots). We used correlation coefficients to examine associations among the pheromone trap, egg mass and larval counts. Pearson's product moment correlation (r) (Sokal and Rohlf 1995) was used to analyze the majority of the data. Logarithmic and square root transformations were used when appropriate to meet the assumption of normality. Correlations using pheromone trap counts only (between years) used data from the 68 traps across Kellogg Forest. Correlations of pheromone trap counts with egg mass counts used data from the 33 sites where both types of data were collected; Spearman's nonparametric rank correlation (r_s) (Sokal and Rohlf 1995) was used because of the high frequency of zeros for egg mass counts. We only used data from the 9 treatment plots for product-moment correlations involving larval counts.

Analysis of variance (ANOVA) was used to test for differences in mean *E. maimaiga* resting spore densities among the 1996 soil samples tested by Dr. Hajek. All statistical analyses were conducted with JMP Statistical Software (SAS Institute 1995) using a significance level of alpha = 0.05.

RESULTS

Contrary to expectations, gypsy moth population levels remained low during the 3 yrs of this study. Pheromone trap counts and egg mass densities are summarized in Table 2. We conducted annual defoliation surveys in the study plots. Although we observed gypsy moth feeding in many plots, defoliation never exceeded 1% during this study.

Gypsy moth larval densities in the treatment plots were roughly associated with abundance of preferred hosts (Figures 1 - 3, Table 1). Plot 16, an English oak plantation (a preferred host), had the greatest number of larvae. A maximum of 103 larvae were observed under the 18 burlap bands during a single sampling period. We found only 3 larvae in the yellow birch stand (plot 23) during the 3 yrs of sampling. Yellow birch is not a preferred host for gypsy moth (Liebhold et al. 1995). Other stands with high larval counts had high proportions of oak species.

Pheromone trap counts from consecutive years were significantly correlated ($r=0.798$, $p<0.0001$ for 1993 and 1994; $r=0.402$, $p=0.001$ for 1994 and 1995; $r=0.422$, $p=0.018$ for 1995 and 1996). Similar trends were observed for egg mass counts from consecutive years ($r_s=0.583$, $p<0.001$ for 1993 and 1994; $r_s=0.901$, $p<0.001$ for 1994 and 1995). Correlations between pheromone trap counts and counts of egg masses laid in the same year were significant for 1993 and 1994 ($r_s=0.564$, $p=0.0006$ and $r_s=0.645$, $p<0.001$, respectively), but not for 1995. Pheromone trap counts from 1994 were significantly correlated with counts of egg masses laid in the previous year ($r_s=0.438$, $p=0.011$), but this association did not hold true for correlations of 1995 and 1996 trap counts with preseason egg mass density. Counts of total larvae under burlap bands in each treatment plot were not significantly associated with pheromone trap counts or with egg mass counts in those plots. Larval counts from 1994 and 1995 were significantly correlated with each other (r_s=0.689, p=0.040), but correlations between larval counts in 1995 and 1996 were not significant (r_s=0.653, p=0.057).

Parasitoids, predators and pathogens

It is difficult to accurately determine parasitism rates in low density gypsy moth populations since few larvae can be collected. Therefore, these results should be interpreted with caution. Since we collected only the larvae that appeared to be dead or dying, parasitism rates presented here may underestimate actual rates if some parasitized larvae under the bands appeared healthy or if parasitized larvae remained in the tree canopies.

We counted 2,122 gypsy moth larvae under burlap bands at Kellogg Forest during this study. A total of 453 gypsy moth larvae were collected under the bands, with 194, 92, and 167 in the years 1994, 1995, and 1996, respectively. Larvae collected in 1994 and 1995 were examined for parasitoids and pathogens, but larvae from 1996 were only examined for pathogens. Of the 286 gypsy moth larvae that were collected in 1994 and 1995, 128 were killed by parasitoids, predators, or pathogens. Another 129 died from unknown causes, and 29 were healthy. The largest individual source of larval mortality was the tachinid fly *Compsilura concinnata* (Meigen). This parasitoid was reared from 36 larvae in 1994 and in 19 larvae in 1995. Its highest parasitism rates during a sampling period were 27.3% on 28 June 1995 in plot 40 (3 of 11 gypsy moth larvae seen), and 13.4% on 30 June 1994 in plot 25 (11 of 82 larvae seen). Other tachinid flies collected infrequently from gypsy moth larvae were *Parasetigena silvestris* (Robineau-Desvoidy) (7 specimens), *Eusisyropa virilis* (Aldrich and Webber) (2 specimens), and *Blepharipa pratensis* (Meigen) (1 specimen). *Parasetigena silvestris* also emerged from 4 gypsy moth pupa collected under burlap bands. The encyrtid wasp *Ooencyrtus kuvanae* (Howard) was observed each year parasitizing gypsy moth egg masses in many parts of the forest. Phorid fly larvae were commonly found in dead late instar larvae and pupae. Phorids are primarily scavengers, and Sabrosky and Reardon (1976) considered them to be of no importance.

