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Diapause and Gypsy Moth Management: Status, Applications, and Research

Edited by:

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Thomas M. ODell
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Blacksburg, Virginia
October 2-3, 1991

COVER

Caricature of a gypsy moth (*Lymantria dispar*) egg in diapause. Carol A.B. ODell was the artist.

DIAPAUSE AND GYPSY MOTH MANAGEMENT: STATUS, APPLICATIONS, AND RESEARCH

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PREFACE

A dominant topic in insect ecology has been the analysis of insect adaptations to seasonal environments. Seasonal environments pose two classes of non-trivial challenges to cold-blooded organisms. The first challenge is to withstand the inhospitable conditions of wintertime freezing temperatures. The second involves timing of critical life history events to coincide with the seasonal ebb and flow of essential habitat resources, such as high quality food, appropriate ovipositional sites, etc. Diapause is central to the mechanistic response of insects to both of these environmental challenges. As such, diapause research has occupied a central role in insect physiology and insect ecology. A mechanistic understanding of diapause also is essential to formulation of effective control strategies for many economically important insects. For example, knowledge of environmental factors that mediate diapause would enhance development of predictive models for forecasting egg hatch over broad geographic areas and, thus, benefit scheduling of control programs. The economic applications of diapause research are fully explored in this proceedings. For both basic and applied reasons, a significant research effort currently is underway on gypsy moth diapause. These efforts involve three federal agencies and several universities in both the United States and Canada.

Because of the number of different administrative units involved, the communication and interaction among research scientists working on gypsy moth diapause are less than ideal. In response to this perceived lack of dialogue, we conceived the idea for a workshop on the theme of gypsy moth diapause at the 1991 gypsy moth review in Annapolis, Maryland. Subsequently, we contacted everyone we knew who might be interested in such a workshop. Our intent was to consider all aspects of gypsy moth diapause. The research scientists we contacted expressed interest and supported our efforts to arrange such a workshop. From these initial contacts, a critical mass of research scientists was able to commit the time and resources to attend a 3-day workshop that was held on the campus of Virginia Polytechnic Institute and State University, Blacksburg, Virginia, the following fall.

The objectives of the workshop were to: (1) facilitate dialogue and communication between research scientists working on various aspects of gypsy moth diapause. It was hoped that by providing a common forum, individuals specializing in one area of diapause research would gain an appreciation for other aspects of this multi-faceted problem. (2) Allow individual scientists the opportunity to present their latest results in gypsy moth diapause research. (3) Identify future research needs and priorities. Presentations by workshop attendees were scheduled for the afternoon of the first day and the morning of the second day. During the afternoon of the second day, we broke into subgroups organized around key issues of gypsy moth diapause. The specific charge to the breakout groups was to evaluate current research efforts and to make recommendations for future research directions. The morning of the third day was devoted to discussion of summary presentations made by the breakout groups.

Individual participants were offered the option of providing an abstract or a more substantive manuscript on their research. Summaries of the breakout group discussions also are included in these proceedings. The overall goal of publication is to provide a benchmark of our knowledge regarding gypsy moth diapause research as it was at the time of the workshop, and to provide an enlightened view of future directions for research on this key topic. Because of unforeseeable complications that included responding to the introduction of the Asian gypsy moth, relocation by one of us, and completion of a Ph.D. degree by another, there was an unfortunate delay between the workshop and the fruition of publication. However, in retrospect we are of the opinion that the papers and evaluation contained herein are equally valuable today as they were when originally presented in 1991. Unquestionably, advances in diapause research have occurred in the ensuing 3 years. However, other events, in particular the introduction and apparent establishment of the Asian gypsy moth, have accentuated the potential impact of this pest. If anything, the topic of these proceedings is even more timely today than when the workshop originally was held.

ACKNOWLEDGMENTS

We owe a particular note of thanks to Rose Norris-McClellan and Quintin McClellan for their help in organizing and implementing the local arrangements for this workshop, and to Katherine McManus for coordinating the publication of this proceedings. The Expanded Gypsy Moth Research Program of the USDA Forest Service, Northeastern Forest Experiment Station, provided support for the Workshop (through a cooperative research agreement with Virginia Polytechnic Institute and State University, FS-23-383) and for publication of the proceedings.

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DISCUSSION SUMMARY

Modeling Subgroup

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OBJECTIVES

The modeling group discussion began with the disclaimer that a model of gypsy moth diapause is not "real" in the same sense of diapause as a physiological process. The somewhat philosophical (and rhetorical) question was asked, "does a model exist with respect to an external reality?" The answer was, "no, it doesn't," and that a model of diapause is to some degree artificial and manufactured. Models are constructed not discovered, and they should be defined by the objectives of their intended use(s). Therefore, for a model to be of value, the intended objectives of the model must be stated clearly. To a large extent, model research and development should be driven by the other research agendas relating to gypsy moth diapause.

The goal of a gypsy moth diapause model is to predict egg hatch in nature. The importance of this goal is based on several aspects of gypsy moth control and requires meeting several specific objectives:

(1) It is necessary to model the pattern of egg hatch in order to start within-season phenology models that may be used to improve timing of gypsy moth management activities, such as *Bacillus thuringiensis* (*Bt*) application or pheromone trap placement and removal. Once a starting distribution is provided, subsequent phenological prediction can proceed with well-tested procedures and existing models (Logan et al. 1991). The first objective can be stated as: Produce a model that provides the initiating distribution for subsequent phenology models.

(2) A model of egg development that include diapause is needed for several aspects of F_1-sterility production. Such a model could increase efficiency of laboratory production of F_1-sterile individuals by providing the basis for procedures that would allow more efficient "stockpiling" of eggs for anticipated periods of peak demand. Also, such a model could be used to improve application of the F_1-sterile technique. Efficacy of the F_1-sterile field application is dependent upon coincident emergent patterns of F_1-sterile and wild types. Clearly, such timing depends on the pattern of F_1-sterile egg hatch as well as subsequent phenology. A second objective then, is: Produce a model of F_1-sterile egg diapause and development.

(3) A third objective relates to basic diapause research. The interaction of diapause and embryogenesis results in complex and often enigmatic patterns of egg hatch. A robust model of diapause and egg development could assist in egg physiological research in several ways. Such a model could be used to generate testable hypotheses regarding the effects of various temperature treatments on the observed pattern of egg hatch. Empirical experiments could be designed to test these hypotheses and, by implication, the underlying, physiological mechanisms. A diapause model also could serve a valuable function as an objective means to evaluate the state of existing knowledge. A third objective then, is: Produce a diapause model with a mechanistic basis and sufficient realism to be useful in basic diapause research.

CURRENT STATUS

At the present time there are two models used to predict gypsy moth egg hatch. The first of these was published by Johnson et al. in 1983 and the second by Lyons and Lysyk in 1989. Both models take the generally accepted approach to modeling insect phenology by accumulating physiological (thermal) age according to a parameterized developmental rate function. The primary difference between the two models is in the shape of the developmental rate curve (linear degree-day in Johnson et al., and a quadratic in Lyons and Lysyk) and the starting date for heat accumulation (January 1 in the Johnson et al. model, and April 1 in the Lyons and Lysyk model). The most recent version of the gypsy moth life system model, GMPHEN (Sheehan 1991), allows initiation of egg hatch by either model. Distribution of egg hatch pattern is determined by the number of cold days in the winter (Masaki 1956) for the Johnson et al. model, or by a Weibull distribution fitted to the normalized emergence times for the Lyons and Lysyk model.

Both models were validated for the geographic area in which they were developed, and prediction from either model should work well for their geographic region of origin. Applications of these models beyond their geographic region of origin, however, have led to disappointing results. For example, application of the Johnson model as the phenological initiation point for GMPHEN in Utah resulted in predictions that were 25 days in advance of those observed in the field (S. L. Smith, personal communication). As another example, the best starting date determined by Lyons and Lysyk for their model was April 1; a date that is after the initiation of egg hatch in Georgia (personal communication, C. W. Berisford). The reason for lack of geographic robustness in the existing diapause models is most likely due to either inadequate mechanistic or phenomenological representation of the diapause process. In both models, diapause is treated as a "black-box" process. Adequacy of prediction, therefore, depends upon how well the arbitrary starting date for heat accumulation in the model corresponds to the actual starting time of physiological processes that result in egg hatch in the field. The real challenge is, then, to remove the opaqueness of the black box used to model the diapause portion of the egg stage.

JUSTIFICATION

The requirement for a unified modeling framework that is robust to geographic distribution results from aspects of gypsy moth distributional ecology and the interaction of human activities with this ecology. Gypsy moth populations are distributed over a wide geographic and climatological range. It seems unlikely that research resources in time, money, or personnel would ever allow building a model for each location of the gypsy moth range. Exacerbating the impracticality of location-specific models is the fact that human activities, both commercial and recreational, provide ample opportunities for long-range dispersion of gypsy moth eggs. For example, almost 500 interceptions of gypsy moth eggs are made at the California border each year (McFadden and McManus 1991). Clearly, it is conceivable that eggs from Maine could be transported to Georgia during the appropriate time for egg hatch. It also appears that the timing mechanism for hatch could result in the appropriate coincident phenology between transported eggs and local host trees. International commerce poses additional threats. How likely is it, for example, that egg masses deposited on a container ship originating from a Siberian port could result in appropriate hatching in a North American destination? Finally, gypsy moth control strategies previously discussed, such as F_1-sterile approaches, require accurate prediction of egg hatch over a wide geographic area for eggs that are produced at a centralized location.

For all of the above reasons, there is a clear need for a process level model that represents the individual phases of egg development.

MODEL FORMULATION - ALTERNATIVE HYPOTHESES

In order to meet the goal of a geographically robust model of diapause and egg hatch, it will be necessary to represent the physiology that results in diapause and arrested development at an appropriate level of mechanistic realism. Our intuition is that the appropriate level of realism will result in a phenomenological model, i.e., one that does not necessarily represent enzyme kinetics, but also one that is not a simple curve fitting exercise for a purely empirical model. The model that represents diapause successfully must: (1) incorporate the underlying diapause mechanism at a descriptive level, and (2) cannot be in violation of the physiological processes that result in the expression of diapause and arrested development. In our discussion of the possible alternative formulation of phenomenological models, we have adopted the following terminology:

- **Stage** refers to the egg stage in the same sense as any other life stage, (the egg stage, the larval stage, etc.).

- **Phase** is defined as an observed period in the progression of the individual through the egg stage. As such, phase may or may not coincide with an actual physiological state or process. There are three phases of egg development: Phase I - pre-diapause embryogenesis or embryonation; Phase II - diapause development; and Phase III - post-diapause development or incubation.

- **State** is defined as a "true" physiological state in the developmental process. There are, therefore, at least two states in the egg stage--diapause and embryogenesis. There also may be three states, corresponding to the three phases of the egg stage.

Primarily, we have limited our discussion to the thermal response of egg development since there is no clear indication of a significant photoperiodic influence on diapause (Tauber, et al. 1990). However, we do acknowledge that it may be necessary at some future date to incorporate photoperiodic effects if further research indicates an influence that is not currently appreciated.

Four general classes of alternative model formulations were identified by the modeling subgroup.

Three-state, Obligatory, Sequential Model

The first, and conceptually simplest, model of the egg developmental process is a three-state, obligatory, sequential model. In this model, there are three distinct developmental states, each with an unique controlling temperature-developmental rate curve. The embryogenesis curves have a high temperature optimum and the diapause rate curve has a low temperature optimum. The three phases of egg development occur in an obligatory fashion, the pre-diapause (embryonation) phase must be entirely completed before the diapause phase, which, in turn, must be entirely completed before post-diapause development and egg hatch can be completed.

This model has several points that make it an attractive starting point for diapause model development. First, it is conceptually the simplest model of the diapause process, and it is clearly the most straightforward to apply from a modeling prospective. The model also seems to correspond to the historical view of diapause development. Finally, from an observational point of view, the phases of development directly correspond to the physiological state of development. This model is the most conservative with respect to knowledge required to apply an egg-hatch model in the field.

The implications of this model are important. Two possibilities exist. The first is that Phase II development is always completed in the winter before the occurrence of spring temperatures above the threshold for Phase II development. In this case, the population has completed diapause development before spring and are in a "refractory" (quiescent) state ready to begin development as soon as temperatures are warm enough for Phase III embryogenesis to begin. Predicting spring emergence, then, is simply a matter of determining the temperature threshold for post-diapause development and the correct shape of the post-diapause developmental rate curve. The model is initialized at some time in the winter before the time when micro-habitat temperatures are above the threshold for post-diapause development. For an application of this concept, see Logan et al. 1979. Therefore, in order to predict egg hatch there is no requirement for complete thermal history of the egg mass, nor is there any particular need to characterize the form of developmental rate curves for Phase I or Phase II development.

A second possibility with the three-state, obligatory, sequential model is that broad overlap exists between the Phase II and Phase III developmental rate curves, and that Phase II development has not been completed before the time when micro-habitat spring temperatures rise above the Phase III developmental threshold. In this case it is necessary at least to be able to predict when Phase II is completed in order to provide a starting rule for Phase III development. Characterization of at least Phase II and Phase III developmental rate curves would be necessary for accurate prediction of egg hatch.

Two-state, Three-phase, Obligatory Sequential Model

A second model form is a two-state, three-phase, obligatory sequential model. In this model, the developmental rate curves for Phases I and III are indistinguishable; however, there is a developmental heuristic that requires Phase II to be completed before embryogenesis can proceed beyond a specific critical value. The critical developmental value or index for gypsy moth is approximately 90 percent completion of egg development. Conceptually, this model can be thought of as a special case of the previous model with the addition of a heuristic relation of two developmental rate curves rather than three distinct curves. This model is empirically indistinguishable from the previous model with identical Phase I and Phase III curves. Since the current model is a special case of the previous model, model implications will not be discussed further. The reason this model is included in the discussion is to introduce the idea of a heuristic that relates developmental states resulting in a rule-based (as opposed to a rate-curve-based) definition of developmental phases. The two-state, three-phase obligatory sequential model is included as a transition model.

Two-state, Potentially Simultaneous, with Phase-activation Heuristic Model

The third model formulation is that of a two-state, potentially simultaneous, model with a phase activation heuristic. In this model, the requirement for sequential completion of the three developmental phases is relaxed. If conditions are favorable, both embryogenesis and diapause development can proceed simultaneously. The model allows conditions under which diapause development could be completed without the outward expression of arrested development typically associated with diapause. An application of this model is provided in Hilbert, et al. 1985. Their model formulation consisted of two developmental rate curves, one with a low-temperature optimum that regulated diapause development and the other with a high-temperature optimum that regulated embryogenesis. These two curves were related by a simple heuristic that stated: development of the two stages could proceed simultaneously provided temperature conditions were appropriate, and embryogenesis was less than 80 percent completed. The model as formulated and parameterized by Hilbert et al. provided an excellent fit to a complex, historical data set in the grasshopper literature, and reconciled apparent contradictions from the *Melanopuls sanguinipes* literature (e.g., the occurrence of multivoltinism in a species with obligatory diapause).

The two-state, simultaneous, heuristic model has several features that are attractive from both a biological-ecological and a modeling point of view. The model provides a parsimonious, flexible modeling construct. Depending on the relationship between characteristics of the developmental rate curves (thermal minimum, maximum, optimum, etc.), and the rule that relates developmental characteristics to state activation, the model can describe a wide variety of observed diapause (arrested development) patterns. These patterns include no external manifestation of diapause if the relationship between temperature thresholds and the heuristic that related them are appropriate. It is, in fact, possible to simulate many different patterns of arrested development simply by tuning the heuristic that regulates the potential for simultaneity. The heuristic often can be tested empirically; for example, the 80 percent development is arrested in diapausing individuals. The corresponding value for gypsy moth is 90 percent completion of embryogeneses. The flexibility of this modeling structure is illustrated in the ability of the previous model to be recast as a special case.

One of the consequences of this model is that complete thermal history of the egg mass is required for application in the field. This fact implies the necessity for describing the distribution of oviposition as well as temperature monitoring for the time interval from oviposition until egg hatch. Also, the data required for parameterization of this model are relatively data intensive. As more complete physiological information becomes available, data requirements are anticipated to become less demanding.

Phase III Continuously-Variable Temperature Response Model

The next model considered is a model that includes a Phase III continuously-variable temperature response. Conceptually, this model is the same as the first model (the three-state, obligatory, sequential model), with the exception that temperature response during Phase III is assumed to be dependent upon physiological age. The typical approach to modeling insect developmental processes is that the rate of development for a particular life stage is assumed to be constant. For many examples, this assumption has worked well. However, "recovery" from diapause and the resumption of embryogenesis is physiologically complex, and there is no *a-priori* reason to assume that Phase III is not age- as well at temperature-dependent. In fact, that is exactly what Tauber, et al. (1990) hypothesized as an explanation for their experimental results. The constant, age-insensitive rate assumption may work only by coincidence for other life stages of gypsy moth (and other insects) where environmental conditions are approximately uniform over the duration of the life stage. The ephemeral nature of other life stages is in obvious contrast to the egg stage that encompasses all four annual seasons, and Phase III that covers the transition from winter to spring. A temperature-age-dependent model has been developed by Régnière for description of post-diapause development in spruce budworm.

Information for field application of this model is the same as that for the first model described. If some valid "biofix" exists for gypsy moth populations (as Régnière found for spruce budworm), then it should be possible to predict spring egg hatch based on winter-spring temperatures alone.

Integrated Photoperiod Model

A final model is an integrated photoperiod model. This model is included for the sake of completeness. Since gypsy moth has been shown to be, at most, weakly photosensitive, it seems only reasonable first to model the temperature requirements for diapause, embryogenesis, and egg hatch. Having said this, there is concern that photoperiod response might be related to the hatch sequence.

IDENTIFICATION OF CRITICAL RESEARCH NEEDS

To summarize the technical requirements for a model of the egg developmental process that satisfies the objectives of an egg hatch model, the model does not necessarily need to represent the mechanistic basis of diapause at the enzyme or the hormonal level; however, it needs to represent the individual phases of diapause and embryogenesis at a phenomenological level and it clearly cannot violate constraints imposed by underlying physiological processes. It will be necessary, most likely to represent the complete developmental process (all three phases of egg development) in order to meet the requirement of geographic robustness. A model that is mechanistically independent of geographic area of application is needed due to the vast geographic distribution of gypsy moth and the important questions centered on transport of gypsy moth eggs over large-scale geographic regions.

The basic requirements of an adequate model suggest several "critical" questions and experiments. This list is by no means considered to be complete or exhaustive, nor is importance indicated by order.

Phase Relationship and Developmental Heuristics. The outward manifestation of diapause (i.e. arrested development results) derives from the relationship between developmental thresholds of rate curves that describe the temperature response of the various states of development. Ordering rules between states, such as obligatory sequential vs. potential simultaneity, also may play an important role. For example, the critical question of whether gypsy moth populations are capable of multivoltinism in the southern region of the potential North American distribution may depend on such developmental heuristics. Hilbert et al. (1985) rationalized an obligatory diapause with multivoltinism through implementation of an empirically motivated developmental heuristic. Such a model has been proposed for gypsy moth (Logan, work in progress). The real challenge is to devise experiments that will provide unambiguous discrimination between competing models (hypotheses). In particular, the second model (Two-state, potentially simultaneous, with phase activation heuristic) and the third model (Phase III Continuously-variable temperature response) could result in similar predictions under many circumstances, and yet conceivably could lead to dramatically different predictions regarding the potential for multivoltinism.

Continuously Variable or Distinct Phases. Determining the nature of the temperature response with relationship to developmental stage is basic to model formulation. Two competing hypotheses recently have been published. Tauber et al (1990) hypothesized a continuously variable (age- as well as temperature-dependent) rate curve for diapause development. Conversely, Gray et al. (1991) hypothesized temperature-dependent but age-independent (within state) curves. Conceivably, there may be a combination of the two concepts, with one (or more) state being age-independent, and one (or more) state being age- dependent. A model incorporating the conceptual model of Tauber et al. has been in development for some time (subsequently published as Sawyer et al. 1993) and Gray et al. (1991) currently are testing an age-independent model. Laboratory/field validation experiments are critically needed.

Developmental Biofix for Egg Hatch. Existence of a "biofix" (Welch et al. 1978) or "developmental zero" for model initiation would vastly simplify the prediction of egg hatch. If a valid, stable biofix can be determined, then it will not be necessary to model the entire process of egg development. A model that predicts egg hatch could be developed based only on those processes that occur after the biofix date. Régnière (1990) found such a biofix for modeling spruce budworm egg hatch, and experimental procedures described in that publication should be applied to gypsy moth.

Diapause--Non-diapause Strain. The rapid selection of a non-diapause strain could result from selection for parameters that allow diapause to proceed without an obvious or a long-term phase of arrested development. This is in contrast to the general perception of a non-diapause strain in which the capacity for diapause has been selectively removed. If the first is a reasonable representation of the underlying physiology, then it should be possible to back select for diapause, whereas if the second is representative it would not be possible to back-select for diapause. The back-selection experiment should be done because of the important implications for basic model formulation and because of the implications for multivoltinism.

Distributions of gypsy moth egg masses and temperature in the microhabitat. Successful application of any egg development model requires accurate representation of temperature as the driving variable. It is, therefore, necessary to represent both the spatial distribution of egg masses and the temperatures that impinge upon them. Models of microhabitat temperature distributions as a function of some easily measured variable (i.e. standard meteorological station measurements) is essential for field application of validated phenomenological models of egg development and diapause.

Characterization of F_1-sterile diapause characteristics. Accurate prediction of egg hatch and subsequent phenology of F_1-sterile individuals is essential to the successful application of this technology. Although our intuition suggests that mechanistically the diapause process is constant regardless of strain and rearing conditions, this assumption needs to be tested explicityl for F_1-sterile produced eggs. We also anticipate that parameter values for laboratory selected individuals will differ from those of wild-typed individuals.

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MODELING DIAPAUSE DEVELOPMENT: PHASE MULTIPLICITY AND SIMULTANEITY

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ABSTRACT

A simulation model of diapause development is described, based on the hypothesis that diapause is constituted of three distinct processes, or phases. Only the last two phases are explicitly considered. The model incorporates development rate variability in Phases II and III, photoperiod sensitivity during Phase II, and phase simultaneity. Simulation experiments show that partial phase simultaneity is equivalent to having sequential phases with a shorter Phase III. It is also shown that a high degree of phase simultaneity reduces the ability of the diapause process to prevent development completion under adverse conditions and to synchronize development. A model with two sequential phases generated realistic development completion patterns under natural weather conditions using parameters suited for spruce budworm, although accurate estimates of Phase-II parameters were not available. Sensitivity to photoperiod in the latter part of Phase II further reduced the sensitivity of the model to development-rate parameters. Nevertheless, it is concluded that accurate prediction of development completion, particularly in environments where winter temperatures regularly exceed postdiapause development thresholds, requires precise estimates of development rates during the last two phases of diapause. Such estimates may be obtained best from measurements of metabolic rates during diapause.