NPV was found in 26 larvae in 1994 and in 15 larvae in 1995. Polyhedral inclusion bodies (PIBs) were present in small numbers in most of these larvae, but were abundant and presumably responsible for mortality in only 5 larvae.

Many arthropods were observed preying on gypsy moth life stages. Stink bugs (Hemiptera: Pentatomidae) were the predator most often seen attacking gypsy moth on tree boles and under burlap bands. Both immatures and adults were active predators, and were observed feeding on all gypsy moth life stages except eggs. *Apateticus cynicus* (Say) was the predaceous species that we found most often, followed by *Podisus modestus* (Dallas). Other pentatomids were *P. maculiventris* (Say), *P. placidus* Uhler, *P. serieventris* Uhler, and *Dendrocoris humeralis* (Uhler). All of these species are generalist predators, and all have previously been reported to prey on gypsy moth except *P. maculiventris*, which is known to have a very large host range (McPherson 1982). All of these pentatomid species are active during the period of the year when gypsy moth larvae are present (McPherson 1982), and we found all 6 species under burlap bands.

Ants and spiders were only rarely observed feeding on gypsy moth larvae. Many other predators were collected under burlap bands, but were not observed preying on gypsy moth. These included spiders, harvestmen, and the carabids *Calosoma scrutator* Fabricius and *C. frigidum* Kirby. These predators are opportunistic, feeding on whatever prey or carrion they encounter (Smith and Lautenschlager 1978, Cameron and Reeves 1990).

Cotesia melanoscela

Emergence rate of *C. melanoscela* adults at the time of release was 47%, based on our examination of 100 cocoons. Females comprised 54% of the adults that emerged (50 adults examined). Recovery of *C. melanoscela* in the field was low. All of the *Cotesia* we recovered had already emerged from their hosts under burlap bands or on tree trunks or foliage; none emerged from the gypsy moth larvae we collected. At least 38 cocoons were found in treatment plots in 1994. The actual number may be as high as 55, because cocoons could not always be collected and therefore may have been counted during more than 1 sampling period. Thirteen cocoons were seen on tree boles and leaves, while the rest were found under burlap bands. Nine to 15 cocoons were found in plot 15, 27 to 38 in plot 16, 1 in plot 25, and 1 in plot 53 (see Table 1 for plot descriptions). It is not surprising that *Cotesia* were found in 2 non-release plots (plots 16 and 25); they were within 225 m of a release plot and both plots had a greater number of gypsy moth larvae than the release plots. Only 3 cocoons were found in 1995, 1 each from plots 15, 16 and 53.

Entomophaga maimaiga

None of the larvae collected in 1994 and 1995 were infected with *E. maimaiga*. Six infected larvae were collected in 1996, but only 1 was within a treatment plot (plot 15). Four were collected under burlap bands on a small group of trees separated from the nearest *E. maimaiga* release plot by about 100 m of grass. One infected larva was collected on a tree bole near plot 53. All larvae infected with the fungus were collected on 8, 10 or 15 July.

Quantification of resting spores in soil. Resting spore densities in the soil samples were determined by Dr. Ann Hajek's laboratory according to the methods described in Hajek and Wheeler (1994). Soil for the 1994 introduction contained 62.3 ± 13.1 (mean ± SE) resting spores/g of dry soil. Soil for the 1995 introductions contained 1676 ± 186 resting spores/g.

Low numbers of resting spores were found in all 9 samples of soil collected in May, 1996 at the W. K. Kellogg Forest. The highest counts (22.2 ± 8.7 resting spores/g) were in soil from around the trees where resting spores were introduced in the first 2 yrs of this study (18 trees total in the 3 release plots). Soil from the 3 control plots, however, had similar counts (20.1 ± 7.0 spores/g). Lowest counts (9.5 ± 4.2 spores/g) also came from *E. maimaiga* release plots, but from around trees where no resting spores had been intentionally introduced. There were no significant differences in mean resting spore density among samples from the 3 categories (F=0.959, df=26, p=0.4). Limited resources prevented us from quantifying soil resting spore density in the *Cotesia* release plots.

Bioassays. In the bioassay that used soil from the 1995 *E. maimaiga* release, 7 larvae were dead and producing *E. maimaiga* conidia as early as 29 May, 6 d after being placed on the soil. Of the first 15 larvae removed from the soil, 10 were infected with *E. maimaiga* and died, and 5 were successfully reared to adults. Of the second group, 9 larvae were killed by *E. maimaiga*, 3 survived to the pupal or adult stage, and 3 were killed by NPV, presumably contracted from the soil. All larvae that were infected with the fungus died by 4 June.

All of the larvae in the 1996 bioassay died except for 3 that pupated. Slides were made of 146 of the 260 specimens. NPV was found in 34 of the larvae, but *E. maimaiga* was not observed in any of the larvae. When NPV was present in the larvae, only small numbers of PIBs were present in larvae reared on soil from Kellogg Forest. Large numbers of PIBs were present in many of the larvae reared on soil from the Huron-Manistee National Forest and on soil from the 1994 and 1995 fungus releases. Larvae reared on these soil samples often had very high numbers of PIBs. NPV survived in soil that was saved from the 1994 introduction, even after being frozen at -15°C for 2 years. The Massachusetts soil (1995 release) was stored in a refrigerator (4°C) for the 11.5 months between the time when we obtained it and when we conducted the bioassay. Since no *E. maimaiga* infection was noted in the larvae used in the bioassay, it appeared that resting spores did not germinate in any soil sample, even in the samples where we knew they were present. They may not have received the appropriate amount of moisture while in the petri dishes, or may not have had adequate time to break diapause. The cause of death could not be determined for 112 of the larvae using our evaluation techniques.

High larval mortality in the 1996 bioassay may have been caused by improperly prepared artificial diet in the individual cups.

DISCUSSION

Introduction and establishment of *C. melanoscela* and *E. maimaiga* proved to be difficult in the low-density gypsy moth populations at the W. K. Kellogg Forest. By the end of the 3-yr study, it was still not clear how well they were established. The number of *C. melanoscela* recovered declined after the release, and *E. maimaiga* was first recovered in 1996, although in very low numbers.

The inability of *C. melanoscela* to impact gypsy moth populations is usually attributed to 2 factors, hyperparasites and poor host synchronization (Grimble and Palm 1976, Weseloh 1976). Second-generation cocoons are vulnerable to attack from over 30 native hyperparasite species from July until the following May (Van Sickle and Weseloh 1974). Weseloh (1983) found the survival rate of overwintering cocoons in Connecticut to be only 2.3%. High mortality resulted from hyperparasitism (66.4%), predation (23.4%) and unknown causes (7.9%). Even with the low number of cocoons recovered at Kellogg Forest, 2 were found to be hyperparasitized. We have also seen frequent hyperparasitism of *C. melanoscela* in the Huron-Manistee National Forest (northwest Lower Michigan), where *Cotesia* is often quite abundant.

Cotesia melanoscela adults emerge from their overwintering cocoons when the gypsy moth are first and second instars, and are quite successful at attacking these larvae. But development of the second generation is not completed until most gypsy moth larvae are fourth instars (Weseloh 1976). Successful attack rates decrease dramatically with fourth instars because these larvae have long hairs and can shake off attacking wasps (Weseloh 1976, 1981). Wasps of the second generation started emerging from their cocoons around 7 June at Kellogg Forest. At this time gypsy moth larvae were primarily in the third and fourth instars. *Cotesia melanoscela* appeared, therefore, to have the same problems at Kellogg Forest that it has in other parts of its range.

The lack of success of *E. maimaiga* in 1994 and 1995 is difficult to explain. Soil from the 1994 introduction had a very low concentration of resting spores (62.3 ± 13.1 spores/g), but the inoculum in 1995 had high numbers of resting spores (1676 ± 186 spores/g) that were shown to be viable through a bioassay. Previous introductions in northern Michigan were made with soil that had counts of 937 spores/g (Smitley et al. 1995). Hajek and Roberts (1991) successfully introduced *E. maimaiga* in Ithaca, New York using soil with 320 resting spores/g. Given this data, soil used in the 1995 introduction at Kellogg Forest had an ample amount of resting spores to cause infections.

During the 1989 outbreak of *E. maimaiga* in New England, high rates of mortality were only observed where total May and June rainfall totaled at least 20 cm (Elkinton et al. 1991). May and June rainfall at Kellogg Forest totaled less than 19 cm in 1994 and 1995, and 22 cm in 1996 (Table 3). Rainfall during the first 2 yrs of the study may have been too low to result in successful infection of larvae. Although we periodically watered the area where soil was released between precipitation events, the soil often remained dry for 2 or 3 consecutive days. In 1996, infected larvae were found at Kellogg Forest between 8 and 15 July. Rainfall in July was sparse, totaling only 3.8 cm. The most significant precipitation event in 1996 was 3 consecutive days of rain from 17 - 19 June. This rainfall may have resulted in infection of at least some gypsy moth larvae.