INTRODUCTION

Few models have been developed to simulate diapause development in insects. Most phenology models assume that diapause is over in late winter, at a time when temperatures are still too low for postdiapause development to occur. This approach works well for univoltine species with an "obligate" winter diapause, in environments where winter temperatures drop and remain below development thresholds for prolonged periods. In such cases, a date for the initiation of postdiapause development summation can be chosen objectively (e.g. Lyons and Lysyk 1989; Régnière 1990). However, to model postdiapause development in species with facultative diapause or in environments where temperatures regularly exceed postdiapause development thresholds throughout the year, diapause processes must be simulated directly. This is due to the impossibility of choosing an objective, constant point in time where diapause can be considered satisfied, and postdiapause development summation can begin. The modeling approach has been to view the diapause phenomenon as consisting of two processes, a concept proposed and developed by several authors (Lees, Mansingh, Hodek and Zalslavski, as cited in

Zaslavski 1988). The two processes, or phases, were thought to occur sequentially (Logan et al. 1976) or simultaneously (Hilbert et al. 1985).

In this paper, the first phase of diapause, prediapause development, is not considered explicitly. Rather, it is assumed that individuals have reached the diapause state (Phase II). The behavior of a general model of diapause and postdiapause development is investigated, taking into account individual variability in development rates during both phases, partial or complete phase simultaneity, and photoperiod sensitivity in late diapause. The differences are emphasized between sequential and simultaneous phases in terms of the model's ability to synchronize the completion of development and to prevent it from occurring under adverse conditions. The model is also applied to the simulation of spruce budworm diapause development under natural conditions of temperature and photoperiod.

MATERIALS AND METHODS

Model Description

In the model, no attempt is made to simulate diapause induction, although this is a very important process in insects with a facultative diapause. Rather it is assumed that diapause is obligate, or that it has been induced. Diapause is viewed in this model as consisting of two distinct processes (phases II and III), each with its own thermal responses (Fig. 1). Phase II has been called diapause development (Andrewartha 1952), reactivation (Danilevski 1961 as cited in Zaslavski 1988) or diapause regulating process (Hilbert et al. 1985). It often is seen as a slow, cool-temperature process. Phase-III development has been called postdiapause (Tauber and Tauber 1976), morphological development (Hilbert et al. 1985) or activation (Zaslavski 1988). It is most often characterized by a faster, warm-temperature response. Strictly speaking, Phase III occurs after Phase II only when the two processes are completely sequential.

Development rates in both phases are functions of temperature, T. In Phase II,

$$d_1 = P_{1,1}\left[\frac{1}{1+e^{P_{1,2}-P_{1,3}\tau_1}} - e^{(\tau_1-1)/P_{1,4}}\right] \qquad [1]$$

where $P_{1,1}$ to $P_{1,4}$ are parameters, and

$$\tau_1 = (T_{1,\max}-T)\,/\,(T_{1,\max}-T_{1,\min}) \qquad [2]$$

In Phase III,

$$d_2 = P_{2,1}\left[\frac{1}{1+e^{P_{2,2}-P_{2,3}\tau_2}} - e^{(\tau_2-1)/P_{2,4}}\right] \qquad [3]$$

where $P_{2,1}$ to $P_{2,4}$ are also parameters, and

$$\tau_2 = (T - T_{2,\min}) / (T_{2,\max} - T_{2,\min}) \qquad [4]$$

$T_{1,\min}$ and $T_{2,\min}$ are used as lower development-threshold temperatures, and $T_{1,\max}$ and $T_{2,\max}$ as upper thresholds.

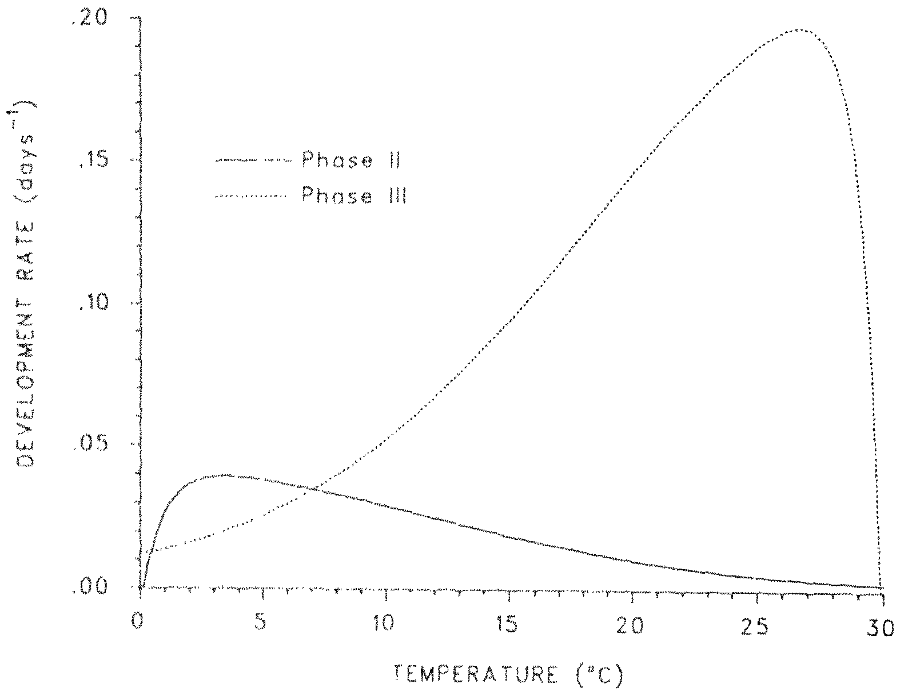

Figure 1. Relationships between temperature and development rate during the last two hypothesized phases of diapause development. Phase II described by equations (1) and (2); Phase III by equations (3) and (4).

There is a certain amount of individual variability associated with each of these rate functions that can be described by a cumulative distribution of development rates relative to the median (δ) (Fig. 2a). One such distribution can be assigned to each phase (Régnière 1984):

$$Y_1 = g_1(\delta) = \left(\frac{1}{1 + e^{[-K_1(\delta - 1)]}(.5^{Q_1} - 1)} \right)^{1/Q_1} \qquad [5]$$

and

$$Y_2 = g_2(\delta) = \left(\frac{1}{1 + e^{[-K_2(\delta - 1)]}(.5^{Q_2} - 1)} \right)^{1/Q_2} \qquad [6]$$

The simulation approach used in the model is to partition the population into n equal classes (or subpopulations), each with its relative Phase-II development rate $\delta_{1,i}$ (Fig. 2b):

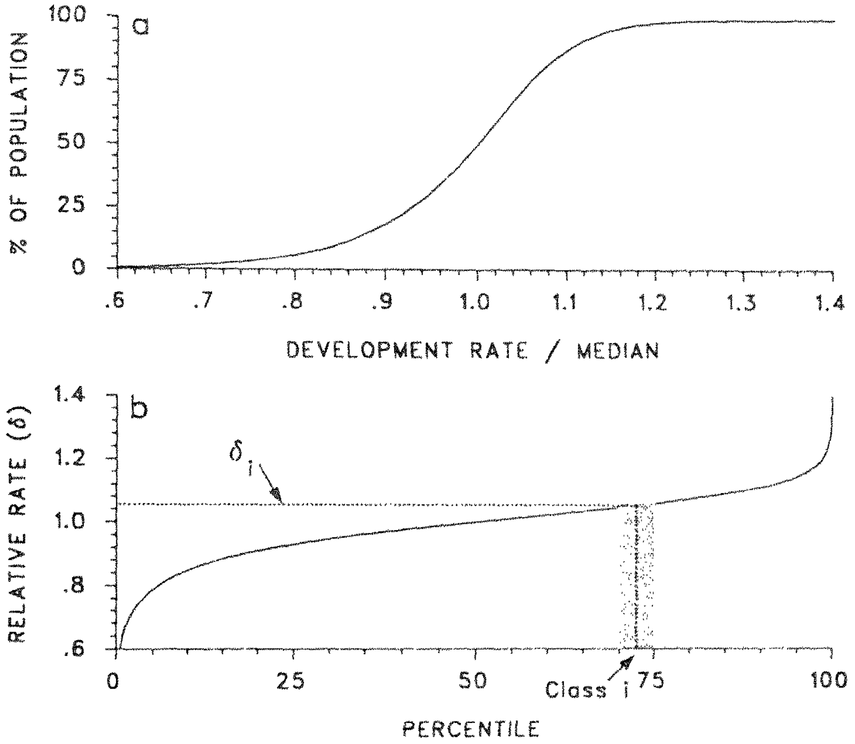

Figure 2. (a) Example of cumulative distribution of development rates, relative to the population median; (b Inverted distribution used to determine the relative rate for any percentile of the population (a given subpopulatio or class is assigned the rate corresponding to the center percentile of the class).

$$\delta_{1,i} = g_1^{-1}(Y_i) \qquad [7]$$

where Y_i is the percentile corresponding to class i. Each subpopulation is further divided int m sub-classes (sub-subpopulations) each with its relative Phase-III development rate $\delta_{2,j}$

$$\delta_{2,j} = g_2^{-1}(Y_j) \qquad [8]$$

where Y_j is the percentile corresponding to subclass j. Phase-II development is accumulatc separately for each class as long as $D_{1,i} < 1$:

$$D_{1,i}(t) = D_{1,i}(t-1) + d_1 * \Delta t * \delta_{1,i} \qquad [9]$$

where Δt is the time step (days). Within each class i, Phase-III development also is accumulat for each subclass j until $D_{2,i,j} = D_{2,max}$. Here, $D_{2,max}$ is a limit that Phase-III development cann exceed unless $D_{1,i}=1$:

$$D_{2,i,j}(t) = D_{2,i,j}(t-1) + d_2 * \Delta t * \delta_{2,j} \qquad [10]$$

Thus, the model can simulate the whole spectrum between strictly sequential ($D_{2,max}=0$) and strictly simultaneous ($D_{2,max}=1$) phases.

To describe the effect of photoperiod on diapause termination, the existence of a critical photoperiod (P_{crit}, length of photophase in hours) was assumed, above which Phase-II development is considered complete by the insect. P_{crit} is a function of Phase-II physiological age, D_1 (Fig. 3):

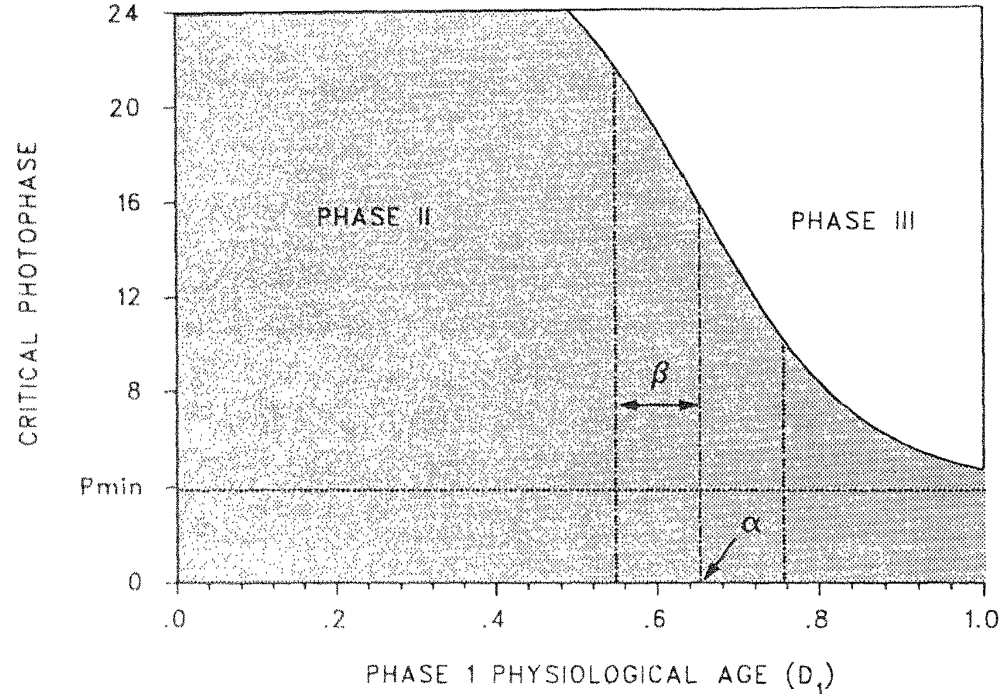

Figure 3. Example of relationship between photoperiod and Phase-II physiological age. As soon as daylength exceeds the critical photophase corresponding to a given subpopulation's physiological age, Phase II is complete and the insects enter Phase III.

$$P_{crit} = P_{min} + \frac{P_{max} - P_{min}}{1 + e^{(D_1 - \alpha)/\beta}} \qquad [11]$$

where P_{min} and P_{max} are lower and upper critical photoperiods. Parameter α is the physiological age at which P_{crit} is half-way between P_{min} and P_{max}, while β determines the spread of D_1 over which P_{crit} changes. This function is sufficiently flexible to describe photoperiod-insensitive cases

(P_{min} and $P_{max} > 24$) as well as a whole spectrum of photoperiod responses (Fig. 3). The model sets $D_{1,i}=1$ as soon as photophase $\geq P_{crit}$.

The process of diapause simulation at the core of the model is illustrated in Figure 4.

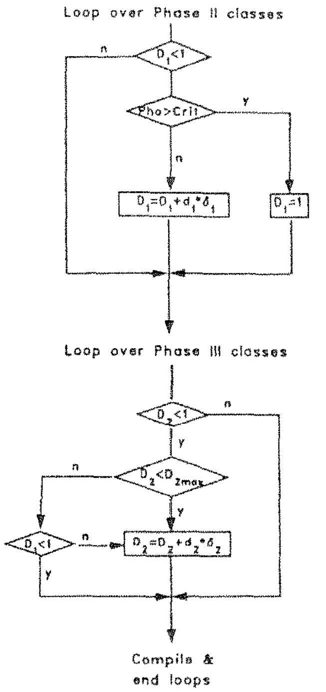

Figure 4. Flow chart of the two-phase simulation process at the core of this model of diapause development.

Model Behavior

To investigate the behavior of this model, four simulation experiments were conducted. First, the effect of cold-storage duration on the pattern of development completion (e.g., emergence of larvae in spruce budworm, egg hatch in gypsy moth) was tested. Simulated populations were exposed for various periods (from 0 to 100 days) to 5°C, and then transferred to 20°C until completion of development. Five different values of $D_{2,max}$: 0 (strictly sequential phases), .2, .5, .8 and 1 (strictly simultaneous phases) were used. Other parameter values used were:

$$P_{1,1}=0.05,\ P_{1,2}=3.0,\ P_{1,3}=5.0,\ P_{1,4}=0.03,\ T_{1,min}=0.0,\ T_{1,max}=30.0$$

$$P_{2,1}=0.25,\ P_{2,2}=3.0,\ P_{2,3}=5.0,\ P_{2,4}=0.03,\ T_{2,min}=0.0,\ T_{2,max}=30.0$$

$$K_1=K_2=23.0,\ Q_1=Q_2=2.0$$

$$P_{min}=P_{max} > 24 \text{ h}$$

The parameters of equations [1] and [3] produce the thermal responses illustrated in Fig. 1, with a Phase-II optimum near 3°C, and a Phase-III optimum near 28°C. Variability-function parameters were the same for both phases. The values of P_{min} and P_{max} ensured that photophase would remain below P_{crit} so that no effect of photopheriod on Phase II termination was introduced in the simulations.

In a second experiment, the development rates (length) of Phase III ($P_{2,1}$=.15, .25 .35 .45.) were varied, in a strictly sequential model ($D_{2,max}=0$), using the same set of experimental conditions (cold-storage durations, temperatures) and parameter values as in experiment 1.

In a third experiment, the diapausing insects were exposed to 20°C for periods of 1 to 30 days before putting them at 5°C for 45 days. Then the insects were returned to 20°C until completion of development. I used values of $D_{2,max}$= 0, .2, .5, .8 and 1. Other parameter values were the same as in experiment 1.

In the fourth experiment, the insects were kept for 16, 18 , 20 or 22 days at 5°C, and then transferred to 20°C at photoperiods of 10L:14D, 13L:11D or 16L:8D. Development-rate function parameters were the same as in experiment 1, with $D_{2,max}=0$ (strictly sequential phases) and photoperiod-response parameters set to:

$$P_{min}=4\text{ h},\ P_{max}=28\text{ h},\ \alpha=0.65,\ \beta=0.1$$

These values produced the curve in Figure 3.

Model Performance Under Natural Weather Conditions

The behavior of the sequential model ($D_{2,max}=0$) under natural weather conditions was tested using daily minimum and maximum air-temperature records from Montreal, Quebec, Canada (45°30'N, 73°35'W, 57 m altitude), as input. Daylength was calculated from information provided by Withrow (1959), in relationship with latitude (L) and day of the year (D):

$$Phot = 12 + 0.0018\, L^2 \sin\ [\, 2\pi (D - 80) / 365\,] \qquad [12]$$

A half-cosine function (Régnière 1982) was used to interpolate between minima and maxima, with a 4-hour time step ($\Delta t=0.167$). The entire population was assumed to enter diapause Phase II on August 1, and simulations were run until 100 percent of individuals had completed Phase III. To provide a basis for comparison, development-rate and variability function parameter values reflected those observed in the spruce budworm. For postdiapause (Phase III), these values were taken from Régnière (1987):

$$P_{2,1}=0.194;\ P_{2,2}=3.0,\ P_{2,3}=5.84,\ P_{2,4}=0.03,\ T_{2,min}=2.5,\ T_{2,max}=35$$

and

$$K_2=18.72,\ Q_2=2.22$$

No parameter estimates were available for Phase II. However, there is good reason to believe that some development occurs below 0°C (unpublished), so $T_{1,min}=-2.0$ was used. It is also probable that Phase-II development rates do not exceed approximately 0.02 days^{-1} at the optimum temperature of 3-6°C (unpublished). With this in mind, results from simulations with values of $P_{1,1}=0.015$, 0.020, 0.025 and 0.035 were compared, keeping other parameter values constant at:

$$P_{1,2}=3.0,\ P_{1,3}=5.0,\ P_{1,4}=0.03,\ T_{1,max}=30$$

and

$$K_1=23.0,\ Q_1=2.0$$

A series of simulations was done without consideration of photoperiod sensitivity ($P_{min}=P_{max}=24$). In another series, photoperiod sensitivity in late Phase II was included by setting $\alpha=0.65$, $\beta=0.1$, $P_{min}=8$ and $P_{max}=28$.

RESULTS AND DISCUSSION

Model Behavior

The results of experiment 1 are illustrated in Figure 5. The median time to development completion, including the cold-storage period and time at 20°C, decreased linearly with cold-storage duration between 0 and 28 days, for all values of $D_{2,max}$ (Fig. 5a). This decrease was proportional to the amount of Phase-II development that occurred during cold storage. Beyond 28 days, which was the median duration of Phase-II at 5°C (Fig. 1), development time increased with further increases in cold storage duration, up to a maximum due to completion of development at 5°C. This increase in development time resulted from slow Phase-III development at 5°C, and was inversely proportional to $D_{2,max}$ (Fig. 5a). Therefore, in this experiment, increasing phase-simultaneity resulted in a reduction in the ability of the diapause process to prevent development completion in cold storage.

Variability in development time, expressed here as the range (days) between 10 and 90 percent development completion, was high in all cases of storage duration under 28 days (Fig. 5b), because in these cases Phase-II development was forced to proceed at 20°C. Variability dropped sharply in insects kept 28 days or longer at 5°C. This is a phenomenon often observed, and has been called "synchronization". The model shows that synchronization results from the accumulation (queuing), at cold temperature, of insects in early Phase-III, which proceeds more slowly than Phase II at low temperature (Fig. 1). Once temperature increases, the insects develop rapidly and complete development synchronously. This synchronization was maintained with increasing periods at 5°C, until insects started to complete Phase III in cold-storage. However, the ability of the model to produce consistent synchronization was greatly reduced at higher values of $D_{2,max}$ (Fig. 5b). These patterns are more easily understood by examining in more detail the results of a number of simulations (Fig. 6). In a strictly sequential model ($D_{2,max}=0$), increasing the cold storage period results first in accelerating Phase-II development, and

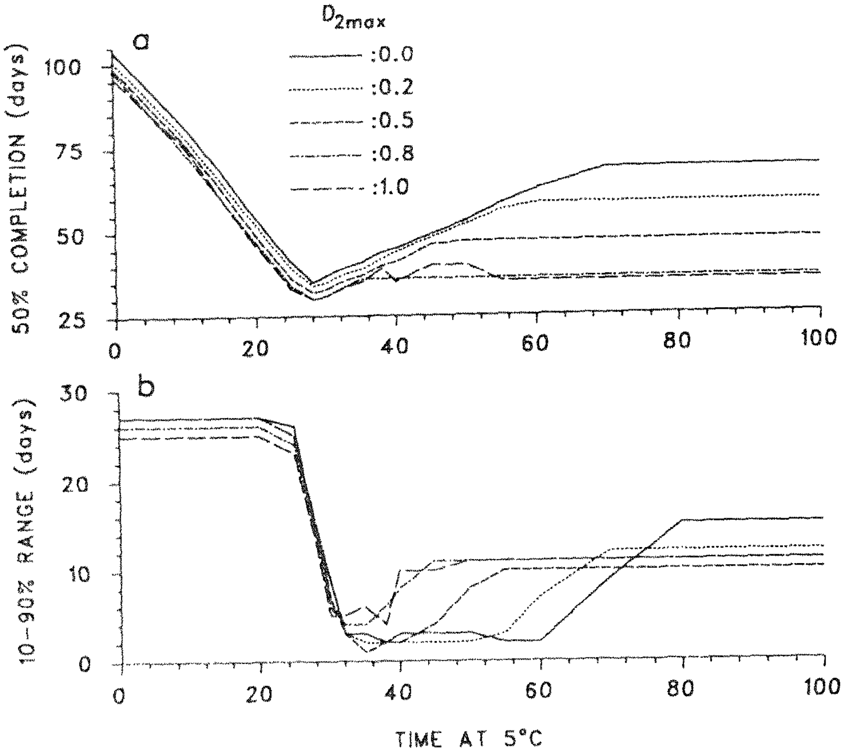

Figure 5. Relationship between the duration of cold storage (at 5°C) and completion of development after transfer to 20°C. (a) Time to completion by 50 percent of individuals. (b) Range in completion time (days) between 10 and 90 percent of the population. Results of experiment 1, changing the value of parameter $D_{2,max}$.

eventually in the queuing of insects in the early stages of Phase III, until transfer to 20°C (Fig. 6a-e). In a strictly simultaneous model ($D_{2,max}=1$), the response to short cold-storage periods is very similar to that of the sequential model, although Phase III is finished well before Phase II (Fig. 6f-h). As the cold storage period increases, however, Phase II tends to be completed before Phase III (Fig. 6h,i). Nevertheless, there is only a brief period during which synchronous development completion is achieved, because insects do not queue in the early stages of Phase III (much of Phase III development occurs simultaneously with Phase II). This reduces the ability of the simultaneous model to "hold" the insects in Phase III until transfer to warmer temperature and to synchronize their development (Fig. 6).