The low numbers of gypsy moth larvae present at Kellogg Forest may also have contributed to the poor establishment of *E. maimaiga*. But Elkinton et al. (1991) found high levels of mortality caused by *E. maimaiga* in plots with similarly low numbers of gypsy moth larvae. Release plots monitored by Hajek and Roberts (1991) had successful establishment of *E. maimaiga* in sites with egg mass densities as low as 600 egg masses/ha. Egg mass densities in our release plots at Kellogg Forest were even lower, with a maximum of 250 egg masses/ha just prior to releases of the fungus. Low larval densities may have led to both a low rate of infection and low probabilities of finding infected larvae during our surveys.

Resting spore counts in 1996 soil samples from Kellogg Forest were unexpected. We expected to find resting spores in the soil collected from around the release trees, but did not expect to find them in the other samples, especially from the control plots. Resting spores may have been transported among the study sites on shoes between 1994 and 1996, since burlap bands were checked quite frequently. Hajek et al. (1995) showed that this can happen, and found approximately 250 resting spores per shoe after walking through an area where an *E. maimaiga* epizootic had occurred the previous year. No infected larvae were found in any of the plots where soil samples were tested for resting spore density (control and *E. maimaiga* release plots). Therefore, we presume that the spores Dr. Hajek found in these soil samples during the spore quantification process were actually from the releases in 1994 and 1995. Another possibility is that the soil examined in these tests may have contained resting spores from another species of fungus. The most likely candidate would be *Entomophaga aulicae* (Reichardt in Bail) Humber, a complex of species of which *E. maimaiga* is a member. The 2 species are indistinguishable without the use of molecular techniques. *E. aulicae* is native to the United States and infects many species of forest lepidoptera, but *E. maimaiga* is the only species of the group that is known to infect gypsy moth (Hajek et al. 1991, Hajek et al. 1996a). However, it isn't likely that *E. aulicae* would be detected at Kellogg Forest unless an outbreak of a host had occurred recently. The only outbreak of a hardwood defoliator at Kellogg Forest in the last 10 yrs was orangestriped oakworm (*Anisota senatoria* (J. E. Smith)) in 1986 in 1 of the English oak plantations (G. Kowalewski, Kellogg Forest Manager, pers. comm.). Therefore, it is likely that resting spores found in our soil samples were *E. maimaiga* rather than *E. aulicae*.

It is impossible to say with certainty that the *E. maimaiga* infections detected at Kellogg Foest were a result of our introduction efforts, since infected larvae were not found within *E. maimaiga* release plots. We didn't repeat the introduction in 1996, but it may be possible that resting spores remain viable in the soil for several years. Observers checking burlap bands in 1995 and 1996 could have inadvertently transferred resting spores on their shoes from a release site to other locations in the forest, where other larvae could have become infected. Airborne dispersal of conidia is another possibility (Hajek et al. 1995). *Entomophaga maimaiga* epizootics were especially prevalent in Michigan in 1996, even present in central Kalamazoo County and some surrounding counties within 40 km of Kellogg Forest (Mott and Smitley, unpubl. data). Conidia may have spread within Kellogg Forest, or to the forest from a surrounding area. Another explanation for the spread is that early instars were infected by resting spores in a release plot, and then dispersed by wind to other locations within the forest. Other larvae could then have been infected by conidia produced by these larvae.

The actual introductions of *E. maimaiga* and *C. melanoscela* were relatively simple and required little effort. Permits from USDA APHIS were needed for both agents. Direct monetary costs for the *E. maimaiga* introduction were very low, consisting primarily of the cost of shipping the soil. Labor included collecting, "releasing", and watering the soil. The soil was also tested for the presence of plant parasitic nematodes. The risk of introducing soil-borne pests to new areas could potentially be alleviated by using infected gypsy moth cadavers instead of soil for *E. maimaiga* introductions. The initial cost for purchase of *C. melanoscela* from a commercial supplier was quite high (ca. $ 0.50 per wasp). Labor included releasing the wasps. Neither agent had a significant effect on the gypsy moth during this study, but their long-term benefits may be more substantial if they become established. *Entomophaga maimaiga* has proven its value in causing gypsy moth population collapses across the northeastern United States and Michigan (Elkinton et al. 1991, Hajek and Roberts 1991, Smitley et al. 1995). Inundative releases of *C. melanoscela* have not been shown to have a significant impact on gypsy moth populations based on egg mass counts, even though increased rates of parasitism have been observed after releases of *C. melanoscela* (Grimble 1975, Hoy 1975, Weseloh and Anderson 1975, Ticehurst and Fusco 1976, Blumenthal et al. 1981, Kolodny-Hirsch et al. 1988). The high costs of purchasing these parasitoids from commercial suppliers may outweigh their later benefits.

Predators, parasites, and pathogens caused mortality in at least 6.5% of the gypsy moth larvae seen under burlap bands in this study. This value is likely an underestimate, since larvae that appeared healthy were not collected, and the cause of mortality for many collected larvae could not be determined. *Compsilura concinnata* provided the biggest single source of gypsy moth larval mortality in this study. Opportunistic predators like pentatomids were frequently observed feeding on gypsy moth larvae. NPV was present, but likely will not cause significant gypsy moth mortality until an outbreak occurs. The egg parasitoid *Ooencyrtus kuvanae*, and several parasitic tachinids specific to gypsy moth were already present in this area. Although no single species appeared to be controlling the gypsy moth population at Kellogg Forest, it is this combination of predators, parasitoids, and pathogens working in concert that may limit the rate of increase of gypsy moth population levels (Reardon 1976).

Table 2. Summary of stand characteristics and biocontrol releases for the nine treatment stands. Basal area, average DBH, density, and height were computed for all trees at least 2.5 cm in diameter.

tand no	Dominant overstory species	Plantation or natural stand	Stand origin	Basal area (m^2/ha)	Avg. DBH (cm)	Density Stems/h	Height (m)	Biocontrol treatment
15	English oak	Plantation	1984	5.3	6.1	1557	5.2	*Cotesia*
16	English oak	Plantation	1985	5.3	6.1	1705	5.2	Control
21	Black walnut, black cherry	Plantation	1942	22.0	13.0	1112	11.0	*Entomophaga*
22	Bigtooth aspen, red maple, sassafras	Natural	1951	32.8	11.4	1952	13.7	*Cotesia*
23	Yellow birch	Plantation	1968	21.4	13.2	1317	15.5	Control
25	Red oak	Plantation	1962	20.9	19.6	667	21.3	*Entomophaga*
40	Red oak, basswood, white pine	Plantation	1932	37.4	12.2	1334	12.5	*Entomophaga*
42	Red oak, red maple	Natural	1946	27.8	10.7	1458	11.0	Control
53	Black oak, black cherry, hackberry	Natural	1921	31.2	10.4	1705	11.0	*Cotesia*

Table 2. Average pheromone trap and egg mass survey counts (moths/trap and egg masses/ha, respectively). "Forest-wide" counts include 68 traps and 33 egg mass survey locations. "Treatment plots" only include the nine biocontrol plots. Egg mass counts are for the year in which the eggs were laid.

Year	Pheromone traps		Egg mass counts	
	Forest-wide	Treatment plots	Forest-wide	Treatment plots
1993	136	278	41	148
1994	72	176	52	93
1995	114	158	87	113
1996	338	275		

Table 3. Total rainfall and deviation from the 50-year average (in cm) for May and June 1994 and 1995 at the W. K. Kellogg Experimental Forest, Michigan.

	Total rainfall		Deviation from average	
Year	May	June	May	June
1994	3.2	14.9	-5.7	+5.2
1995	10.4	8.4	+1.5	-1.3
1996	7.6	14.4	-1.2	+4.7