Under the conditions of experiment 1, intermediate values of $D_{2,max}$ (.2, .4, .6) produced a behavior qualitatively similar to the strictly sequential model: the ability to prevent development completion under adverse conditions and consistent synchronization. In fact, varying $D_{2,max}$ seems to be equivalent to changing the length of Phase III in a sequential model, as suggested by the results of experiment 2, in which the value of $P_{2,1}$ was varied, keeping $D_{2,max}$ = 0 (Fig. 7).

Experiment 3 mimicked more closely the temperate-zone temperature regimes that an insect would experience when diapause is induced in late summer. There, the cold season is preceded by a period of warm temperatures during which Phase II is slowed and, in cases of simultaneity, Phase III is accelerated. The results of the experiment indicate that the qualitative characteristics of the multiple-phase model are maintained with this type of temperature regime. Development is arrested in the early stages of Phase III, particularly in the strictly sequential model, whereas the strictly simultaneous model is even less apt to produce arrested development or synchronous completion of development (Fig. 8). Intermediate values of $D_{2,max}$ produce intermediate results, but the higher the amount of simultaneity, the less the ability of the model to produce synchronous completion upon the return of favorable temperatures (Fig. 9). However, this decreasing efficiency of the multiple-phase diapause model is the result of an effective shortening of the sequential portion of Phase III development. It is interesting to note that a pre-storage

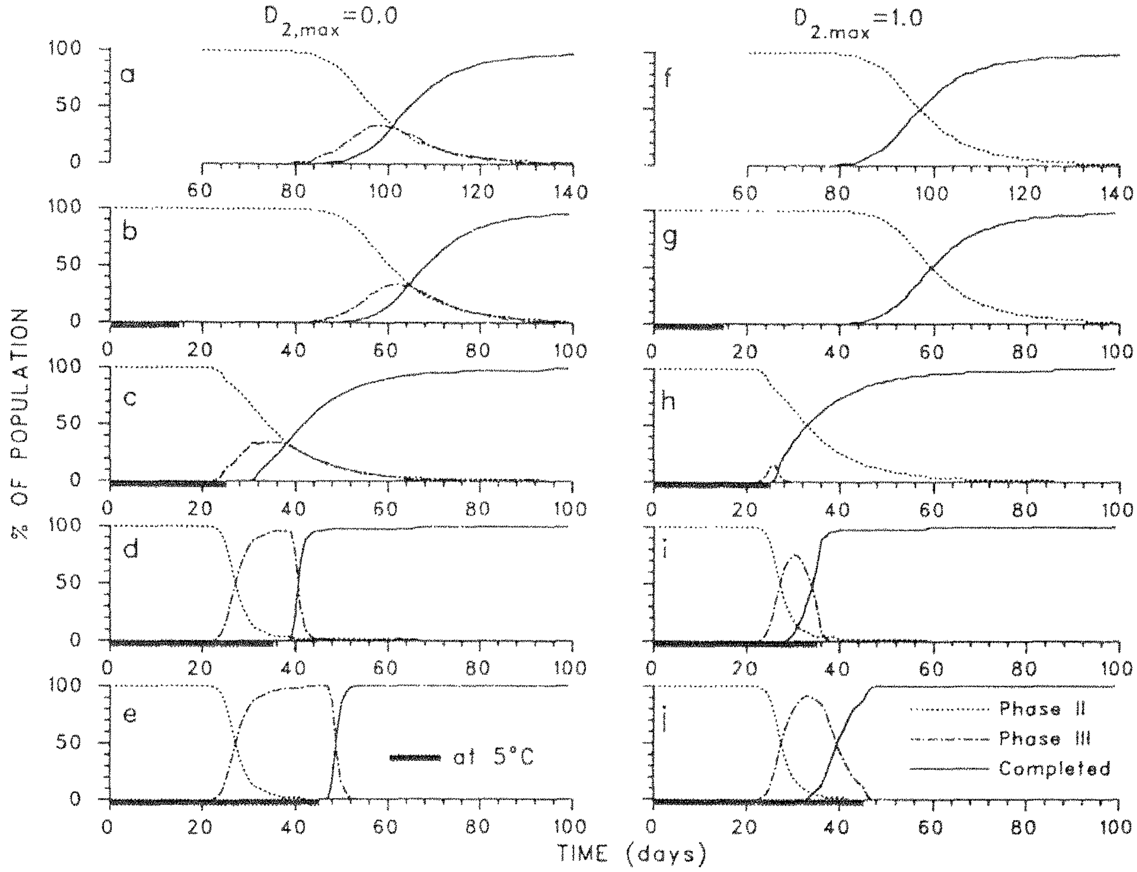

Figure 6. Percent of population in Phase II, Phase III or completing development as a function of time, with increasing durations of cold storage (at 5°C). (a)-(e): strictly sequential model. ($D_{2,max}=0$); (f)-(j): strictly simultaneous model ($D_{2,max}=1$). Results of experiment 1.

exposure to 20°C caused a net drop in total emergence time only in the strictly simultaneous model (Fig. 9, $D_{2,max}=1$).

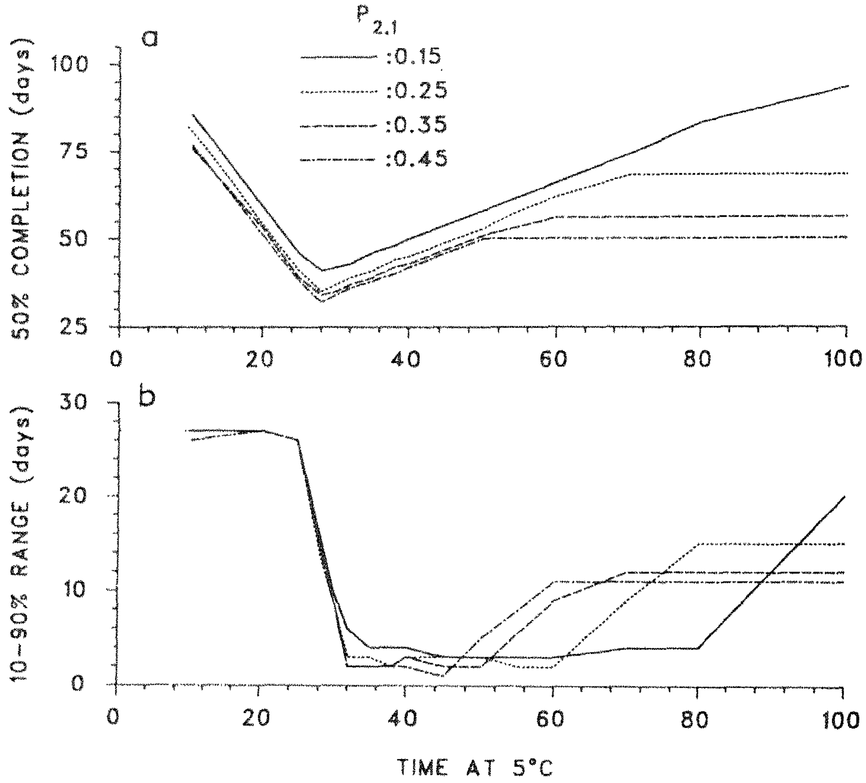

Figure 7. Relationship between the duration of cold storage (at 5°C) and completion of development after transfer to 20°C. (a) Time to completion by 50 percent of individuals. (b) Range in completion time (days) between 10 and 90 percent of the population. Results of experiment 2, changing the value of parameter $P_{2,1}$.

The results of the fourth experiment (Fig. 10) are strikingly similar to the observed reactions of spruce budworm to photoperiod during cold storage (unpublished). In the early stages of Phase II, photoperiod sensitivity is minimal, with a 3-day (10 percent) reduction in total development time for each 3-hour increase in daylength (Fig. 10a). However, after 18 days in cold storage (median Phase-II physiological age $D_1=\alpha=0.65$), the effect of photoperiod was very pronounced (Fig. 10b). This sensitivity decreased with increasing cold-storage duration, as more and more insects completed Phase II and entered Phase III (Fig. 10c, d).

Model Performance Under Natural Weather Conditions

Simulations using parameter values suited for the spruce budworm, and air temperatures from Montreal (Quebec, Canada) produced highly realistic diapause-development patterns (Fig.

11). With all the values of $P_{1,1}$ tested, the model predicted synchronous emergence over a 20-day period from late April to mid-May. These dates are quite realistic for that part of spruce budworm's area of distribution. The model's prediction was relatively insensitive to the value of parameter $P_{1,1}$. These results indicate that the multiple-phase hypothesis constitutes a robust diapause-development mechanism not very sensitive to Phase-II development rates. In the absence of photoperiod sensitivity, the best estimate of $P_{1,1}$ was 0.020 (optimum: 65 days or 9.3 weeks at 1°C), because it predicted a shift between Phase II and Phase III in the period December-February (Fig. 11b), which reflects available information on spruce budworm diapause termination (Régnière 1990).

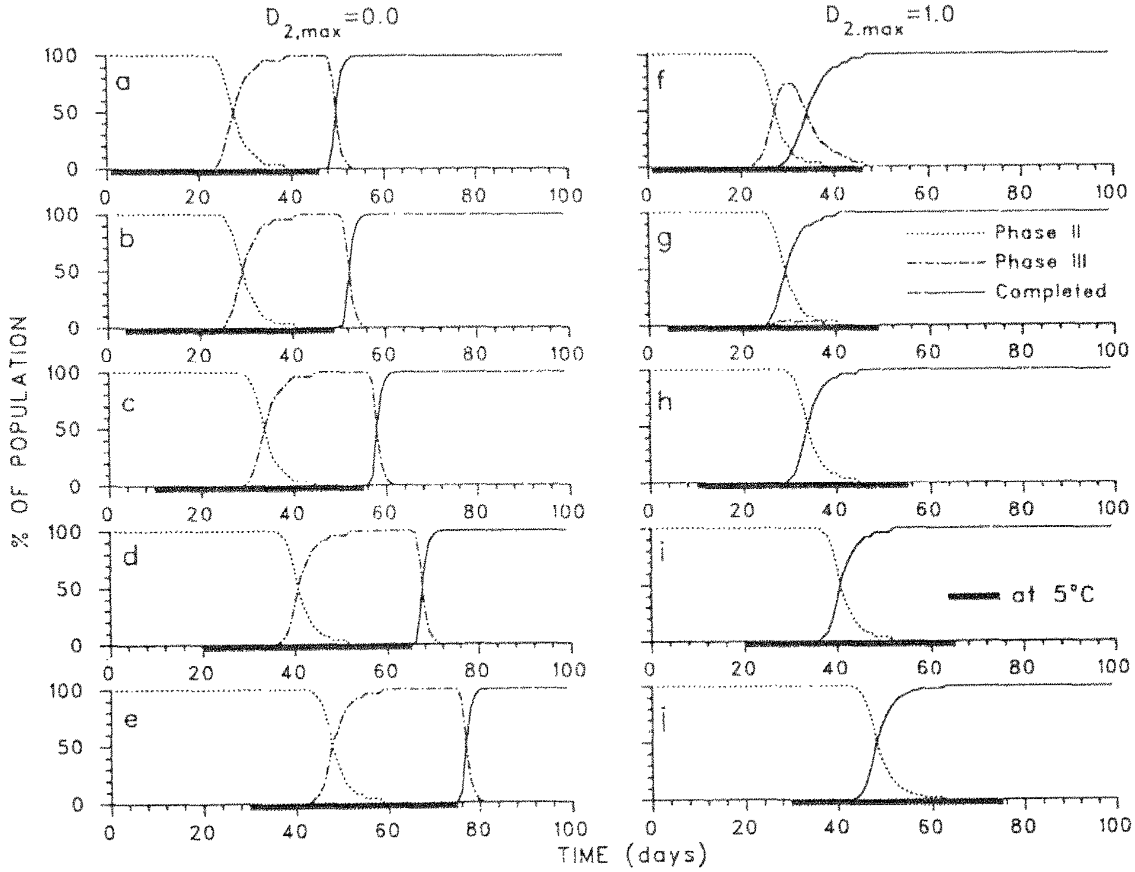

Figure 8. Percent of population in Phase II, Phase III or completing development as a function of time, with increasing durations of pre-storage at 20°C (cold storage: 45 days at 5°C). (a)-(e): strictly sequential model ($D_{2,max}$=0); (f)-(j): strictly simultaneous model ($D_{2,max}$=1). Results of experiment 3.

Photoperiod sensitivity in late Phase II (α=0.65) increased model robustness by further reducing its sensitivity to Phase-II development rates (Fig. 11d-f). With the parameter values used (equation [11]), the best value of $P_{1,1}$ was 0.015 (optimum: 87 days or 12.5 weeks at 1°C).

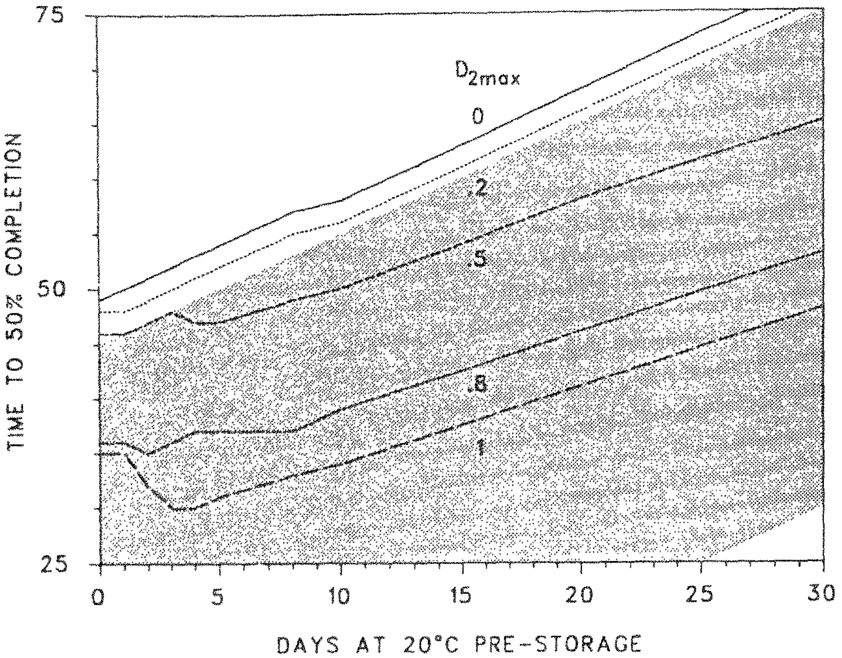

Figure 9. Relationship between the duration of pre-storage at 20°C and time to development completion by 50 percent of the population, with increasing values of $D_{2,max}$. Shaded area: development completion in cold-storage. Results of experiment 3.

CONCLUSION

The simulation experiments conducted with this multiple-phase model of diapause development indicate that phase simultaneity decreases the ability of the diapause process to prevent development completion under adverse conditions and to produce synchronous development. It has an effect similar to a reduction in the duration of postdiapause development. For these reasons, strictly simultaneous phases ($D_{2,max}=1$) probably are not common in nature. Hilbert et al. (1985) proposed the idea of phase simultaneity to explain results obtained under controlled conditions with eggs of the migratory grasshopper, *Melanoplus sanguinipes* (Parker 1930, as cited in Hilbert et al. 1985). It is possible that a sequential multiple-phase model of diapause development could explain Parker's observations if thermal responses during Phase III were not assumed constant, but gradually changing, as was found in the spruce budworm (Régnière 1990). The partly simultaneous model ($D_{2,max}=0.8$) of Hilbert et al's (1985) adequately predicted the occurrence or absence of diapause in various geographical locations. From the present analysis, however, it seems that similar results could be obtained with a sequential-phase model with a short second phase. It also seems likely that modeling the occurrence of facultative diapause in insects will require consideration of diapause-induction processes, which undoubtedly are involved in determining diapause intensity, defined in terms of Phase-II development rates. Important developments have been made in modeling the endocrine processes involved in diapause induction and maintenance (Zaslavski 1988).

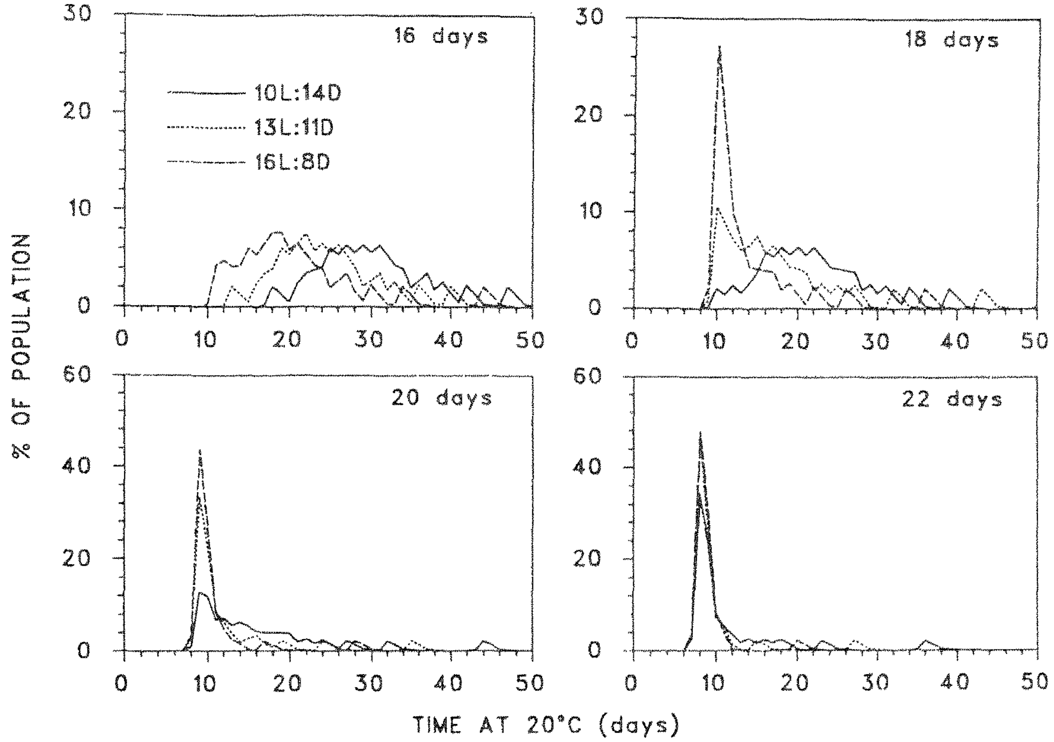

Figure 10. Relationship between time at 20°C and the rate of development completion (percent of population) at three photoperiods, after (a) 16, (b) 18, (c) 20 and (d) 22 days storage at 5°C. Results of experiment 4.

A multiple-phase model of diapause processes can produce highly realistic simulations of diapause development, even when Phase-II parameter values are not accurately estimated. In the case of spruce budworm, these values were arrived at with relatively simple experiments in which cold-storage duration, temperature and photoperiod were varied (unpublished). More accurate estimation would require observation of the shift between phases in individuals under diverse environmental conditions. In cases where there is no clear morphological or behavioral sign of such a change in individuals, this observation may require the use of more sophisticated experimental methods such as the measurement of metabolic rates and thermal responses through respirometry. Based on the results of the simulation experiments reported here, it would be difficult to distinguish partially simultaneous from strictly sequential phases on the basis of measurements of development duration under various temperature regimes. Again, analysis of changes in metabolic rates and responses to temperature throughout diapause would provide an appropriate basis for this distinction.

A multiple-phase model of diapause development seems to produce generally valid predictions of diapause-development completion times. These predictions were relatively insensitive to Phase-II development parameters. Photoperiod sensitivity in late Phase II further increased the model's robustness by reducing optimal Phase-II development rates. Such a reduction diminished the likelihood that insects enter Phase III in the fall and complete development under adverse conditions. Photoperiod sensitivity would then allow the insects to enter Phase III in early spring, bypassing further Phase-II development. However, the simulations done here reflected a cold-winter climate. To simulate diapause development in milder areas (e.g. southern United States), where winter temperatures often exceed postdiapause development thresholds, more precise parameter estimates would be required for Phase-II development rates, variability and photoperiod sensitivity. It would seem adaptive for insects living in such environments to display wider separation in the ranges of temperatures at which each phase can proceed, and to avoid phase simultaneity. Photoperiod sensitivity also would enhance the likelihood of a timely switch between phases, although variation in daylength is less pronounced at lower latitudes.