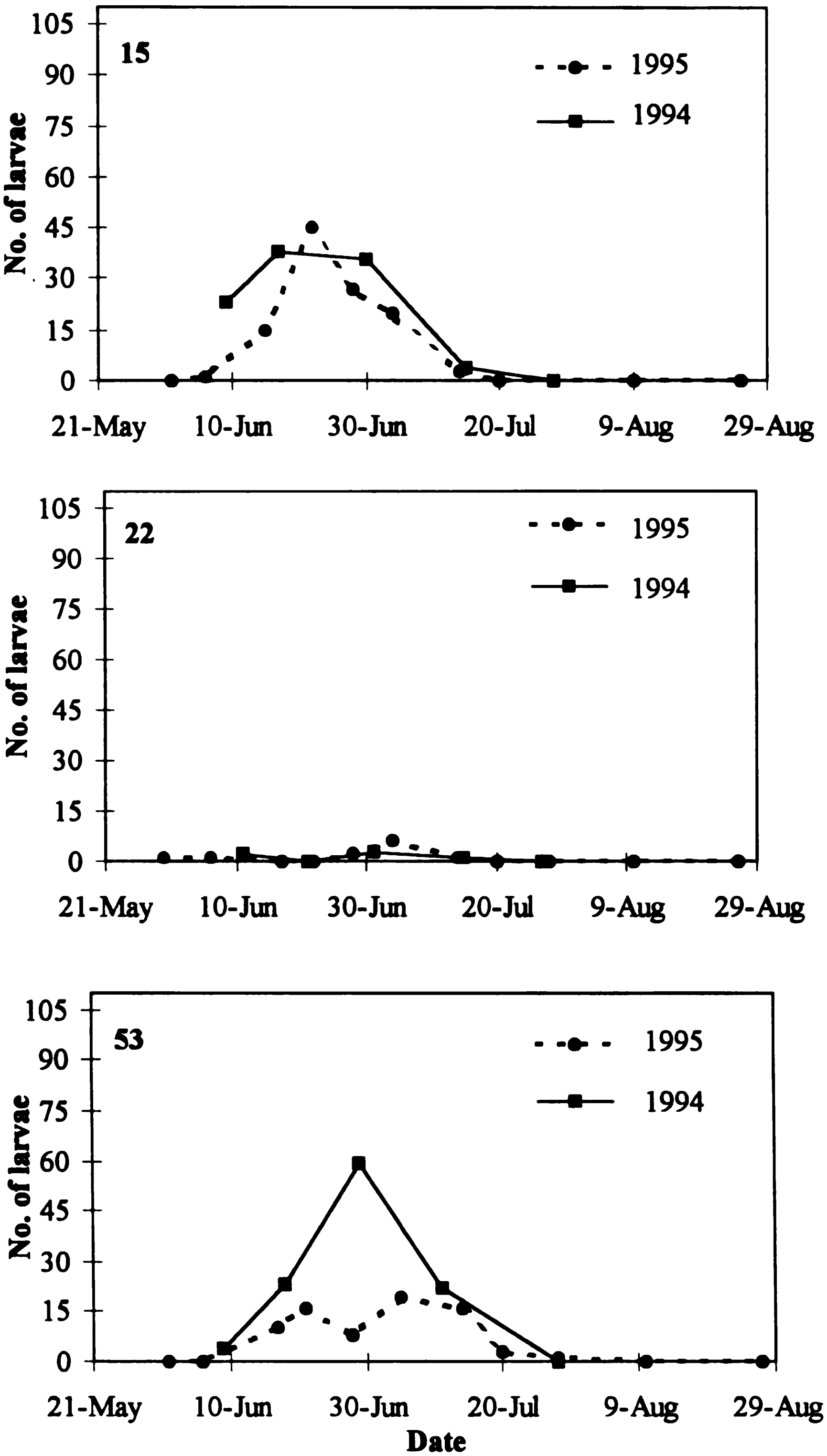


Figure 1. Total counts of live gypsy moth larvae under burlap bands in the *C. melanoscela* release plots at Kellogg Forest in 1994 and 1995. Plot identification numbers are in the upper left corners. n = 18 bands.

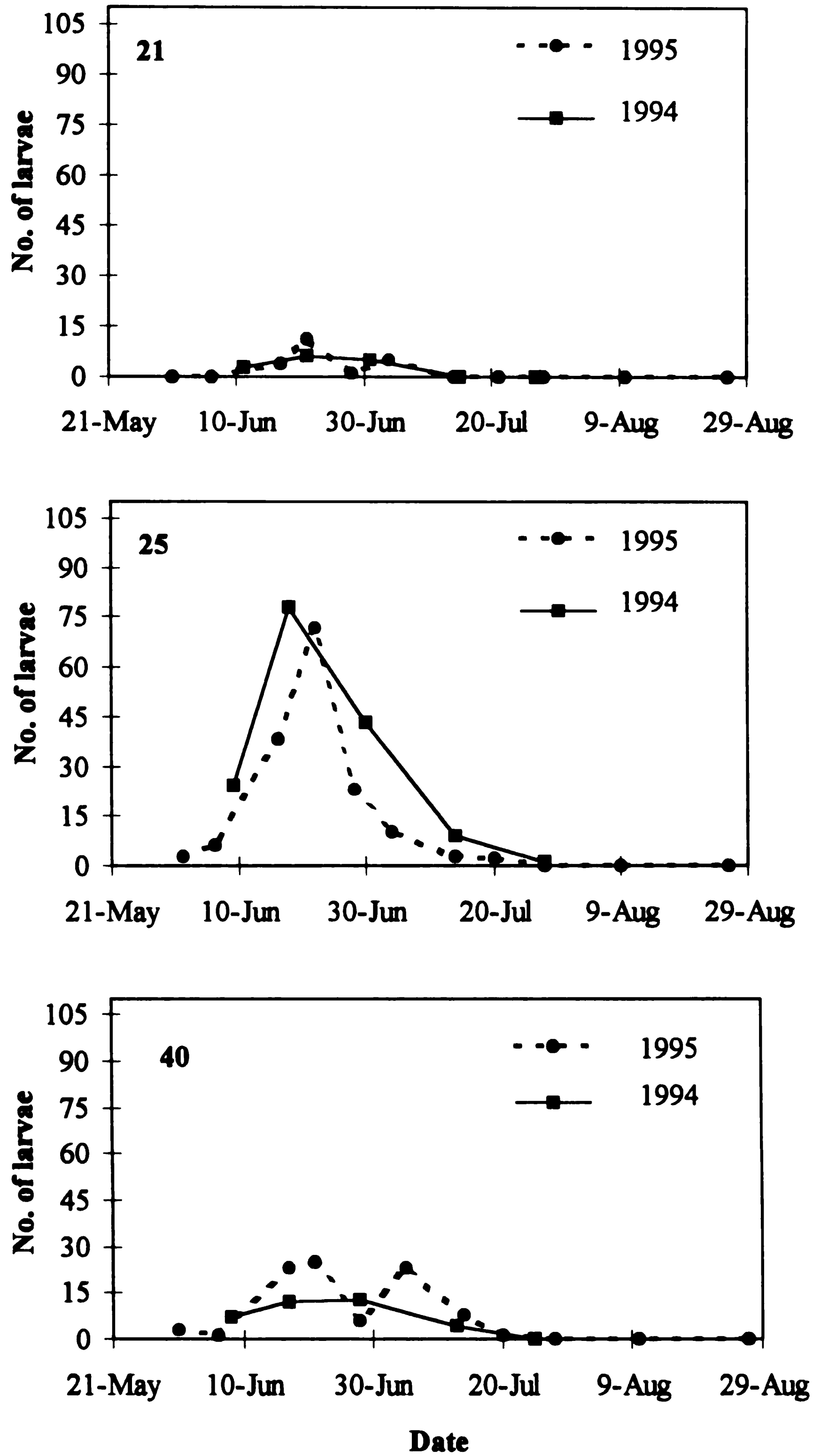


Figure 2. Total counts of live gypsy moth larvae under burlap bands in the *E. maimaiga* release plots at Kellogg Forest in 1994 and 1995. Plot identification numbers are in the upper left corners. n = 18 bands.