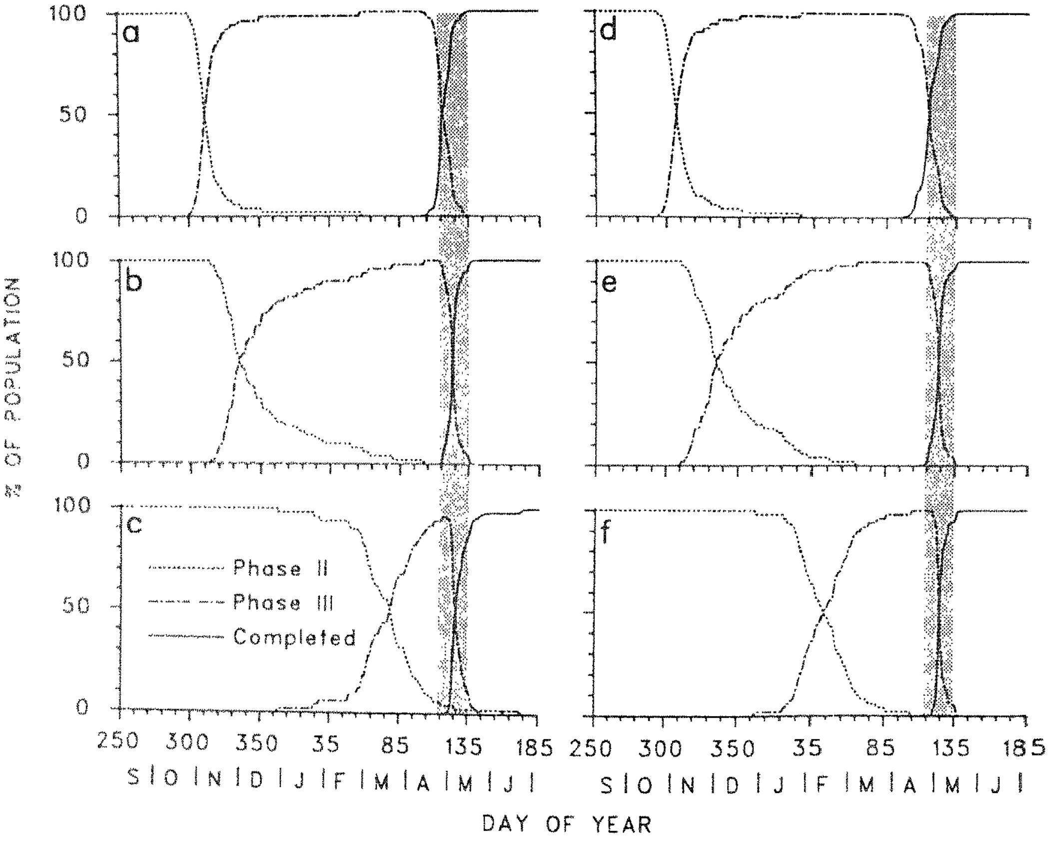

Figure 11. Percent of a simulated population in Phase II, Phase III, or completing development as a function of day of the year, under a natural air-temperature regime from Montreal (Quebec, Canada), with decreasing values of $P_{2.1}$: (a) 0.035, (b) 0.025, (c) 0.02 and (d) 0.015. Shaded area: 20-day period from late-April to mid-May during which emergence of spruce budworm can be expected to occur at that latitude.

Several insects have evolved additional mechanisms by which the diapause process can be fine tuned to insure that development resumes in a timely fashion. Photoperiod sensitivity early in postdiapause would increase the likelyhood that postdiapause development proceeds only as daylength increases in the spring (McNeil and Stinner 1983, McNeil and Fields 1985). A gradually changing response to temperature during postdiapause probably also constitutes such a mechanism (Régnière 1990). It is not known at this time whether such changes are common. They would best be detected, once again, through direct measurement of metabolic rates during postdiapause.

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MICROCLIMATIC EFFECTS ON THE PHENOLOGY OF EGG HATCH OF THE GYPSY MOTH, *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE)

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ABSTRACT

Simulation of the phenology of egg hatch of gypsy moth, *Lymantria dispar* (L.), indicates that substrate temperature and microhabitat distribution of egg masses affects our predictions of hatch phenology. The influence of the position of egg masses of the gypsy moth on a tree bole on their hatching time, therefore, was investigated at field sites in Ontario and Pennsylvania. Female gypsy moths deposited egg masses predominantly on the eastern sides of tree boles but egg hatch was most advanced in those masses located on southern sides. There was a weak relationship between height on the tree bole and rate of egg hatch but not on the lower portion (< 2 m) of the bole. Eggs deposited under bark flaps experienced a significant delay in hatching compared with eggs deposited on the bark surface. Within egg masses, eggs from the bottom third of the mass hatched slightly later than those from the middle or top thirds of the mass. Understanding the microclimatic variables that influence hatch times will improve the accuracy of prediction of the phenology of this forest pest.

INTRODUCTION

Understanding the phenology of the gypsy moth, *Lymantria dispar* (L.), is critical for effective application of control programs against this important pest of hardwood forests because suppression methods must be targeted against specific life stages (Lewis et al. 1979). To facilitate the timing of control treatments, attempts have been made to develop predictive models of phenology (Casagrande et al. 1987, Gray et al 1991, Logan et al. 1991). Timing of egg hatch is the most difficult phenomenon to predict (Masaki 1956, Johnson et al. 1983, Waggoner 1984, Lyons and Lysyk 1989). This difficulty arises in part from an inability to determine when diapause terminates and development proceeds and because weather in spring is extremely variable and subject to additional variation resulting from the microclimate in the microhabitats where egg masses occur.

At constant temperatures, most variation in the time of egg hatch occurs within rather than between egg masses (Lyons and Lysyk 1989). These differences in hatch times within an egg mass may be related to within mass differences in egg size with the last eggs deposited in the mass being the smallest (Leonard 1970). In the field, however, most variability in the time of egg hatch is among different egg masses. Lyons and Lysyk (1989) found that there were small but significant differences in hatch times for eggs collected in the spring from two heights (< 1 m and > 1 m) on the tree bole and subsequently reared in the laboratory. Much of this variability was thought to be due to the influence of microclimatic differences associated with the position of the egg mass in the field. Johnson et al. (1983) found no relationship between egg-mass position and hatching times, but attributed this to their small sample size. If the position of egg masses in the field influences their hatch phenology, and position is, in turn, influenced by population density (Montgomery 1990), then the phenology of gypsy moth populations might be influenced by the density and stage of the outbreak.

This paper describes further simulations of egg hatch using the Lyons and Lysyk (1989) model to examine the effect of microhabitat temperature on hatch phenology. The use of this model to provide input to a more comprehensive model of gypsy moth larval and pupal development (Casagrande et al. 1987) also is demonstrated. Our goal was to examine and describe the relationship between the timing of egg hatch and egg-mass position including: egg position within the egg mass, vertical position of the egg mass on the tree, and compass orientation (aspect) of the egg mass on the tree bole. In addition, we describe a preliminary survey of the distribution of egg-mass positions in the field.

MATERIALS AND METHODS

Egg Development Simulation

The egg hatch model developed by Lyons and Lysyk (1989) was used to simulate the development of gypsy moth eggs using the temperature data from Mont-St.-Hilaire, Quebec (39°48'N, 79°40'W) in 1988 to 1990. Individual hourly temperatures (T) were estimated from daily maximum and minimum temperatures using the algorithm developed by Régnière (1982). Development rates, for each hour of the day, were determined from the temperature-dependent solution to the following rate equation:

$$\mathrm{rate}(T) = -0.1079 + 0.0145T + (-0.00024T^2) \qquad (1)$$

Calculated rates were accumulated and used to solve the variability function. Variation in hatch around the mean developmental times was described using a cumulative Weibull function [2], where F(x) is the proportion of the population that had completed development by normalized time x.

$$F(x) = 1 - \exp(-((x - 0.7519)/0.2704)^{2.9442}) \qquad (2)$$

Additional simulations were run using bark temperatures as model input to investigate the effects of microclimate on egg hatch. Bark temperatures were estimated from ambient air temperature using the following function (Lyons and Lysyk 1989):

$$\mathrm{Ln}(\text{bark temperature}) = 0.061 + 1.004 * \mathrm{Ln}(\text{air temperature}) \qquad (3)$$

This same model, without bark temperature correction, was used to predict the hatch phenology of eggs in southeastern Ontario using weather data collected at Godfrey (44°32'N, 76°41'W). The temporal distribution of predicted hatch was used as input for a model of larval and pupal development of gypsy moth (Casagrande et al. 1987). Predicted development was compared with observed development of larval and pupal populations at Northbrook, Black Lake, Bon Echo Provincial Park, and Coxvale, sites all within 57 km from the Godfrey weather station.

Position Within Egg Mass

To determine the effect of the position of an egg within the egg mass on the time of egg hatch, egg masses collected near Kaladar, Ontario (44°39'N, 77°20'W), on 15 September 1988 were used. Gypsy moth egg masses are usually teardrop shaped, with the last eggs deposited at the apex. Typical egg masses were collected intact in the field and placed on sticky paper, with their orientation recorded on the paper. They were then returned to the laboratory, where they were trisected horizontally and each portion placed in a separate plastic vial. These portions were stored for 6 months at 0°C. They were then incubated at a constant temperature of 21°C. Egg masses were examined daily and newly eclosed larvae were removed and counted. Hatching rates (the reciprocal of hatching times) were calculated for individual eggs and mean rates for the three within-mass positions were determined. Mean rates were compared using the analysis of variance procedure, PROC ANOVA (SAS Institute 1985).

Spatial Distribution

The effect of the spatial distribution of egg masses on the rate of egg hatch was examined at study sites in southwestern Pennsylvania (39°48'N, 79°40'W) and southwestern Ontario (42°41'N, 80°28'W). At the Ontario site, line transects were run through a hardwood woodlot. At each tree, one egg mass in each cardinal direction was marked. This was continued until 30 egg masses in each cardinal direction quadrant were marked, for a total of 120 egg masses. At the Pennsylvania site, 21 sample points were located in a grid with approximately 30 m between points. At each point, the nearest six or fewer egg masses were marked. The position above the ground and the aspect (compass direction, in degrees) was determined for each egg mass. For these egg masses, whether or not the masses were under bark flaps was also noted. Hatching dates of egg masses under bark flaps and egg masses on the bark surface were compared with a *t*-test using PROC TTEST (SAS Institute 1985). Hatching dates of egg masses under bark were not stratified by position for analysis because of the small sample size. In Ontario, egg masses were examined daily; in Pennsylvania, egg masses were observed at 2- to 3-day intervals. In Ontario, only egg masses less than 2 m above the ground were examined; in Pennsylvania, egg masses up to 5 m above the ground were marked for examination. At both locations, a Tangletrap® barrier was placed around each mass to prevent the dispersal of newly eclosed larvae. Egg masses were examined throughout the emergence period and newly eclosed larvae were counted and removed.

For each data set, a sine wave function of the form:

$$\text{MEAN HATCH} = A * [B + \text{SIN}(2\pi * (\psi - C)/360)] \tag{4}$$

was fitted to the mean hatch times (days) using a nonlinear regression procedure, PROC NLIN (SAS Institute 1985), for each egg mass, where ψ = aspect (degrees), and A, B, and C are estimated parameters. Mean hatch date also was regressed as a linear function of height above the ground using PROC REG (SAS Institute 1985). For Pennsylvania data, only egg masses found on the surface of the bark were used in the regressions.

Aspect of the Egg Masses

Because the position of egg masses on the tree may affect hatch phenology, a survey was conducted at two woodlots in Ontario to determine the aspect orientation of egg masses on the lower portion of tree boles. The two woodlots had different egg-mass densities. The woodlots were separated by about 3.5 km and were located within the Deciduous Forest Region (Hosie 1979). One location, Backus Woods, was part of a Conservation Authority's managed woods and the other, St. Williams Nursery, consisted of woodlands adjacent to a provincial forest tree nursery. In order to adequately represent the woodlots, 0.01-ha fixed-radius plots were sampled until 1000 egg masses had been recorded. Plot locations were selected from maps before visiting the sites. Plot centers were identified with flagging tape and two ropes, with their center points marked, were used to determine plot boundaries. Egg masses on all trees greater than 7.5 cm in diameter within the plot were included. Compass aspect (orientation) was recorded for each egg mass. Since the position of egg masses around the tree bole represents a circular distribution (i.e., 0° = 360°) the distribution was described using the length and angle of mean vectors (Batschelet 1981).

RESULTS

Egg Development Simulation

The results of the computer simulations for egg hatch at Mont-St.-Hilaire in 1988 to 1990 are shown in Figure 1. The predicted mean hatch was approximately 5 days later than observed hatch for 1988, approximately 2 days later than observed hatch for 1989 and 3 days following egg hatch in 1990. The incorporation of the bark temperature correction function into the model considerably improved predicted hatch in all years. In all cases the simulations that used bark temperatures predicted hatch within 1 day of the observed time.

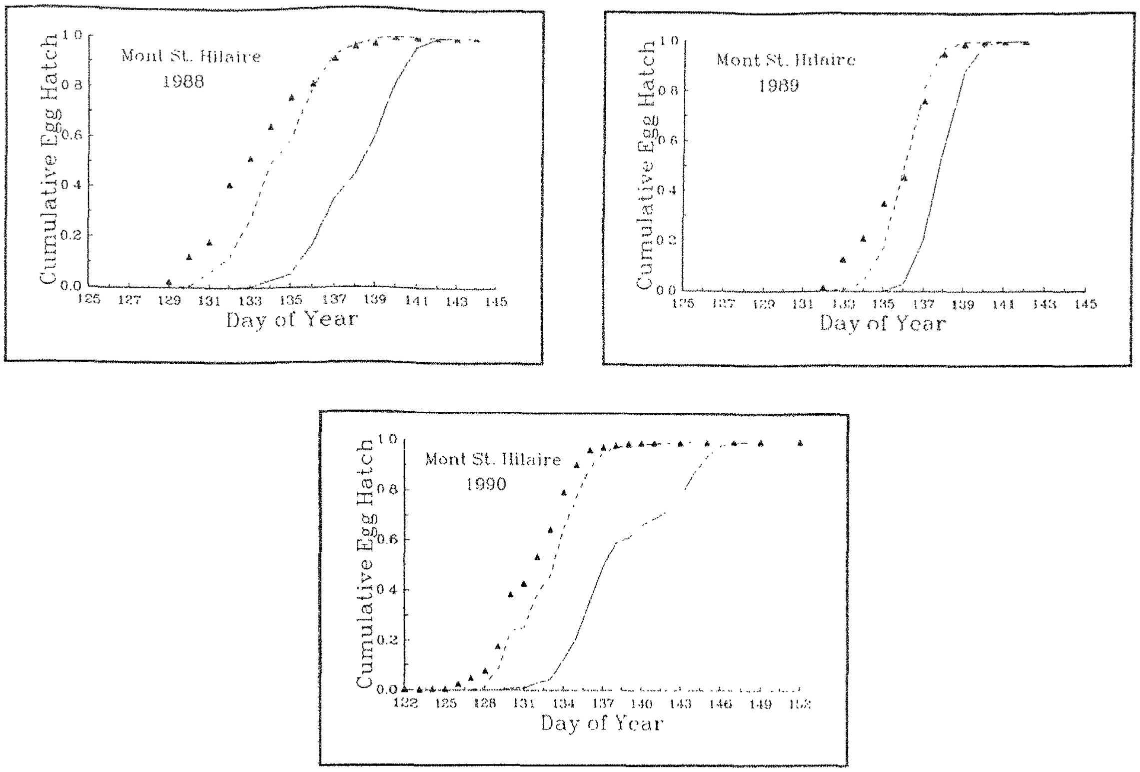

Figure 1. Results of computer simulations of egg hatch for Mont-St.-Hilaire, Quebec, in 1988, 1989, and 1990. Triangles show observed egg hatch. Solid lines represent uncorrected predictions and dashed lines are predictions incorporating bark temperature corrections.

Using the predicted egg hatch from the Lyons and Lysyk (1989) model as starting values for the Casagrande et al. (1987) model, the simulated larval and pupal development agreed closely to observed development in all locations (Fig. 2). The bark temperature correction function was not used in the simulation because of the great distances between sites.

Position Within Egg Mass

The first-laid eggs from the bottom of the masses had significantly ($F = 16.69$; $df = 2, 5758$; $P < 0.0001$) slower development rates than did those from the middle and top (Table 1).

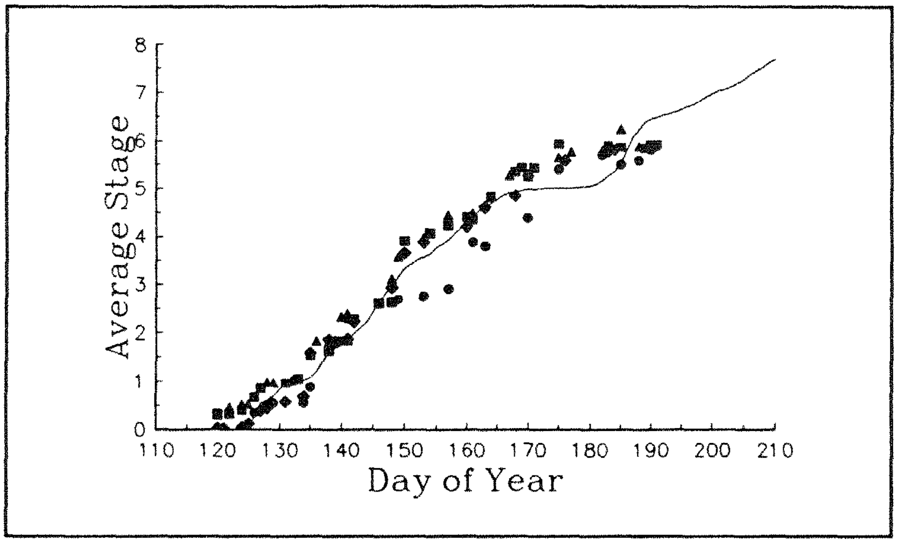

Figure 2. Simulated development of gypsy moth by the Casagrande et al. (1987) model for weather data collected at Godfrey, Ontario, using egg hatch predicted by the model of Lyons and Lysyk (1989) as starting values. The circles, squares, triangles, and boxes show observed development at Northbrook, Black Lake, Bon Echo, and Coxvale, respectively, while the solid line is the predicted development.

Table 1. Mean development rates for trisected egg masses of gypsy moth reared at a constant temperature.

Egg mass portion	n	Mean[a] development rate (1/days)	SE
top	1325	0.0957a	0.00032
middle	2392	0.0960a	0.00024
bottom	2044	0.0940b	0.00026

[a] Means followed by the same letter are not significantly different (Tukey's studentized range test [SAS Institute 1985]; $P > 0.05$).

Spatial Distribution

There was no relationship between mean egg hatch time for each egg mass and the height of the egg mass above ground at the Ontario site (Fig. 3A) ($F = 0.251$, $df = 118$, $P =$

0.6174), although all egg masses examined were below 2 m on the tree boles. In Pennsylvania, however, egg masses were observed over a greater range of vertical distances on the tree boles, and there appeared to be a slight delay in hatching for the highest egg masses relative to egg masses lower on the bole (Fig. 3B). Fitting a regression line to the data points supported this observation ($F = 6.511$, $df = 65$, $P = 0.0131$). As indicated by the linear regression for mean hatch times (Y) as a function of height on the tree bole (X) ($Y = 0.0133X + 129.10$), the slope was only slightly positive and the regression explained only 9 percent of the variability ($r^2 = 0.0911$). Egg masses above 2.5 m had a mean hatch date of 14 May (day of the year = 134.8, $n = 16$, $SE = 0.737$), whereas those below this height had a mean hatch date of 12 May (day of the year = 132.7, $n = 77$, $SE = 0.636$).

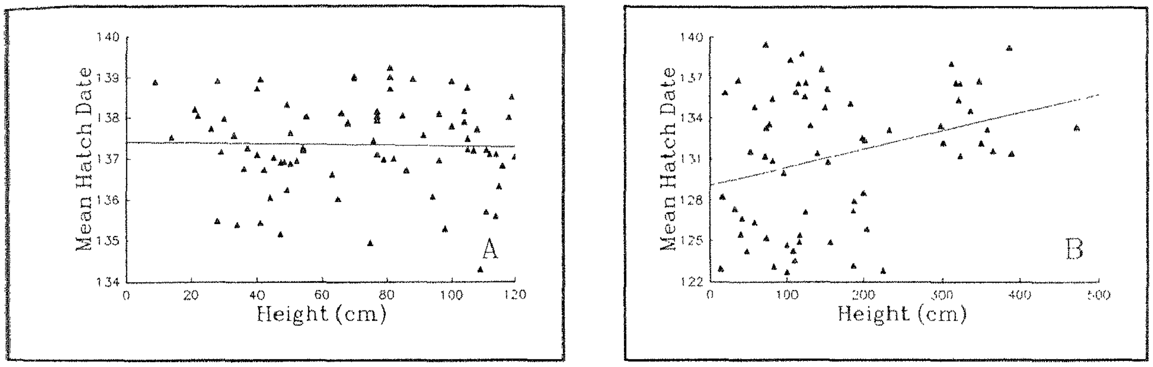

Figure 3. Mean hatch dates of gypsy moth egg masses as a function of height on the tree bole for (A) Ontario and (B) Pennsylvania populations. Triangles are observed points and the lines represent the results of linear regressions.

Egg-mass hatch times also varied with the aspect (orientation) of the egg mass (Fig. 4). Figure 4A shows the sine wave function fitted to the Ontario data and demonstrates that eggs on the southern side of the trees hatched first and those on the northern side hatched last, with a sine-wave gradient from south to north. For the Pennsylvania data, a similar but weaker relationship was apparent (Fig. 4B). Table 2 lists parameter and R^2 values for the regression lines.

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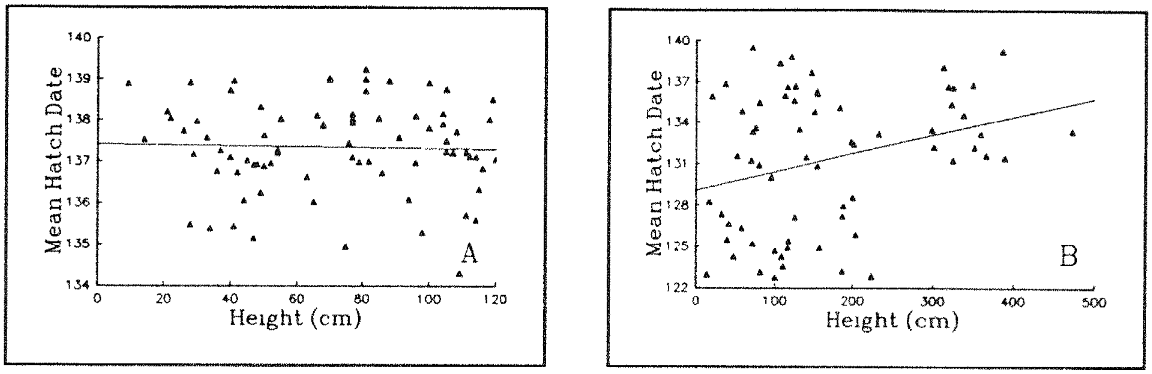

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populations levels. The mean number of eggs on the lower 2 m of tree boles per 0.01 ha was 7.0 for the Backus Woods site (*n* = 142, *SE* = 0.78); at the St. Williams Nursery site the mean number of egg masses was 92.4 (*n* = 11, *SE* = 42.3). The former site was designated the sparse population site, and the latter site was designated the dense population site.

The frequencies of the egg masses in each site were plotted by aspect, and a discernible pattern was apparent, with egg masses concentrated in one direction for both populations (Fig. 5). The lengths of the mean vectors were significant, indicating that the distributions were not random (Table 3). The angle of the mean vectors (Table 3) for both circular distributions indicated that the egg masses for both populations were concentrated on the eastern sides of the trees.

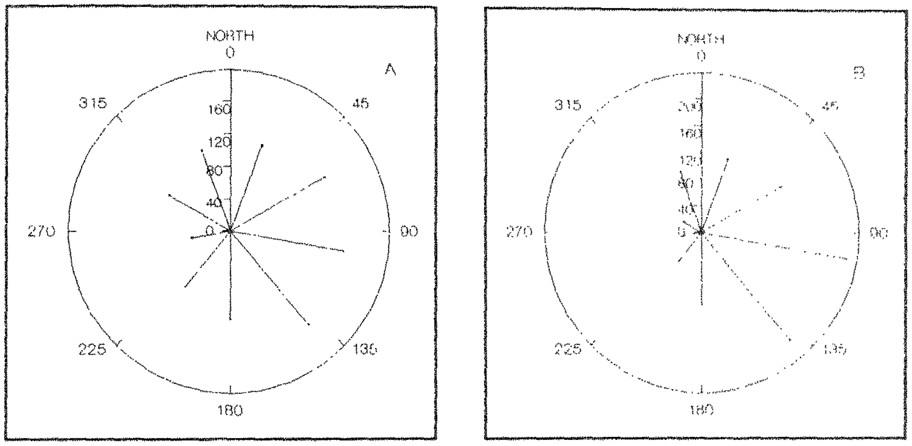

Figure 5. Frequencies of gypsy moth egg masses at 40° class intervals distributed around tree boles for sparse (A) and dense (B) populations.

Table 3. Circular statistics for spatial distribution of gypsy moth eggs around the tree bole, by aspect.

Population density	n	Angle of mean vector (angular deviation)	Length of mean vector
sparse	982	92.3° (73.5°)	0.1777[a]
dense	1016	99.0° (62.3°)	0.4079[a]

[a] not random (Rayleigh test, $P < 0.001$)

DISCUSSION

Further simulations of egg hatch using ambient air temperature as input to the Lyons and Lysyk (1989) model demonstrates the utility of the model for predicting this phenological event. Temperatures in the microhabitat where egg masses reside, however, often are considerably different from ambient air temperatures recorded in shelters that house weather-sensing equipment. For example, temperatures on the surface of the bark often are considerably higher than ambient air temperatures (Nicolai 1986) and would influence the development of eggs that have a bark substrate. The incorporation of the bark temperature correction function into the model considerably improved the agreement between predicted and observed hatch for all 3 years tested. The Lyons and Lysyk (1989) egg hatch model also performed very well in providing an egg hatch distribution over time for use in the Casagrande (1987) larval and pupal development model. Large distances between the gypsy moth populations and the weather station from which the temperature data were obtained would explain some of the disparity between observed and predicted development. Use of the bark temperature correction function would not be warranted to explain microclimatic variability at this scale of resolution.

The improved prediction of egg hatch using a bark microclimate correction function suggested that to better understand egg hatch at a finer scale, one must understand the spatial distribution of egg masses in their microhabitat and how this relates to egg hatch phenology. Toward this end we investigated the influence of position within the egg mass and position on the tree bole, with respect to cardinal direction and height, on hatch phenology.

Slight but significant differences in egg hatch were observed in eggs from the bottom of masses. However, the differences in mean development times were less than 1 day. These differences were, therefore, too small to be of relevance in predicting hatch in the field. The bottom eggs are the largest, as described by Leonard (1970), and their size would vary directly with population quality. Although the data show that the position within an egg mass affects hatch times, we did not compare egg-mass size with hatch times and, therefore, cannot make judgments about female resource allocation. Size differences may have influenced egg hatch times.

Vertical distribution on the tree had only a slight effect on hatch date. The weak relationship between egg-mass height above ground and hatch phenology support the observations that only slight vertical temperature (Christy 1952) and radiation (Anderson et al. 1990) gradients or variability exist in the lower portion of a bare deciduous canopy, and that these differences have little significance for egg hatch.

The insulating effect of snow cover in reducing gypsy moth egg mortality is well known (Leonard 1972, Madrid & Stewart 1981). Snow cover also changes the microclimate of the egg masses and consequently influences hatch phenology. Small differences in hatch phenology in eggs collected from low and high on the tree bole by Lyons and Lysyk (1989) may have been due to the presence of a winter snow cover. For both field locations in the present study, however, there had been virtually no snow cover during the overwintering period to influence hatch times.

Bark surfaces experience higher than ambient temperatures as a result of solar radiation (Nicolai 1986) and egg masses exposed to direct solar radiation hatch sooner than unexposed egg masses (Leonard 1968). This explains the earlier egg hatch on the southern (180°) side of the trees. The similarity in the values of the phase lags (parameter C) of the sine wave regressions describing populations in both Ontario and Pennsylvania was remarkable, and indicates similarity in the relationship between position and mean egg hatch.

Eggs on the surface of the bark at the Pennsylvania site hatched before those under bark flaps. Presumably, microclimatic conditions under the bark flaps delayed hatching of egg masses in comparison with egg masses exposed to solar radiation and ambient temperatures. Temperatures under bark plates are considerably lower than those on the surface of the plates (Nicolai 1986). Differences in the variances between egg masses under bark flaps and those on the bark surface suggest that egg masses under the bark are buffered from extremes of temperature resulting from differential solar radiation.

A survey of egg mass distribution indicated that the majority of gypsy moth egg masses was found on the eastern sides of the trees. Bellinger et al. (1989) found a preponderance of egg masses on the sides of trees facing the edges of woodlots. Since the plots surveyed in this study were distributed throughout the woodlots, we are confident that the distribution of the egg masses was influenced by some factor other than an edge effect. Eggs on the eastern side of the trees are not the first eggs to hatch. Since females mostly eclose in the early part of the photophase (ODell 1978, Ma et al. 1982), the position of egg masses might merely reflect female activity in response to morning sunlight. This would, however, be contrary to Doane's (1968) observation that females become negatively phototropic when searching for oviposition sites or that oviposition occurs late in the day. Females deposit eggs in a variety of locations. The selection of oviposition location may be inadvertent on the part of the female or may be limited by available space, but is probably primarily the result of female choice. Egg deposition preference, therefore, may reflect a tendency of females to select for intermediate hatch times as a hedge against asynchrony with the host plant. The protracted hatch observed at the two field locations supports this supposition.

From an applied perspective, the required resolution for phenological predictions is dependent on the scale at which the pest manager is interested. The evidence presented here indicates that variables such as position within the egg mass, location under bark flaps, aspect and to a slight degree height above the ground, influence egg hatch phenology at the microhabitat level. At a local scale, however, incorporation of a bark correction function into an egg hatch model is more than adequate to simulate temporal distribution of hatch. As we move further up the scale to regional predictions, using weather data collected at considerable distance from the gypsy moth population of interest, then even this added resolution becomes unnecessary.

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GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) POPULATION SIZES AND THE WINDOW OF OPPORTUNITY IN SPRING

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ABSTRACT

Temporal limitations on the opportunity for establishment on hosts may contribute to insect population dynamics. Unpredictable weather and variable synchrony with host budburst in spring influence the time available for host-seeking by larval gypsy moth. Female gypsy moths do not fly, and dispersal occurs by ballooning of newly hatched larvae in the spring. First instar larvae require warm and dry conditions for host-seeking. Models were used to predict the timing of egg hatch and budburst of hosts from weather variables, the rate of starvation of newly hatched larvae as a function of temperature, and thus the maximum potential opportunities for host-seeking by a population of larvae. The greater the potential for host-seeking in spring, the greater the population size in the fall. This suggests that the window of opportunity for host-seeking has a significant impact on gypsy moth population size; thus year-to-year variation in spring weather may contribute to the variability of population sizes of spring-feeding forest Lepidoptera.

INTRODUCTION

Many outbreaking species of forest Lepidoptera, including the gypsy moth, *Lymantria dispar* L., begin feeding in the spring around the time of budburst of their host trees (Nothnagle and Schultz 1987; Hunter 1991). Spring-feeding, outbreaking species also tend to have flightless females that are indiscriminate in the choice of oviposition sites (Nothnagle and Schultz 1987; Barbosa et al. 1989; Hunter 1991). Host selection in these species is by passive wind dispersal of larvae. There is a potential connection between this suite of traits and numerical variability. The degree of synchrony between spring eclosion and budburst of host trees may be an important contributor to the population dynamics of these species. The contribution of the phenology of establishment on ephemeral high quality spring foliage to population size variability of the gypsy moth was examined.

Gypsy moth caterpillars must hatch, select hosts, and begin feeding in a relatively short period in the spring, since the acceptability and suitability of host foliage decline rapidly after budburst (Raupp et al. 1988; Hunter and Lechowicz 1992a). Life tables indicate that a large proportion of generation mortality occurs at this stage (Montgomery and Wallner 1988). Two factors could have large impacts on the time available for host-finding: weather during the host selection period and synchrony between egg hatch and host leaf emergence. Gypsy moth larvae are inhibited by cool temperatures and rain (Leonard 1971) from seeking hosts. Larvae that hatch too early with respect to leaf emergence will starve. Late-hatching larvae will not encounter acceptable hosts due to declining host quality. Successful establishment of larvae depends on their hatching when young leaves are available, and on weather events that influence the ability of larvae to get to those leaves.

Thus, the degree of synchrony with host leaf emergence and weather conditions during the establishment period may contribute to population size variability. To assess the contribution of the establishment period to population dynamics, long-term records of population size, weather, egg hatch dates, and budburst dates at a site are necessary. These do not exist. Here, models that predict the time of budburst and egg hatch and the rate of starvation of larvae have been used to seek correlations between change in population size and conditions during the establishment window of gypsy moth larvae at sites where there are records of population size.

METHODS

Gypsy Moth Egg Hatch Model

Several models of gypsy moth egg hatch have been developed. The model of Johnson et al. (1983) predicts the time of egg hatch based on accumulated thermal sum from 1 January. Lyons and Lysyk (1989) used the relationship between development rate and temperature to predict the timing and progress of egg hatch. They fit a quadratic equation to the temperature response and, for comparison, they adapted the linear Johnson model to this format. However, most insect development rate curves are S-shaped rather than quadratic (Wagner et al. 1984a). Lyons and Lysyk failed for various reasons to observe hatch at temperatures below 11°C, which could explain why the quadratic fit their rate data better. Thus, as an alternative to these two models, two points on the Johnson et al. (1983) linear curve (at 6 and 7.5°C) were added to the data of Lyons & Lysyk, and the curve was remodeled using the SAS program of Wagner et al. (1984a). This will be called the biophysical model, since it is based on temperature-dependent enzyme activities. The form of the model is

$$R(T) = \frac{\mathrm{RH025}\ \dfrac{T}{298.15}\ \exp\left[\dfrac{HA}{R}\left(\dfrac{1}{298.15}-\dfrac{1}{T}\right)\right]}{1+\exp\left[\dfrac{HL}{R}\left(\dfrac{1}{TL}-\dfrac{1}{T}\right)\right]+\exp\left[\dfrac{HH}{R}\left(\dfrac{1}{TH}-\dfrac{1}{T}\right)\right]} \tag{1}$$

where r(T) = mean development rate at temperature T (°K), R = the gas constant (1.987 cal degree^{-1} mole^{-1}), RHO25 = development rate at 25°C, HA = enthalpy of activation of the reaction catalyzed by a rate-controlling enzyme, TL = temperature (°K) at which the enzyme is half active and half low temperature inactive, HL = change in enthalpy associated with low temperature inactivation, TH = temperature (°K) at which the enzyme is half active and half high temperature inactive, and HH = change in enthalpy associated with high temperature inactivation of the enzyme (Wagner et al. 1984a). The models all include a cumulative Weibull function to approximate variability around the mean development times (Wagner et al. 1984b; Lyons and Lysyk 1989).

These competing models were evaluated with data on daily egg hatch from Mont-St.-Hilaire, Quebec (45.3°N, 73.2°W). Daily maximum and minimum air temperatures were extracted from hourly temperatures recorded by an Omnidata datapod (Logan, Utah) located about 250 m from the egg masses monitored in the forest. Egg masses were encircled with tanglefoot and hatching larvae were counted and removed daily. Performance of each model was assessed by graphical comparison with the actual hatch pattern in 4 years, 1989-1991.

Tree Budburst Model

In some years gypsy moth egg hatch may be synchronized poorly with host budburst. Leaves of red oak remain highly acceptable to newly hatched gypsy moth larvae for several weeks after budburst, as do those of another preferred host, trembling aspen (Hunter and Lechowicz 1992a). Extrapolating this pattern to other oak species implies that usually only very early hatch relative to budbreak, and not declining foliage quality, will reduce establishment opportunities, at least on favored hosts.

Degree-day models for predicting oak budburst dates were developed from an 18-year record of temperature and budburst dates at Wauseon, Ohio (41.3°N, 84.1°W Hunter and Lechowicz 1992b). Starting dates and threshold temperatures for degree-day accumulations for mature trees cannot be determined by the usual experimental methods (Hunter and Lechowicz 1992b), so predictions were made using all possible combinations of starting dates at 4-day intervals and threshold temperatures at 1°C increments between -10 and 10°C. The combination giving the lowest standard error of prediction was used (Hunter and Lechowicz 1992b). Models were developed for white oak (*Quercus alba* L.), black oak (*Q. velutina* Lamarck), bur oak (*Q. macrocarpa* Michaux), and swamp white oak (*Q. bicolor* Willdenow).

The models were evaluated for their ability to predict budburst dates at other sites in eastern North America where phenological observations were available in the literature. For this purpose, predictive models for 22 other hardwood species (Hunter and Lechowicz 1992b) also were used.

Starvation Rate as a Function of Temperature

Eggs collected from an outbreak near Barrie, Ontario (44.3°N, 79.5°W) in February were stored at 5°C until 22 April, then incubated at 25°C until hatch. Larvae that were one day old or less were allocated randomly to 10 temperature treatments (3.3, 7.0, 9.8, 12.7, 15.7, 19.7, 21.8, 24.9, 25.5, and 28.1°C). Incubator temperatures were measured at each sampling time. Each treatment received 20 parafilm-covered test tubes, with 10 larvae per tube. Survival was monitored twice daily in the three highest temperature treatments, daily in intermediate temperatures, and every day-and-a-half in the low temperature treatments, so that the number of observations would be similar across temperatures (Wagner et al. 1984a). To assess their status, larvae were gently prodded with a fine paintbrush if they were not already moving, and the number of mobile larvae at each sampling interval was recorded. These data were analyzed with the SAS program of Wagner et al. (1984b) to determine the Weibull distribution of the starvation rates, and a linear regression was fit to the rate data.

Window of Opportunity for Establishment

The egg hatch model was used to determine the number of larvae hatching each day (Fig. 1a). The rate of starvation of each daily cohort of larvae was calculated from hourly temperatures and distributed according to the Weibull distribution. The number of larvae that could seek hosts on a given day is thus the sum of all surviving larvae in the cohorts (Fig. 1b). The larvae will not seek hosts if the temperature is below 8°C or if it is raining (Leonard 1971). The number able to seek hosts is the sum of the numbers alive on days with warm, dry weather (Fig. 1c). This number was divided by the total number of larvae-days of survival, which varied from year to year with temperature (larvae-days are analogous to man-hours, and equal to the total area of all the bars). In these estimates, no larvae are removed from the host-seeking population due to establishment on hosts. The estimate is thus the maximum potential host-seeking.

Gypsy Moth Population Sizes and Weather

Two long-term records of gypsy moth population size were analyzed: a 14-year record (1976-1990) at Mont-St.-Hilaire, Quebec (45.3°N, 73.2°W) and an 11-year record (1971-1981) at Mohonk Lake, New York (41.5°N, 74.1°W). Both records are of overwintering egg mass densities; see Lechowicz (1983) and Skaller (1985) for site descriptions and census methods. No population control measures have been used at Mont-St.-Hilaire, and only spot spraying and manual removal from picnic areas have been employed at Mohonk Lake since 1957 (Skaller 1985). Weather data from St. Hubert Airport (45.3°N, 73.3°W) and the Mohonk Preserve weather station were used.

To evaluate the possible contribution of spring events to the population dynamics of gypsy moth, the logarithm of egg mass density and rate of increase were correlated against the arcsin square-root transformed proportion of larvae able to seek hosts.

RESULTS

Evaluation of Egg Hatch Models

With the exception of 1990, the biophysical model gave the best predictions of egg hatch phenology (Fig. 2). In 1990, the Johnson et al. (1983) and Lyons and Lysyk (1989) models

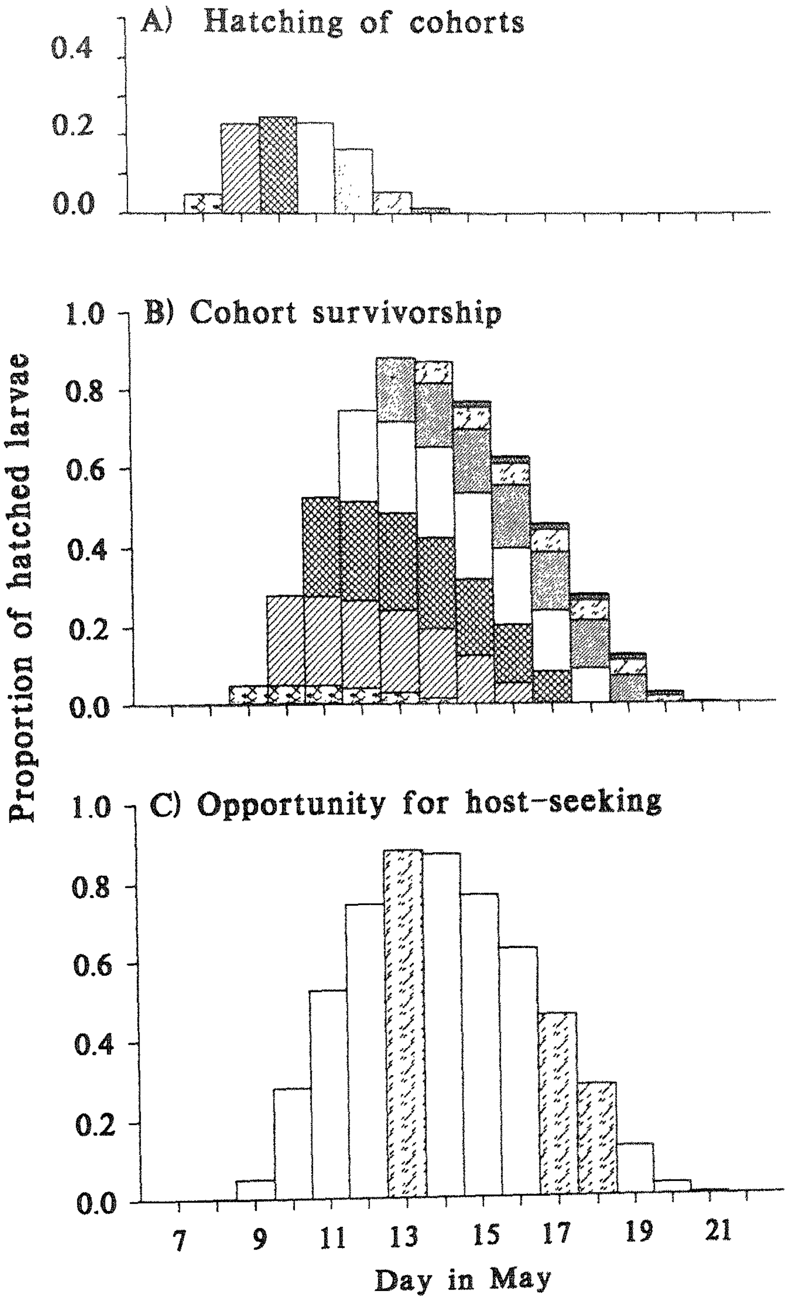

Figure 1.--Hypothetical example illustrating the estimation of the proportion of larvae able to seek hosts is estima A) The hatch pattern is given by the egg eclosion model. A cohort represents the number of larvae that hatc on a given day. B) The host-seeking-population on a given day shows the number alive on that date, given by temperature-dependent starvation of the cohorts. C) The proportion able to seek hosts gives the number alive warm, dry days (striped bars). Open bars indicate cool or wet days.

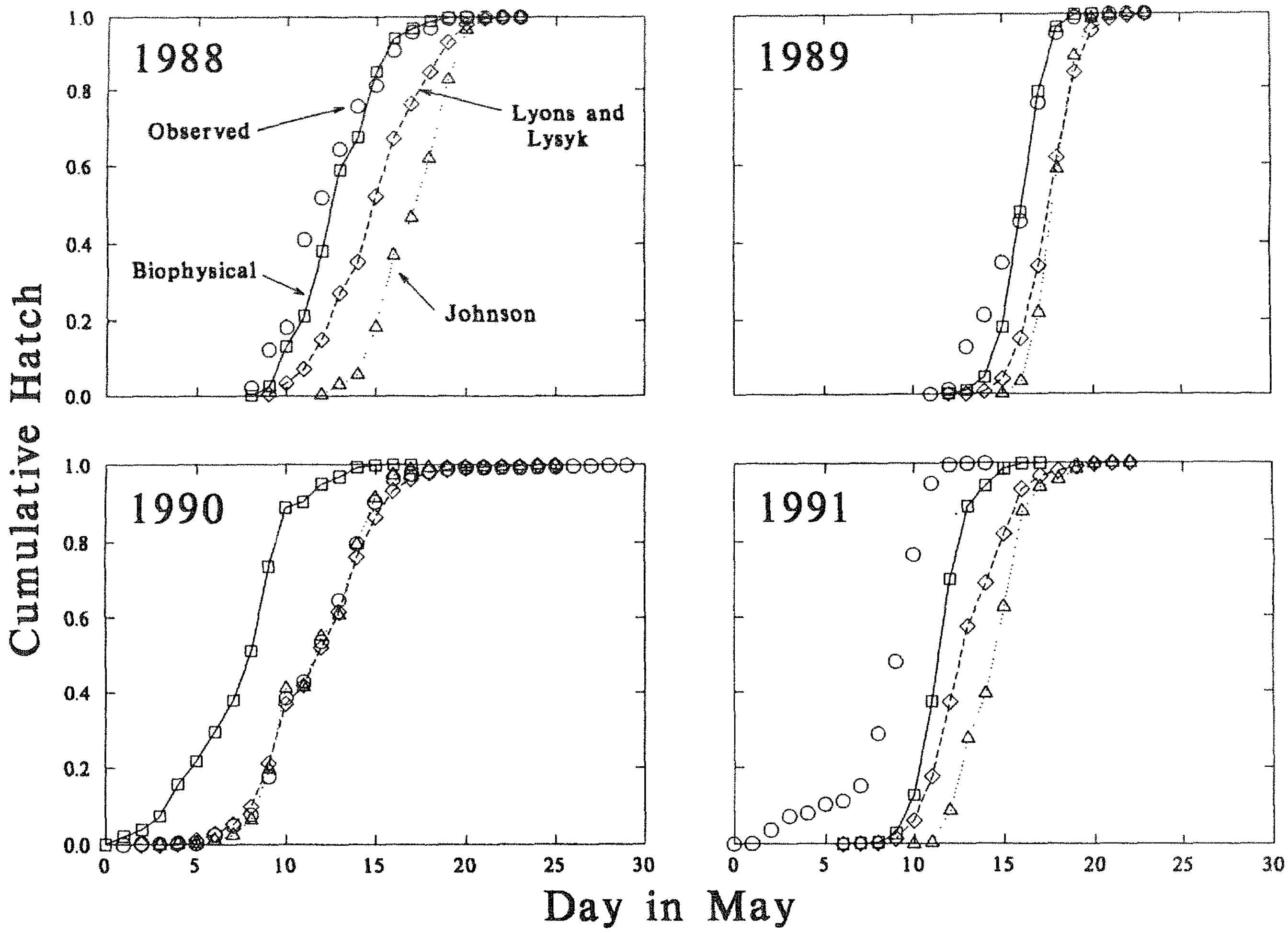

Figure 2.--Comparison of model predictions to observed (circles) egg hatch patterns at Mont-St.-Hilaire, Quebec, Canada. The Johnson model (triangles), Lyons and Lysyk quadratic model (diamonds), and biophysical model (squares) are compared.

were virtually identical in their predictions and very close to the actual hatch pattern. The last week of April 1990 was among the warmest in the 53-year record at St. Hubert. The spring of 1991 also was unusually warm. All the models predicted hatch later than it occurred but the biophysical model was closest to the actual phenology. The biophysical model was used to predict hatch phenology for subsequent analyses. The parameters of the biophysical model (Equation 1) are: RHO25=0.1184, HA=9646.0, HH=48501.2, HL=-60254.9, TH=306.6, TL=284.2.