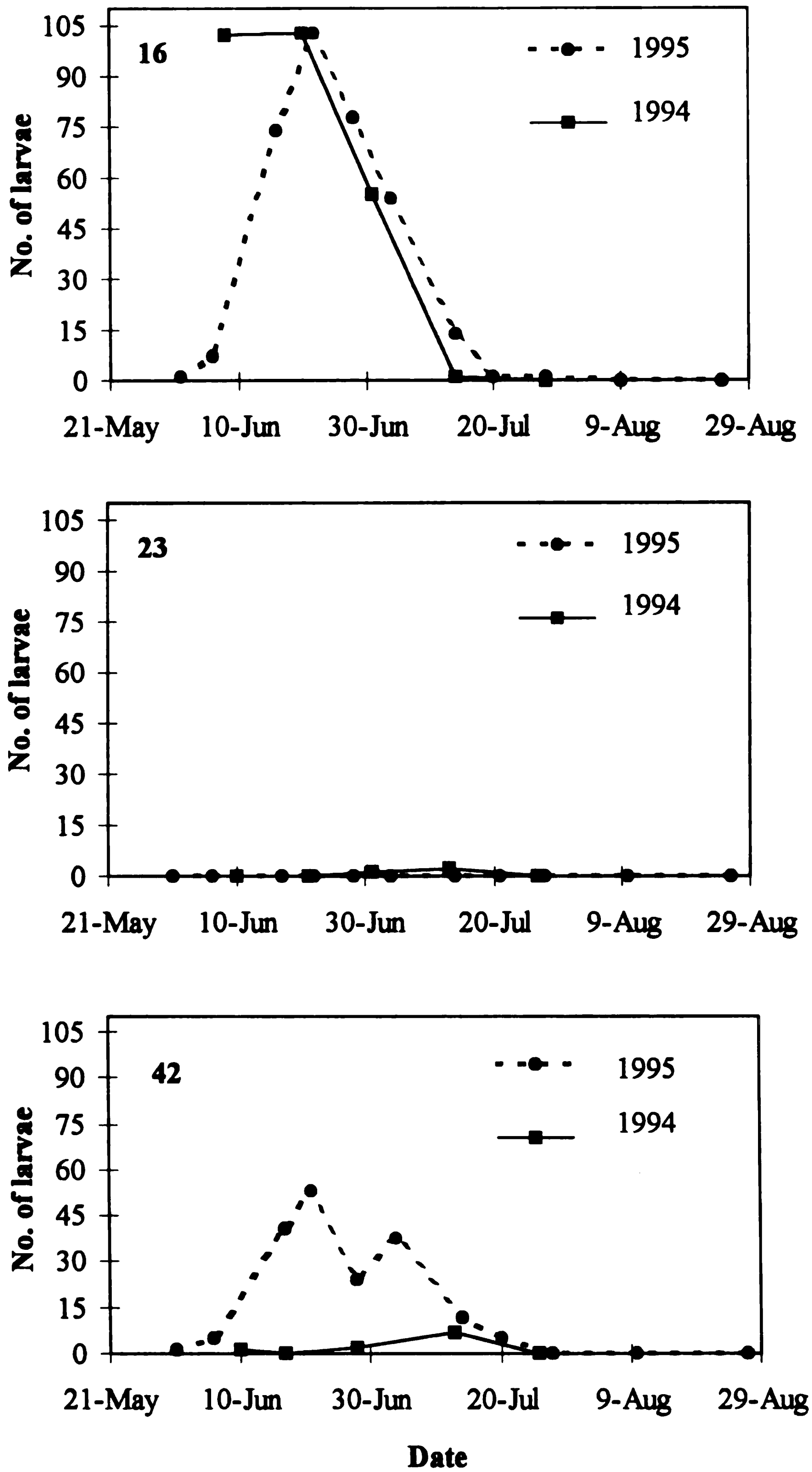


Figure 3. Total counts of live gypsy moth larvae under burlap bands in the control plots at Kellogg Forest in 1994 and 1995. Plot identification numbers are in the upper left corners. n = 18 bands.

SUMMARY

Results from our evaluation of 3 egg mass survey methods showed that egg mass counts from the fixed-radius plots and 100-tree plots were significantly associated with defoliation, but timed walk counts were not. However, when we used common egg mass density thresholds to evaluate the study stands for hypothetical suppression, the fixed-radius plots and timed walks yielded similar results. The 100-tree plot method provided fewer overspraying errors than the other 2 methods. The time required for each method varied substantially: the timed walk always required only 10 min, while the fixed-radius and 100-tree plots required 18 and 30 min, respectively. *Entomophaga maimaiga* and NPV were present in most stands and likely contributed to unexpectedly low defoliation levels.

The Sunfleck Ceptometer was a cost-efficient method of determining LAI. Since it measures transmittance with 80 sensors at once, we collected data representing 25,600 point samples in each plot in an average of only 14 min. Stand LAI estimates were relatively consistent during the period of full leaf expansion, suggesting that the ceptometer could be used to consistently quantify insect defoliation.

Establishment of the biocontrol agents proved to be difficult in the low-density gypsy moth populations at Kellogg Forest. Few *Cotesia melanoscela* were recovered, possibly because of hyperparasitism or difficulty in successfully attacking late instars due to poor

sychronization with the gypsy moth life cycle. Difficulty in locating hosts in the low gypsy moth densities may also have contributed to the poor establishment of *C. melanoscela*.

Although *E. maimaiga* was introduced at Kellogg Forest via resting spores in soil in 1994 and 1995, we did not find infected gypsy moth larvae until 1996. Soil used in the 1994 introduction had a very low concentration of resting spores (62.3 ± 13.1 spores/g), but the inoculum in 1995 had high numbers of resting spores (1676 ± 186 spores/g) that were shown to be viable through a bioassay. May and June rainfall at Kellogg Forest was highest in 1996 with 22 cm, compared to less than 19 cm in 1994 and 1995. Since infected larvae weren't collected in the same plots where the fungus was released, we can not be sure that the infections were the result of our introductions. Resting spores may have been transported on the shoes of workers, or conidia may have blown in from surrounding areas where *E. maimaiga* epizootics were present.

Predators, parasites, and pathogens caused mortality to at least 6.5% of the gypsy moth larvae seen under burlap bands in this study. *Compsilura concinnata* provided the largest single source of gypsy moth larval mortality in this study. Other natural enemies that we found included several other parasitic tachinid species, pentatomids, *Ooencyrtus kuvanae*, and NPV.

The high cost of purchasing biocontrol parasitoids like *C. melanoscela* may not be economically justified for low-density gypsy moth populations like those at Kellogg Forest. *E. maimaiga* may be more practical, partly because of its low cost of introduction.

APPENDIX 1

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1997-1

Title of thesis or dissertation (or other research projects):

Evaluation of Three Egg Mass Survey Methods and Two Biological Control Agents For Gypsy Moth Management

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)

Lyle Jay Buss

Date 18 August 1997

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Included as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Lymantria dispar L.	Mich: Kalamazoo Co. and Ingham Co., July-Aug. 1994, July-Aug. 1995		5			5	5		
Compsilura concinnata (Meigen)	Mich: Kalamazoo Co., Augusta, 23 June 1995, from gypsy moth larvae					5	5		
Cotesia melanoscela (Ratzeburg)	From National Gypsy Moth Mgmt. Group, Landisburg, PA. 10 May 1994					5	5		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Lyle Jay Buss

Date 18 August 1997

Voucher No. 1997-1

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

[illegible] 22 Aug 1997

Curator **Date**

LIST OF REFERENCES

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