Evaluation of Tree Budburst Models

Although the degree-day models were poor predictors of budburst dates at southern sites, they predicted dates to within 3-4 days on average at latitudes similar to those of Ohio, the site of the data from which the budburst models were constructed (Fig. 3). Trees of different provenance are known to have requirements for budburst that correspond to climate patterns at the site of origin (Perry 1971; Kriebel et al. 1976). The Ohio-based budburst models can be used as indicators of budburst dates under climatic conditions similar to those in Ohio. The mean of the budburst dates of the four oak species were used as an indicator of the time of leafing relative to the time of egg hatch. Larvae that were alive on days before budburst are not included in the proportion capable seeking hosts.

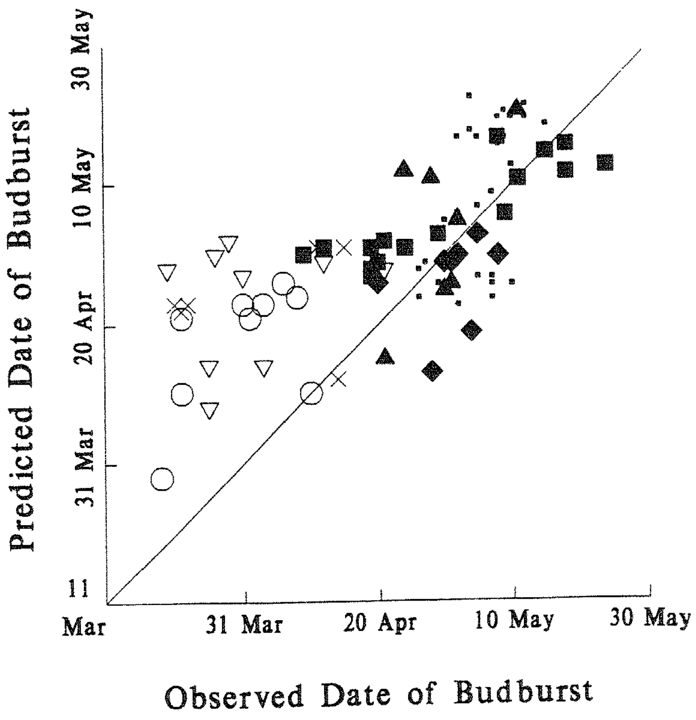

Figure 3.--Comparison of observed dates of budburst with predictions from degree-day models developed from data from Ohio: Athens, Georgia (circles), Oak Ridge, Tennessee (open triangles), Savage River, Maryland (diamonds), Columbia, Missouri (crosses), Cream Hill, Connecticut (filled triangles), Wisconsin Dells, Wisconsin (filled squares), Mont-St.-Hilaire, Quebec, Canada (points). Budburst date predictions are for 26 species of hardwoods based on degree-day models from Wauseon, Ohio. Line indicates 1:1.

Rate of Starvation as a Function of Temperature

Above approximately 10°C, larvae starved more quickly at higher temperatures (Fig. 4). At low temperatures the pattern was complex, first rising then falling as temperature decreased. It was difficult to maintain incubators at the lowest temperatures, so the lowest rates are uncertain since they may include time spent at other temperatures. In the starvation model, a constant rate of starvation below 10°C (Fig. 4) was discussed.

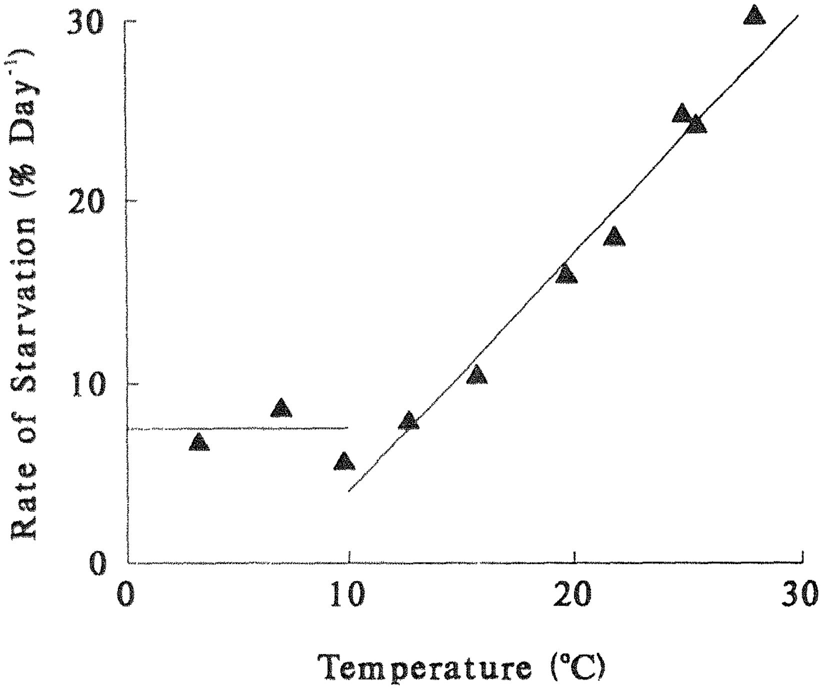

Figure 4.--Median starvation rate of newly hatched gypsy moth larvae as a function of temperature. Line shows relationship used to model starvation.

Relation Between Population Dynamics and Host-seeking Opportunity

The proportion of larvae capable of seeking hosts in the spring is positively correlated with the egg mass population size in the fall at Mont-St.-Hilaire (R=0.56, P=0.04, N=14), and at Mohonk Lake (R=0.77, P=0.02, N=9) (Fig. 5). Potential host-seeking is not correlated with the rate of change between years (Fig. 5). The population cycles of the gypsy moth are strong, so that the rate of change is not greatly affected by the potential for host-seeking (Fig. 6).

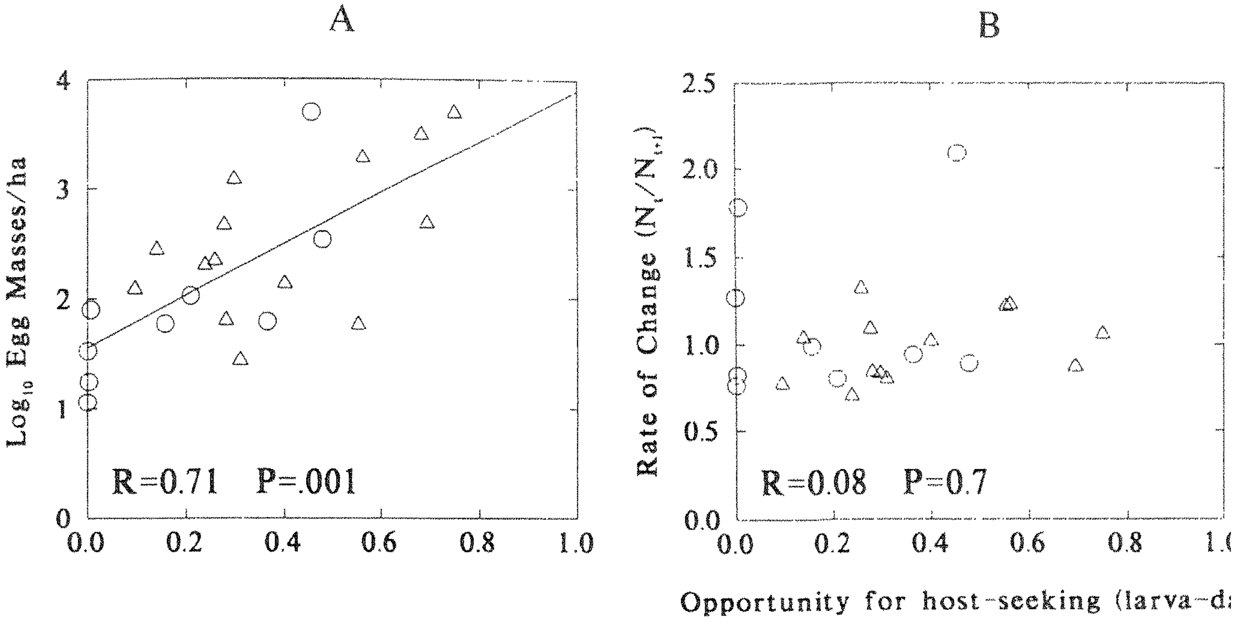

Figure 5A--Logarithm of egg mass density in fall and B) rate of change of population sizes versus estimated proportion able to disperse in spring at Mont-St.-Hilaire, Quebec, Canada (triangles) and Mohonk Lake, New York (circles).

DISCUSSION

Gypsy moth egg mass densities at the end of the season at Mont-St.-Hilaire and Mohonk Lake are related to the estimated maximum potential host-seeking in the spring. Warm, dry weather at the time of egg hatch is required for host searching behaviour. This finding supports the conclusion of Miller et al. (1989) that the area defoliated in Massachusetts and Connecticut is related to 10-day and 30-day average temperatures during April and May, the time of hatch and host-seeking of gypsy moth larvae. The lack of relation to the rate of increase means that although good weather during the host-seeking period is associated with high population densities in the fall, it does not necessarily cause an increase in population size from year to year. Host-seeking opportunities in spring probably have a limited modifying effect on population cycles of the gypsy moth. However, we have insufficient data to test whether the cycles have been modified by the host-seeking opportunities.

Studies in both North America and Eurasia have found correlations between gypsy moth outbreaks and spring weather (Montgomery and Wallner 1988), indicating that warm, dry conditions are favorable for population increase. All such studies, the current one included,

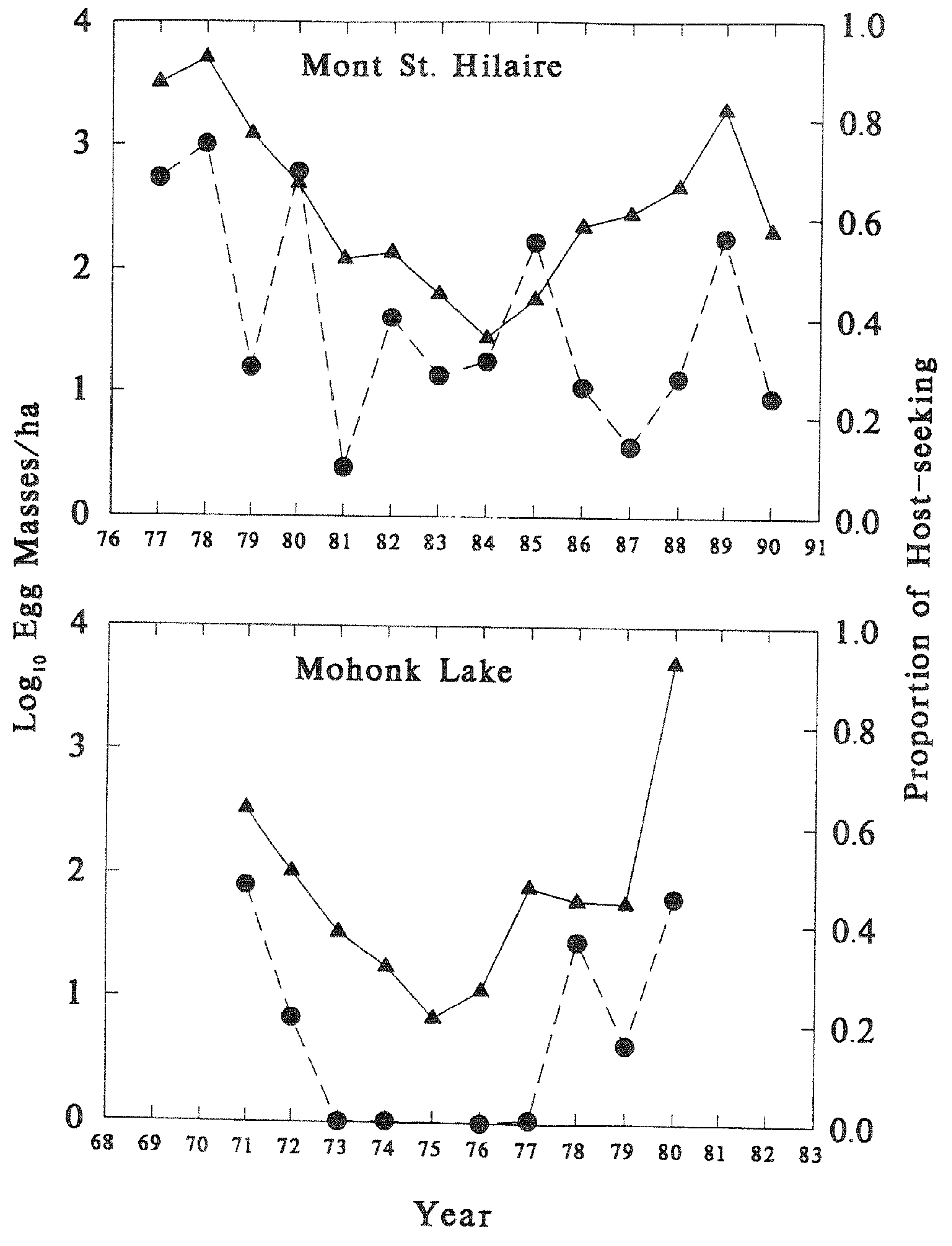

Figure 6.--Egg mass densities (triangles) and estimated proportion of potential host-seeking (circles) at Mont-St.-Hilaire and Mohonk Lake.

share the fault of being correlative; and the correlations may arise through any number of effects of weather. These include, apart from the effects on host-seeking, modification of host quality (Mattson and Haack 1987), efficacy of pathogens (Campbell 1963; Wallis 1957), and influences on predators. However, the estimate of potential host-seeking is very narrowly defined and may not be correlated to these features. The estimate depends strongly on when it rains relative to when peaks in egg hatch occur, so in 2 years with identical temperature progressions large differences in potential host-seeking could result if it rains on different days. The connection between the potential host-seeking and the general weather patterns may be small, so noncausal correlations are unlikely.

The reduction in host-seeking opportunities is due primarily to inclement weather after hatch, rather than poor synchrony with hosts. Although the models showed that in some years hatch began long before budburst, hatch and larval survival also were prolonged in these years, because of cool temperatures after hatch. Inclement weather alone reduced the proportion of larvae capable of seeking hosts by an average of 50 percent at both sites; pre-budburst emergence eliminates only a further 15 percent of the larvae. The use of alternative hosts that break bud earlier than oaks may reduce the effect of asynchrony, but spring weather still will be a major source of variation for gypsy moth establishment.

The gypsy moth egg hatch models worked tolerably well at this northern location. Winters are long enough to ensure diapause termination by mid-winter, and in the case of the model of Johnson et al. (1983), conditions apparently are similar enough at the two sites (New Hampshire and southern Quebec) that the model works reasonably well. However, the models are poor predictors of hatch dates at southern locales (personal observation), and at sites in the Midwest on a similar latitude. Better predictions probably will be possible only with a better understanding of the factors controlling diapause termination. This study shows that phenological models may be useful for analyzing the population dynamics of the gypsy moth, as well as the pragmatic application to biological control efforts.

The population sizes of other spring-feeding Lepidoptera also may be influenced by establishment opportunities and synchrony with their hosts. Larvae of the winter moth (*Operophtera brumata* L.) will starve within about a week, so are relatively susceptible to asynchrony with host budburst (Hunter 1990). The fall cankerworm (*Alsophila pometaria* Harris) suffers high mortality if hatch is too long before budburst and low fecundity if hatch is late (Schneider 1980). A difference of 1 week in the timing of establishment reduces fecundity and development rates of elm spanworm (*Ennomos subsignarius*) (Drooz 1970). Nothnagle and Schultz (1987) argued that outbreaking folivores fall into three groups, one of which is spring-feeders. These spring-feeders have traits that reduce the influence of other factors on population size so that the major source of variation is phenological interactions with the host.

The results reported here support the hypothesis that uncertain weather in spring and the variable opportunity for host-finding and establishment on young foliage can contribute to population size variability in the gypsy moth, although they are not the most important factors in gypsy moth population dynamics.

ACKNOWLEDGMENTS

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DISCUSSION SUMMARY

Physiology Subgroup

M.E. MONTGOMERY, Recorder; D.R. GRAY, R.A. BELL, J. FEHRINGER

> "The problems in defining diapause are similar to those encountered in defining "dormancy" and "quiescence" in seeds, and "hibernation" and "torpor" for mammals and birds; there are several, sometimes conflicting definitions, the diversity of which stems from the numerous approaches used in research on the phenomenon."
>
> (Tauber et al. 1986)

During our working group discussion, it became evident that we were experiencing a similar problem: How to define diapause. While a wide variety of hormonal, behavioral, and metabolic conditions have been associated with the diapause state, there does not exist a variable that is generally accepted as an indicator of the state of diapause. Although it is common to describe an insect as either "in" or "out" of diapause, diapause is a dynamic state with many physiological and biochemical variables undergoing gradual change. Tauber and Tauber (1976) refer to processes of diapause maintenance and diapause termination, which suggests that diapause may be a two-fold process. The diapause state may be maintained by the presence, in sufficient quantity, of biochemical factor(s). The diapause state may be terminated by the partial removal of the "maintaining" factors, and perhaps by the increase in level of some "terminating" biochemical factor(s). The metabolism and catabolism of each set of factors is influenced by the environment, principally photoperiod and temperature (Zaslavski 1988).

At this point, the group began to recognize where our viewpoints differed, Some of us held working definitions of diapause that focused on mediating factors, whereas others focused on manifestation of the diapause process. Mediating factors such as diapause hormone, juvenile hormone, or prothoracicotropic hormone (PTTH) could be examined in a study of diapause maintenance and termination. Conversely, diapause maintenance and termination could be studied by the manifestations of the processes through metabolic rate, respiration rate, thermal responsiveness, reproductive status, or locomotor activity. We recognized that while some viewed diapause as a continuous, dynamic process and others as an abrupt change in state, we all were seeking a way to link the various processes and events.

This led to a discussion of how diapause had been determined by others. Characterizations of diapause include: arrested morphological development, reduced rates of respiration (Harvey 1962, Zaslavski 1988), reduced protein synthesis (Hayes et al. 1972, Venkatesh and Chippendale 1986), increased titre of polyhydric alcohols (Nordin et al. 1984), increased resistance to environmental extremes (Tauber et al. 1986), and changes in the titre of a neurohormone (Chippendale 1988). With regard to gypsy moth diapause, characterization has been based on egg hatch (Masaki 1956, Tauber et al. 1980, Johnson et al. 1983), respiration (Bell 1989, Gray et al. 1991), and the presence of a neurotransmitter (Loeb and Hayes 1980). Unlike most insects, duration and level of cold temperature seem to affect the gypsy moth diapause process to a much greater extent than photoperiod (Leonard 1968, Tauber et al. 1990).

The group was not able to develop a consensus of a definition of diapause, but did agree on attributes that the definition should possess. The group felt that the definition should be versatile. The definition should involve a variable that is easily and accurately measured. The variable should provide a quantitative measure of the diapause state and diapause depth. It should allow for a sufficient sample size to be obtained to define the measure's variability in the study population. Preferably, measurement of the diapause state should be non-destructive so that measurement of a factor of interest (e.g., PTTH level) also can be performed on the same individual or sample. Temperature should be included in the definition since it is a critical factor. Reduced respiration was recognized as a common factor in diapause that can be measured accurately and easily.

With the above stated requirements, we selected the following as our working definition of diapause in gypsy moth:

"Diapause in the gypsy moth is the condition marked by reduced respiration rate at all temperatures."

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A SUMMARY OF RESEARCH ON ENDOCRINOLOGY AND CHEMICAL DISRUPTION OF EMBRYONIC DIAPAUSE IN THE GYPSY MOTH, *LYMANTRIA DISPAR* L.

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The gypsy moth, *Lymantria dispar*, like many other lepidopterous species that feed on forest and shade trees, is univoltine and undergoes a prolonged obligatory diapause that lasts for 8 to 9 months of the annual life cycle. Diapause in the gypsy moth occurs in a late stage of embryonic development as a fully differentiated pharate larva. The endocrine regulation of diapause has been studied intensively in several insect species that undergo diapause in post-embryonic larval, pupal, and adult stages. However, except for the silkworm, *Bombyx mori*, which enters diapause in early embryonic development in response to a special maternally derived diapause hormone, little is known about the endocrinology of diapause in the egg stage of insects. Recently, however, a series of studies has been carried out at the Insect Neurobiology and Hormone Laboratory (INHL) to develop a better understanding of the endocrine regulation of embryonic development and diapause in the gypsy moth. The results of these studies have demonstrated the importance of respirometry as a tool for determining the timing of hormone induced morphogenetic events in embryonic development and the onset and termination of diapause in this species. Further studies showed a strong correlation between the rate of oxygen uptake and the titer of developmental-promoting ecdysteroid hormones, particularly 20-hydroxyecdysone. The onset of diapause was characterized by a low metabolic rate and a deficiency of 20-hydroxyecdysone.

Of special importance was the finding that diapause in the gypsy moth could be prevented or precociously terminated by application of an imidazole terpenoid compound known as KK-42. This compound was reported previously to show anti-juvenile hormone effects in silkworm larvae and to induce termination of egg diapause in the wild silkmoth, *Antheraea yamamai* in Japan. Unlike *A. yamamai*, in which diapause could be terminated by topical application of KK-42 to individual eggs, the gypsy moth showed no response when treated topically, regardless of time of treatment or dosage administered. However, diapause could be prevented by immersion of dehaired eggs in acetone solutions of KK-42 at 4000-8000 ppm. There appears to be a rather brief period (12-24 hr) during embryonic development and just prior to entry into diapause when KK-42 is maximally effective in inhibiting the occurrence of diapause. It also has been established that KK-42 is effective in accelerating the rate of diapause termination and promoting hatch synchrony in eggs that have been chilled for 2-3 months. KK-42 also has been effective in preventing diapause in both wild and laboratory reared strains of the gypsy moth, including a black mutant strain. However, an albino mutant strain showed very little response to the compound even when dosages were increased to 20,000 ppm. Further studies using the albino mutant may be helpful in revealing the mode of action of KK-42 in preventing diapause in gypsy moth eggs. Studies have shown that larvae hatched from KK-42 treated eggs can undergo normal growth, development and reproduction. The mode of action of KK-42 in preventing or terminating diapause is not yet known but previous reports of its anti-juvenile hormone effects strongly suggest that it may be acting, in part, by blocking some unidentified event that requires the action of juvenile hormone (JH). Thus, JH may play a critical role in the regulation of late embryonic diapause in a manner similar to that already reported in post-embryonic larval diapause of certain species such as the southwestern corn borer, *Diatraea grandiosella*. It is concluded that KK-42 provides a practical means by which diapause can be manipulated for improvement of laboratory rearing of the gypsy moth. A particular advantage may be gained from using KK-42 for decreasing timeframes for research, especially for those projects in which experiments involve evaluations over successive generations. Other applications may involve accelerated establishment of wild strains in rearing facilities, improvement of strains via genetic selection for desired traits, and manipulation of diapause for stockpiling of insects for sterile release programs or parasite rearing. Finally, KK-42 should prove useful as a probe for further inquiry into the mechanism of diapause in the gypsy moth and other insect species.

THE USE OF RESPIRATION RATE IN THE EXAMINATION OF GYPSY MOTH EGG DEVELOPMENT AND DIAPAUSE

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ABSTRACT

The pattern of respiration rate was used as an indicator of transition from prediapause to diapause in individual gypsy moth eggs. Prediapause developmental times were estimated and a temperature-dependent developmental rate function was derived. The initiation of diapause caused respiration rates to decline, but thermal responsiveness remained equal to prediapause levels. Approximately 75% of stored triglycerides were depleted by the time diapause was initiated. Temperature had no effect on the amount of triglycerides depleted during prediapause development. Diapause depth (as determined by respiration rate) decreased gradually under constant temperature conditions.

INTRODUCTION

There is a well developed interest in determining the effects of various environmental factors, especially temperature, on the timing of gypsy moth egg hatch. However, the phases of gypsy moth egg development differ sufficiently that a given temperature condition can have opposing effects on hatch if applied to different phases. Warm temperatures during the diapause phase will reduce hatch success, and delay and prolong the time of hatch. The same warm temperature during the postdiapause phase will have the opposite effect of increasing hatch success, and advancing and shortening the time of hatch. Similarly, cold temperatures during the diapause phase will increase hatch success, and advance and shorten the time of hatch. During the postdiapause phase the same cold temperature will reduce hatch success, and retard and prolong the time of hatch. Thus it is essential to know the current developmental phase in order to model the effect.

Gypsy moth egg development is generally described as being comprised of three phases. The first (prediapause) is characterized by rapid morphological development, and high metabolic and respiration rates. Development is favored by moderately warm temperatures. During the second phase (diapause) morphological development is arrested and metabolic and respiration rates are greatly reduced. Development is favored by cold temperatures. The characteristics of the final phase (postdiapause) resemble those of the prediapause phase.

Most published simulation models of gypsy moth egg hatch do not consider the prediapause or diapause phases of development. These models assume that diapause has been completed by an arbitrarily chosen date, and that warm temperatures will result in postdiapause development leading to egg hatch. This postdiapause phase is modeled with either a linear (Johnson et al. 1983) or nonlinear (Lyons and Lysyk 1988) temperature-dependent developmental rate function. The choice of model initiation date (postdiapause initiation) affects the shape and form of the developmental rate curve since eggs may already have spent a greater or lesser amount of time in the postdiapause phase; or they may have a greater or lesser amount of the diapause phase yet to complete. These models have performed poorly in areas climatically different from where they were developed.

More recently Tauber et al. (1990) proposed a model of gypsy moth egg development encompassing the diapause and postdiapause phases. They hypothesized that the relationship between developmental rate and temperature changes gradually between diapause and postdiapause. Their hypothesis was supported by the data of Masaki (1956) and original data. Sawyer et al. (1993) developed a simulation model based on the hypothesis. Their simulation model made no clear distinction between diapause and postdiapause. A single triangular rate function was used to describe the relationship between developmental rate and temperature. Function parameters varied over the course of development. In this way the initial developmental response to temperature has a low temperature threshold and optimum and a low maximum rate. Over the course of development there is an increase in the temperature threshold and optimum, and the maximum developmental rate. Function parameters were estimated by iterative comparison with previously published egg hatch data from laboratory experiments. The date of onset of diapause was similarly estimated and was assumed to be uniform for all data sets. To date, this model has not been validated with independent data.

Gray et al. (1991) interpreted the egg hatch data of Masaki (1956) and McManus (unpublished) as evidence for a discrete phase transition between diapause and postdiapause occurring after ca. 110 days at 5°C. They proposed a three-phase model of prediapause (Phase I), diapause (Phase II), and postdiapause (Phase III) with clear demarcations between the phases, and with each phase being governed by a developmental rate response to temperature unique to the phase. They described a method of distinguishing phases based on respiration rate and described the temperature-dependent rate of development in prediapause.

A significant problem in the development of gypsy moth egg development/egg hatch models has been the absence of a reliable, nondestructive, and simple method of distinguishing and characterizing the developmental phases. An inability to distinguish the diapause and postdiapause phases required that the models of Johnson et al. (1983) and Lyons and Lysyk (1988) assume diapause completion by an arbitrarily chosen date. Tauber et al (1990) relied on observations of egg hatch to examine the effects of temperature and photoperiod on diapause termination, an event temporally removed from their observation. Egg-hatch data used to support a model of gradual diapause-to-postdiapause phase transition also can be interpreted to support a model with three distinct phases. The ability to distinguish the phases would facilitate estimation of the effects of environmental factors on the rates of development during whatever phases exist. Direct examination and quantification of the diapause condition could be used to address questions regarding diapause-to-postdiapause phase transition.

Many researchers have shown that low respiration rate is an indication of the diapause state (Bell 1989, Harvey 1962, Pantyukov 1964). However, techniques have required several hundred eggs per sample in order to measure respiration. The use of several hundred eggs per sample effectively eliminates the use of respiration rate as a useful variable to quantify the diapause state and especially the transition from a diapause to nondiapause phase. An instantaneous phase transition in individuals would be hidden if there were variability in the time of the transition among individuals of the population. Measurements of respiration rate of the population repeated over time would result in a gradual return to a high respiration rate of the aggregate. Only by measuring respiration rate of individuals over time will a clear picture of phase transition emerge.

Gray et al. (1991) developed a quick and simple method of estimating respiration rates in individual gypsy moth eggs that can be used to examine directly phase transition and perhaps to quantify the depth of diapause. Phase transition is indicated by changes in the respiration rate of an individual under constant temperature conditions. Developmental rates within phases can be estimated once the occurrence of a phase transition can be determined. If diapause is characterized by an absence of (or low) thermal responsiveness, some (or higher) thermal responsiveness will be exhibited by eggs less deeply in the diapause state. Therefore, diapause depth may possibly be quantified by estimating the change in respiration rate caused by an increase in temperature (thermal responsiveness).

Respiration rate is a variable that is intimately associated with the ontogenetic processes. As such, it is an extremely useful variable in the examination of gypsy moth egg development and in the development of a multi-phase model.

Respiration Rates and Prediapause-Diapause Phase Transition

Respiration rates of individual gypsy moth eggs are initially low in constant temperature conditions of 10 to 30°C (Fig. 1). Respiration rates increase as the embryos develop and reach a maximum of approximately 4.5 *ul* CO_2 per 24 h at 30°C. The rate of increase is positively temperature-dependent. Rapid increases are seen eight days after oviposition in eggs reared at 30°C, while at 20°C a smaller rate of change is evident only after 26 days. The time after oviposition at which embryos attain maximum respiration rate at each temperature is negatively temperature-dependent. Maximum rate occurs approximately 10 days after oviposition at 30°C, but only after approximately 44 days at 10°C.

Transition from the prediapause to diapause phase is evidenced by a rapid decline from maximum respiration rate for each individual at each temperature. As with the increase to maximum respiration rates, the rate of decrease from the maximum rate also is negatively temperature-dependent. Thus, respiration rates indicate that the prediapause-diapause phase transition occurs without any requisite temperature change and that the transition occurs more quickly at warm than at cold temperatures. Transition probably is completed in approximately eight days at 30°C, but may take as long as 20 days at 10°C. This slower transition at cooler temperatures should be expected; physiological age generally advances more slowly at lower temperatures.

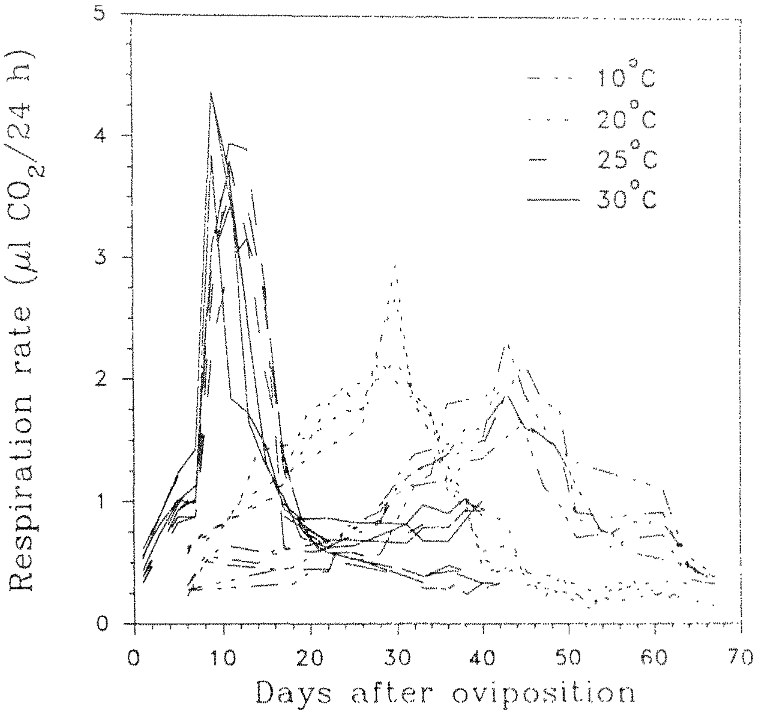

Fig. 1. Sample of respiration rates of individual gypsy moth eggs reared under four different constant temperature regimes.

Under the temperature conditions that are most common immediately following oviposition, transition will be completed quite quickly, and the prediapause-diapause transition can be modeled as an instantaneous event. If we somewhat arbitrarily choose the time when an individual's respiration rate has declined to one-half of its maximum as the completion of prediapause (initiation of diapause), we can measure the time required to complete the prediapause phase under various temperature conditions. We can then construct a developmental rate curve for the phase using

$$R_D(T) = \psi(e^{\rho T} - e^{\rho T_M - \tau}) \qquad [1]$$

where $R_D(T)$ is developmental rate at T°C above the base (lowest examined) temperature, T_M is the lethal, maximum temperature in degrees above the base temperature, ψ is the developmental rate at base temperature, ρ is the rate of increase to optimum temperature, $\tau = {(T_M - T)}/{\Delta_T}$ and Δ_T is the width of the high level boundary layer (i.e. the temperature span over which the developmental rate function changes its behavior) (Fig. 2) (Logan et al. 1976). We also can estimate the variability in prediapause developmental rates in the population by measuring the differences in time to complete prediapause among individuals in identical constant temperature conditions. Normalized developmental times can be calculated by dividing each developmental time by the median developmental time in that temperature regime. Temperature-dependent variability can be described then by a cumulative probability function (Fig. 3).

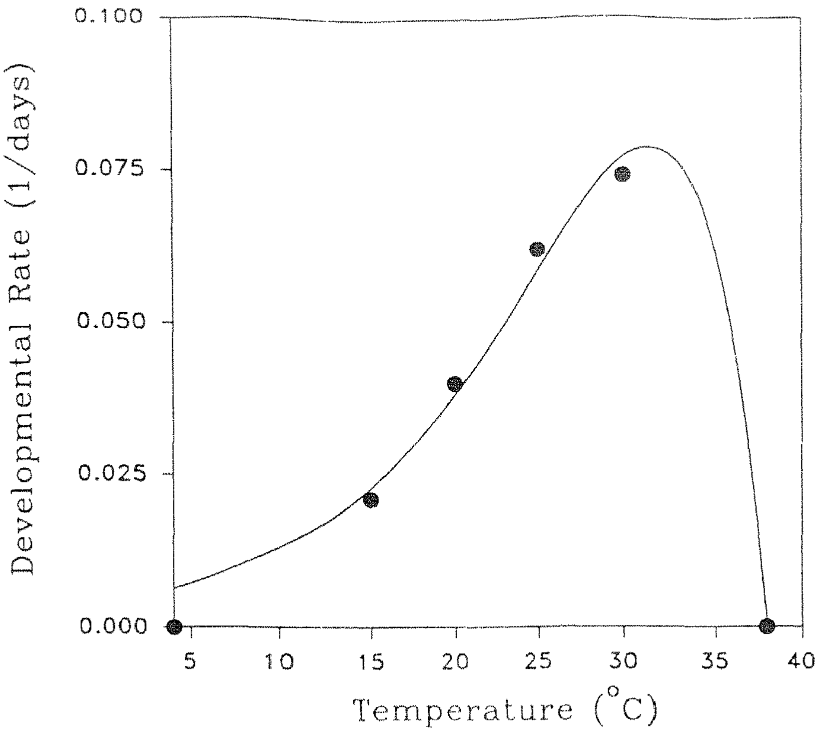

Fig. 2. Relationship between median prediapause developmental rate (days^{-1}) and temperature (°C).

Respiration Rates, Thermal Responsiveness, and Diapause Depth

Gray et al. (1991) proposed measuring thermal responsiveness as an indication of ontogenetic phase. They equated thermal responsiveness with sensitivity to temperature and defined it as the change in respiration rate per degree change in temperature (μl CO_2/24 h/°C). For the following reasons I have redefined thermal responsiveness to equal the change in relative respiration rate per degree change in temperature. A change in physiological state (e.g. from a nondiapausing to a diapausing state) may affect respiration rates in two ways. Firstly, respiration rates may be universally lowered by a constant proportion. Secondly, respiration rates in the diapause state may be less affected by changes in temperature For example, if a change in physiological condition reduces respiration rates at all temperatures by 50%, the relative response to a temperature change is identical between the two physiological conditions. The two physiological conditions would exhibit equal sensitivity to temperature. However, the universally lower respiration rates will result in smaller changes in respiration rate per degree change in temperature (the previous definition of thermal responsiveness). Differences in sensitivity to temperature will be observed only when different temperature changes are required

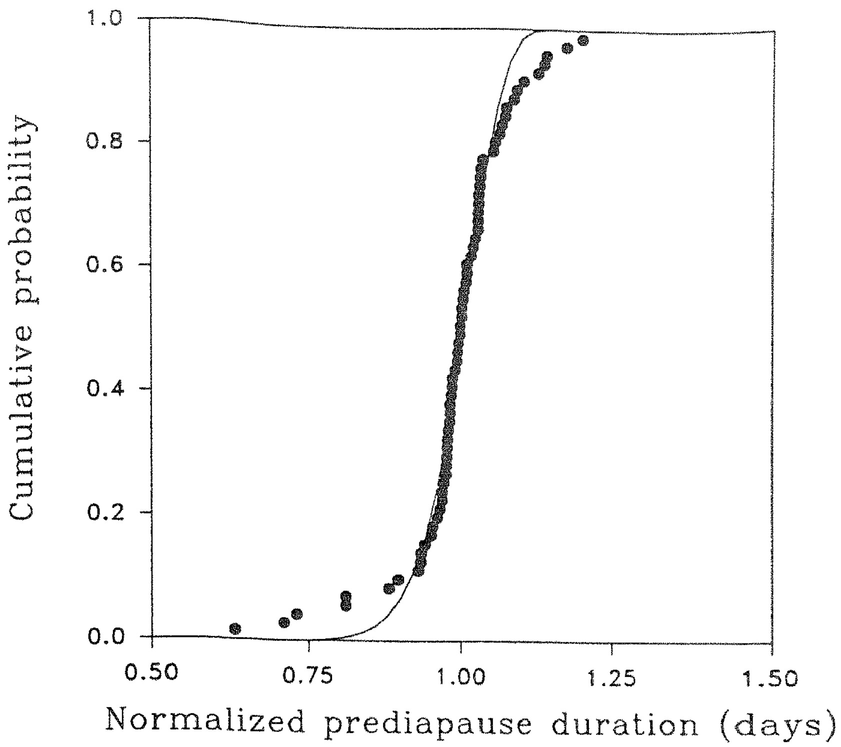

Fig. 3. Cumulative probability function of temperature-independent time (days) to complete prediapause.

to produce a given change in relative respiration rate. Therefore, thermal responsiveness is more accurately examined by first removing any uniform changes in respiration rate that may occur due to a change in physiological condition.

Using this definition of thermal responsiveness, phases of gypsy moth egg development are not characterized by thermal responsiveness. Eggs in diapause exhibit a thermal responsiveness equal to that shown by eggs in prediapause (Fig. 4). Eggs were reared at 25°C immediately following oviposition. Approximately every third day for 40 days samples of eggs were removed and placed in 4, 15, 20, 25, 30, and 38°C temperature treatments with 12:12 photoperiod, and allowed to acclimatize for at least 12 hours before respiration rates were measured. Relative respiration rates for each measurement date were calculated by dividing each respiration rate by the mean respiration rate of eggs of the same mass at 25°C on the same day. Thermal responsiveness was then estimated by the Theil-Sen nonparametric method of linear regression (Hollander and Wolfe 1973) of relative rate on temperature for each measurement date. Therefore, thermal responsiveness has units of $^{\circ}C^{-1}$.

There was a significant thermal responsiveness for all measurement dates as indicated by significant ($p < 0.0001$) positive slopes of all regression functions. There was no distinct pattern to the estimated values of thermal responsiveness during the 40 days following oviposition (Fig. 4). Estimates ranged from 0.0304 $^{\circ}C^{-1}$ on day 22 to 0.0654 on day 38.

Overlapping 95% confidence intervals of estimates indicates there was no significant difference among estimates. Therefore, changes in the relationship between respiration rate and temperature reported by Gray et al. (1991) are due to proportionally equal changes in respiration rate over all temperatures and not to changes in sensitivity to temperature. The onset of diapause is indicated by the sudden decline in respiration rate of individual eggs (Fig. 1) and causes a reduction of respiration rate at all temperatures. Diapause does not, however, render individuals less sensitive to temperature.

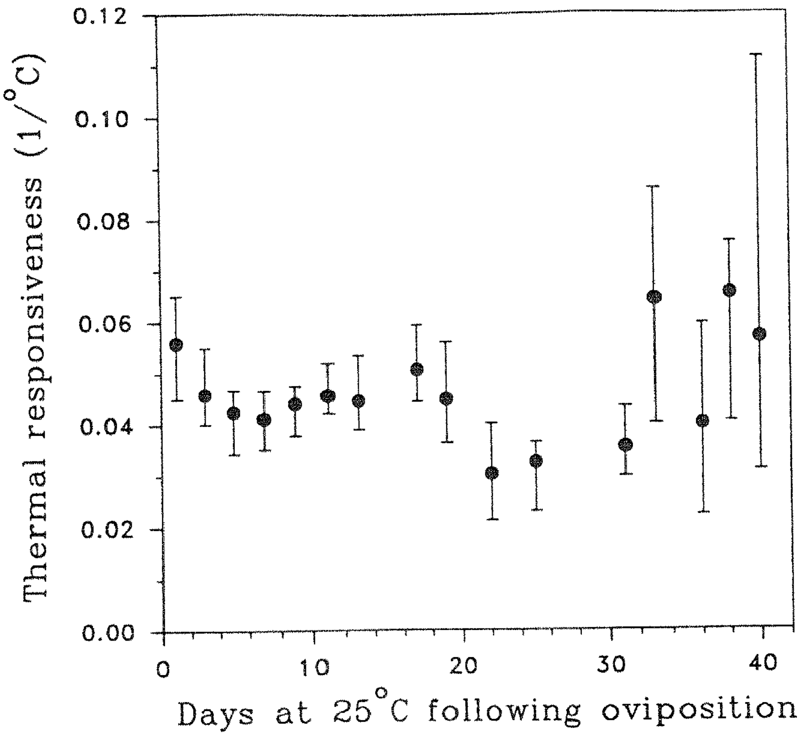

Fig. 4. Estimate (●) and 95% CI of the thermal responsiveness for 40 d following oviposition. Values estimated from the linear relationship between respiration rate (expressed as proportion of the rate at 25°C on the same day) and temperature for each measurement day.

Thermal responsiveness during diapause was investigated in a similar manner, except that linear regression was conducted by the least squares method (Zar 1984). Completion of prediapause was ensured by rearing eggs for 42 days at 25°C and 16:8 (L:D) photoperiod. Eggs were then moved to 5°C and 16:8 (L:D) to promote diapause development. Once per week samples of eggs were removed and placed in 5, 10, 15, 20, and 25°C temperature treatments with 16:8 (L:D) photoperiod, and allowed to acclimatize for approximately 24 hours before respiration rates were measured. There was no trend in the estimated thermal responsiveness for the 88 days following the onset of diapause (Fig. 5). The mean (S.E.) estimate of thermal

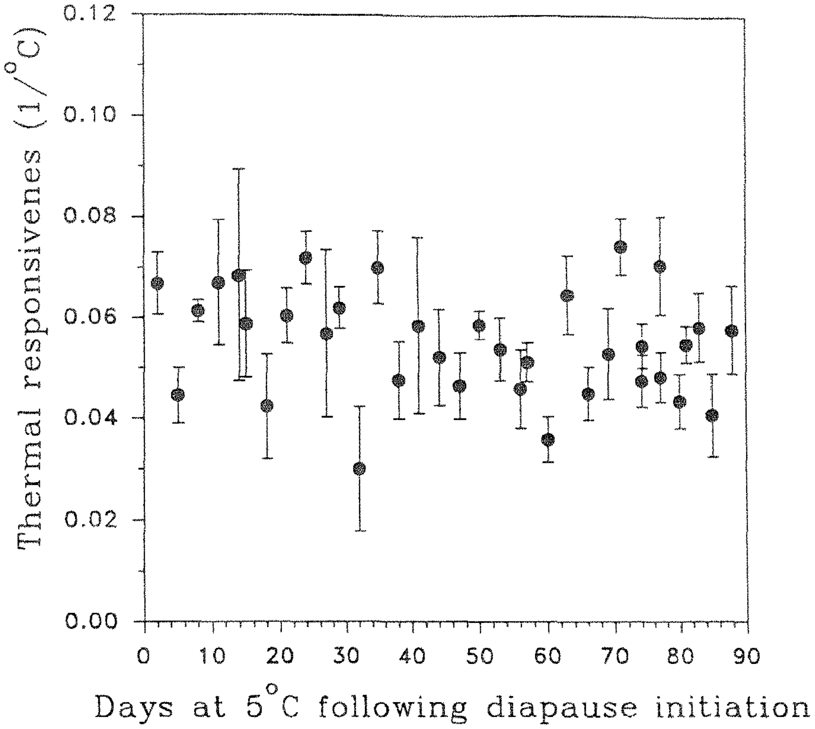

Fig. 5. Estimate (●) and standard error of the thermal responsiveness for 88 d at 5°C following onset of diapause. Values estimated from the linear relationship between respiration rate (expressed as proportion of the rate at 25°C on the same day) and temperature for each measurement day.

responsiveness was 0.055 (0.0018) $°C^{-1}$. Thus, respiration rates at all temperatures were affected in a manner similar to the effect at 25°C (Fig. 6) by advancing physiological age in diapause Eggs were equally responsive to temperature throughout the experiment.

Previous researchers have relied on observation of egg hatch to assess diapause depth. Eggs that take longer to hatch when placed in warm temperatures are said to be deeper in diapause and less responsive than eggs that hatch sooner. However, time to hatch may not be indicative of diapause depth in a three phase process. Two groups of eggs may be equally deep in diapause and therefore display equal respiration rates, but have unequal proportions of the diapause phase to complete. Eggs in both groups may respond identically to the change in temperature, but one group will complete diapause sooner. Differences in phase will, then, certainly cause differences in observed egg hatch.

However, respiration rate may be a reliable indicator of diapause depth. Respiration rate at 25°C was lowest following the initiation of diapause. Thereafter respiration rate exhibited at slight, uniform, and statistically significant daily increase for 88 d (Fig. 6). This suggests that diapause depth may be greatest at its initiation, and at constant and suitable temperatures there may be a gradual decrease in diapause depth. It remains to be seen if this gradual increase persists until a high respiration rate indicative of postdiapause is achieved, or if a distinct diapause-to-postdiapause transition occurs.

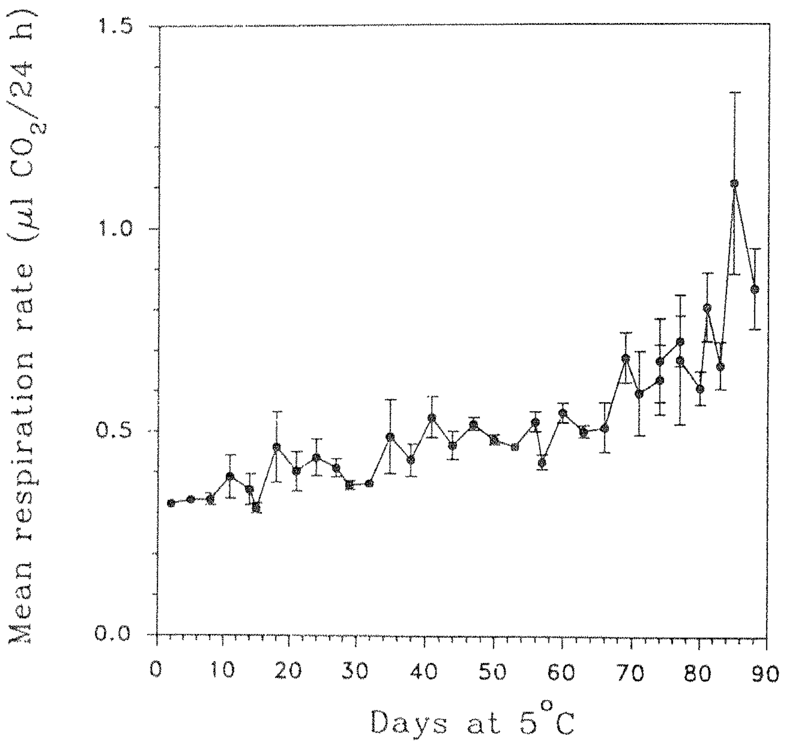

Fig. 6. The relationship between mean respiration rate at 25°C (± S.E.) and physiological age (days at 5°C) for 88 d following onset of diapause.

Depletion of Triglycerides and Survival

Survival of non-feeding life stages of insects is dependent, in part, upon energy reserves within the individual. In gypsy moth eggs this energy may be in the form of triglycerides, as in the closely related *Bombyx mori* (Nakasone 1979).

The mean (± S.E.) triglyceride content (as determined by high performance thin layer chromatography) of 30 eggs sacrificed at oviposition was 117.4 (10.0) μg, 14.2% of live body weight. There was no significant correlation between live body weight and triglyceride content at oviposition, nor a significant difference among egg masses in triglyceride content at oviposition. Mean (± S.E.) triglyceride content per egg at the completion of prediapause was 28.63 (4.73), and temperature had no significant effect on the estimated amount of triglyceride utilized when eggs completed prediapause at 10, 15, 20, 25, 30, or 35°C.

The very large proportion (73% to 84%) of triglyceride reserves utilized during prediapause may have significant consequences for egg hatch and larval survival. Chino (1958) and Nakasone (1979) found evidence that lipids, probably triglycerides, are the major metabolic energy source during diapause of *B. mori*. Prediapause results in a uniform depletion of triglycerides, probably because the phase is completed when a given level of embryogenesis has been completed. However, the volume of triglycerides utilized during diapause may be affected by temperature. While it is known that metabolic activity is minimal during diapause, metabolic reserves are still necessary to survival (Harvey 1962). And Gray et al. (1991) provided evidence that gypsy moth eggs in diapause remain responsive to temperature. While they showed that increasing temperatures cause increasing respiration rates, it has been shown by others (Masaki 1956, see Giese and Cittadino 1977 for a general review) that diapause development is hindered by temperatures above 15°C. Thus, high temperatures may prolong the diapause phase while simultaneously increasing the demand on metabolic reserves. Eggs that enter diapause with low metabolic reserves will be less likely to survive. It has been observed that the advancing infestation in Virginia receded in 1990 following a warm winter (see Roberts et al. 1993 for representation of pheromone trap catches) and Williams et al. (1990) reported a negative relationship between maximum February temperatures and egg viability over a 9 year period. Excessive depletion of triglyceride reserves may lead to post-hatch death. Many gypsy moth neonates disperse before feeding (Doane and McManus 1981).

A Three-Phase Model of Gypsy Moth Egg Development

A model of gypsy moth egg development has been constructed that recognizes the three phases of development. The empirically derived function [1] described above is used to model prediapause (Phase I) development. A developmental rate function for postdiapause (Phase III) also empirically derived from rearing experiments that assumed diapause completion after 111 d at 5°C. A simple polynomial

$$R_D(T) = a + bT + cT^2 + dT^3 \qquad [2]$$

was used to model postdiapause development.

A function estimating diapause developmental rate was "guesstimated" using the hypothesized (Gray et al. 1991) phase transition that occurs after 110 d at 4.4°C or 111 d at 5°C. This function has the form

$$R_D(T) = \frac{R_{MAX}}{1 + e^{\kappa(\mathrm{T}_R - \mathrm{T}_\Delta)}} \qquad [3]$$

where $R_D(T)$ is the developmental rate at temperature T, R_{MAX} is the maximum developmental rate, T_R is the absolute difference between temperatures at which developmental rate is maximum and 0.5 of maximum ($|T_{OPT} - \mathrm{T}_\xi|$), T_Δ is the absolute difference between T and T_{OPT} ($|T - \mathrm{T}_{OPT}|$), and κ describes the steepness in the function (maximum increase in developmental rate per degree increase in temperature). This function describes a temperature-dependent developmental rate that is symmetrical around the optimum temperature and diminishes in a sigmoid fashion as temperature departs from the optimum (Fig. 7).

incorporating an age-dependent factor into the prediapause developmental rate curve is probably not justified.

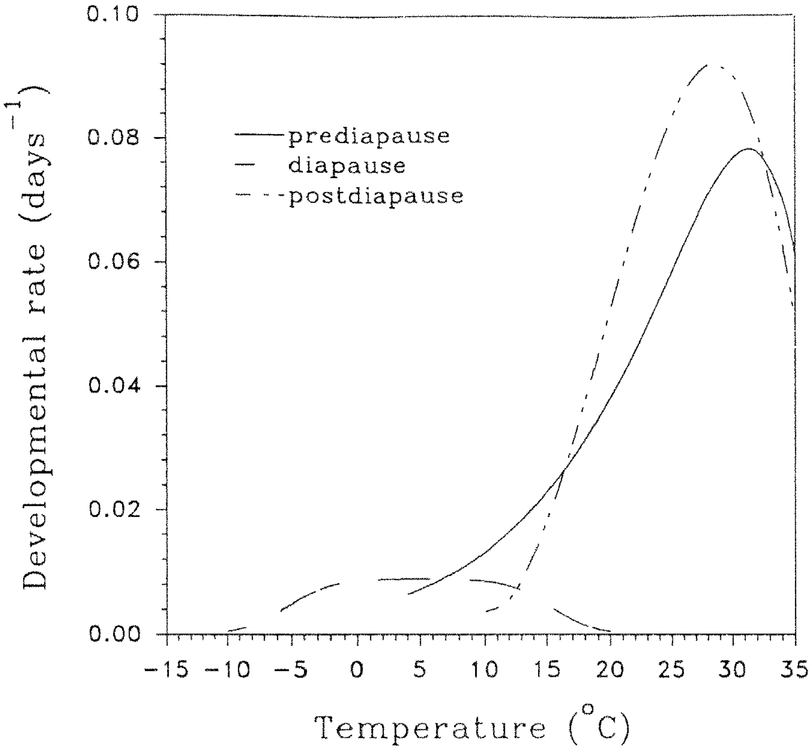

Fig. 7. Relationship between median developmental rate (days^{-1}) and temperature (°C) for the three phase of egg development.

Additional studies are required under different temperature conditions before a function can be derived that will adequately describe diapause development and completion. The slight, but significant, daily increase in respiration rate that occurs during the 88 d following diapause initiation (Fig. 6) suggests that the diapause state may be gradually removed under constant temperature conditions as proposed by Tauber et al. (1990). Thus diapause development may need to be modeled by an age- and temperature-dependent function. The pattern of respiration rate during the developmental period from diapause initiation to egg hatch will provide evidence of the nature of the transition from diapause to postdiapause.

Although model completion awaits the results of additional experiments, the three-phase construct has demonstrated geographic robustness. Under temperature conditions from Montreal, Quebec to Tallahassee, Florida, the three phase model predicted reasonable hatch dates (Gray et al. 1993). In addition, insufficiently cool winter temperatures in the southern range hatch limit hatch success and extend the hatch period, while early and cool autumn temperatures in the northern range limit hatch success.

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DISCUSSION SUMMARY

Management Subgroup

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ABSTRACT

Information required to synchronize gypsy moth management options with the occurrence of stage-specific targets in natural populations are outlined in the context of the F_1-sterile egg program. Development of knowledge for F_1-sterile egg production, storage, acclimation, and timing of application are discussed and testable hypotheses provided. Information developed for the F_1-sterile program should enhance the prediction process for other pest management options.

INTRODUCTION

The Gypsy Moth Management Subgroup recognized that more information was required to synchronize accurately the pest management options with the occurrence of stage specific targets in natural populations. Moveover, the Subgroup recognized that synchronization was dependent on our knowledge of the biological, ecological, and genetic factors that influence phenology of hatch and our ability to utilize this knowledge to predict hatch. In this regard, the development of information on the environmental, genetic, and physiological factors involved in the regulation of gypsy moth diapause had the highest priority and was essential to the prediction process (modeling). The Gypsy Moth Management Subgroup focused on diapause research needs of the F_1-sterile egg release program (Mastro et al. 1989), while recognizing that development of this knowledge would provide information for other management options.

The rationale for emphasizing the needs of the F_1-sterile insect program include:

(1) The F_1-sterile egg technique is being developed for management of low density, building populations over a broad geographical and ecological range; thus, the information developed should be applicable to other management options.

(2) Efficacy of the F_1-sterile insect technique is dependent, in part, on the ability to predict hatch of both the laboratory-reared F_1-sterile eggs (applied material) and the target population, and the knowledge of factors affecting neonate fitness (e.g., vigor) and establishment in the target area.

(3) The New Jersey Standard Strain (NJSS), the laboratory strain used to produce gypsy moth F_1-sterile eggs, has been used for numerous scientific studies, including investigations related to diapause; i.e., the physiology of embryogenesis (Bell 1989), genetics (Keena and ODell 1994), diapause (Gray et al. 1991), and effects of

environmental factors (reviewed in Keena and ODell 1994; J. Tanner, unpublished). Thus, there is a significant base of knowledge with which to integrate new information.

(4) The lack of knowledge of the diapause requirements of NJSS F_1-sterile eggs needed to effect maximum egg survival as measured by percent hatch, hatch synchrony with target population, and optimal neonate fitness as measured by establishment.

OBJECTIVES

The present method for stockpiling, storing, acclimating, and timing of release of F_1-sterile eggs has resulted in sporadic and often only partial synchrony of hatch with the target population. Those factors affecting our inability to effect hatch synchrony include:

(1) The inability to predict hatch of the relatively hidden (obscure) egg masses that characterize the majority of low density natural populations;

(2) The lack of knowledge of the interaction of genetic and ecological factors that mediate diapause and, subsequently, hatch; and how those factors, in particular, influence hatch phenology of populations in new environments (i.e., leading edge, introduced);

(3) The lack of knowledge of how, or if, factors affecting the pre-diapause, diapause, and post-diapause periods interact to affect hatch phenology. This information is critical for optimizing F_1-sterile egg processing (see later), egg and neonate viability, and for predicting hatch phenology of F_1-sterile eggs.

The Management Subgroup approached the overall objective of improving survival and hatch synchrony of F_1-sterile eggs by asking three questions and developing testable hypotheses. These are:

(1) Under what conditions (time, temperature, humidity) should NJSS-F_1-sterile eggs be held in order to achieve synchrony of hatch with wild eggs?

H.1. At 5°-15°C the required chill period for the completion of diapause of most NJSS F_1-sterile "neonates" is 110 days (lab or field), and a minimum of 30 days is required for post-diapause development (incubation) under ambient conditions of the target population.

H.2. Using H.1., synchronization of NJSS F_1-sterile hatch with field strains will not vary geographically or topographically.

(2) What storage conditions provide for optimal survival of F_1-sterile eggs and F_1-sterile neonates?

H.3. Storing NJSS F_1-sterile eggs in chill (5°-15°C) for longer than 140 days will increase mortality of both eggs and neonates.

H.4. Holding NJSS F_1-sterile eggs at temperatures above 25°C or below 20°C during the pre-diapause period (during embryogenesis and prior to chill, or both) will result in increased egg mortality.

H.5. Given H.1. and H.2., holding NJSS-F_1-sterile eggs at >75 percent RH and at field RH, or both, prior to chill increases survival if eggs are laid more than 170 days before estimated application date.

H.6. Given optimal diapause conditions (H.1., H.2., H.3.), NJSS and NJSS-F_1 insects will disperse and establish (survive) in the same proportion as wild larvae.

(3) Do NJSS-F_1-sterile insects hatch and disperse in response to the same temperature, humidity, and photoperiod conditions?

H.7. Hatch and dispersal in NJSS, wild, and F_1-sterile are affected the same way by temperature, humidity, and photoperiod. (Very little information exists on the hatch and dispersal response of F_1-sterile to alternating temperature, humidity, and photoperiod [i.e., lights on]).

F_1-STERILE INSECT TECHNIQUE

Review and Research Needs

Classically, sterile insect programs utilize the release of totally sterile adult males (e.g., Med fly, screwworm fly). These are released in large numbers to mate with females of the target population. Successful suppression or eradication occurs when a high overflooding ratio of sterile-to-wild adult males is achieved, and sterile insects are fully competitive with their wild counterparts. Release of fully sterile adult male gypsy moths successfully eradicated a low-density, isolated population in Michigan after releases in 1980, 1981, and 1982 (Mastro et al. 1989).

The F_1-sterile technique was developed to increase the efficiency and decrease the cost-benefit ratio of the gypsy moth sterile-insect-release program. F_1-sterile egg masses are obtained by treating male pupae with a substerilizing dose of irradiation, adults emerging from these pupae are then mated with normal, laboratory-reared females, and their F_1-sterile eggs are harvested and stockpiled for application. The genetic consequences of the male's substerilizing dose of irradiation is carried over to the generation that hatches from the field applied F_1-sterile eggs; only 35-40 percent of the eggs hatch, all those that hatch are fully sterile, and there is a 2:1 male:female ratio. Success of an F_1-sterile egg release is contingent on achieving a high overflooding ratio of fully competitive sterile-to-wild adult males. Synchrony of hatch of F_1-sterile eggs with the target population, and subsequent establishment of F_1-sterile neonates on primary host foliage are essential for achieving optimum sterile-to-wild adult male ratios. Both variables depend on predicting the patterns of gypsy moth hatch and host plant phenology at the target site, and the ability to produce, store, deliver, and apply F_1-sterile eggs so that F_1-sterile hatch, dispersal, and establishment are in synchrony with these target-site patterns. Interactive models that can integrate target-site patterns with F_1-sterile egg processing are needed to efficiently meet anticipated program demands (i.e., targets in broad and climatologically diverse geographic areas). But knowledge of factors that affect the development, physiological behavior, and viability of gypsy moth during the egg stage (embryogenesis, diapause, post diapause) must be developed for both wild and F_1-sterile populations in order for models to be useful.

Target Population. The inherited sterility egg release technique (IST) is being developed for use in suppressing low-density, increasing gypsy moth populations. Models presently used to predict gypsy moth hatch may not be reliable for these populations because the data used to develop them were primarily derived from egg masses easily observed and located 1 meter or more above the ground. Generally, these locations simulated the distribution of the majority of egg masses in medium- to high-density populations than those targeted for F_1-sterile egg intervention. Egg masses in low-density populations (<50/ha) are generally near the ground in relatively dark, moist locations, in the litter, or under and on bark and rocks (Campbell 1975), and their distribution is patchy in contrast to F_1-sterile eggs that are broadcast on the ground throughout the target site. Quantitative data are needed that describe (1) hatch phenology and viability of egg masses laid in close-to-ground, relatively unexposed locations, (2) the effect of oviposition substrate on hatch phenology and egg and neonate viability, and (3) the relative coincidence of hatch pattern with relative host plant phenology. Studies designed to develop such data also should consider the attributes of patchiness and the potential effect of snow cover.

F_1-Sterile Egg Processing. In order to address efficiently all the factors affecting the F_1-sterile-egg stage, processing will be discussed in four steps: production, storage, delivery, and application. However, it is recognized that each step is likely to affect the next.

Production: A general description of F_1-sterile mating protocols was described earlier. In mass production, 100 male and 100 female pupae are placed in a chipboard rectangular box; adult eclosion, mating, and oviposition usually occur over the next 7-10 days. Egg masses laid on the box are maintained at 25°C, 50-60 percent RH, for approximately 25 days to complete embryogenesis. They then are scraped into a rectangular box and stored at 7-8°C. Each box may contain 1200-1300 egg masses.

The period of embryogenesis prior to entering diapause for both wild and NJSS eggs has been established as approximately 3 weeks (see Giese and Cittadino 1977; Bell 1989; Gray et al. 1991). Thus, the 28-day holding period for embryonation of F_1-sterile eggs appears to be appropriate. However, published and recently completed experiments indicate that holding conditions during embryonation should be reevaluated. Capinera et al. (1977) found that embryonating wild gypsy moth eggs at 27°C, as compared with 15°C (at 75 percent RH) significantly reduced egg yolk content over the 6-week period following oviposition. Robert Bell (personal communication) using NJSS, found that respiration rate and depletion of triglycerides (an energy source) increased as temperatures during embryogenesis increased, but that temperature apparently did not affect time to complete embryogenesis. Tanner et al. (1987)

found that an RH of 80 percent during the embryonation period increased the survival of standard production strain. Considering the experimental evidence presented above, the environmental conditions used in the production step should be investigated to determine if changes in the present system should be modified (i.e., reduce temperature, increase humidity) in order to maximize egg survival, enhance hatch predictiveness, and optimize neonate establishment.

Storage: Storage of F_1-sterile eggs must consider (1) the need to stockpile egg masses for various periods of time to meet program objectives, (2) the need to satisfy diapause requirements, and (3) the need to optimize egg and neonate survival. The present storage methods do not optimize these needs.

During egg mass production F_1-sterile eggs are harvested daily, approximately 29 days after oviposition, and placed (chill date) in a walk-in refrigerator (7-8°C and 100 percent RH) for 150-170 days. If eggs are to be stored for more than 170 days to meet schedule requirements, they are transferred to 5°C after 100 days at 7-8°C; precocious hatch occurs when eggs are held for more than 180 days at 7-8°C (J.A.T., personal communication). Generally, eggs from a range of chill dates are mixed together for each application. F_1-egg masses are selected for site-specific application based on the time in chill and estimated hatch date of target population; i.e., F_1-eggs must be in chill for a minimum of 140 days before application, which is minimally 2 weeks before predicted hatch of the target population. Recent studies have shown that longer periods for stockpiling can be achieved by holding egg masses at warm, naturally occurring temperatures for 1-2 months after oviposition, before chilling (J.A.T., personal communication).

The storage methods described above have been, and are presently, used to stockpile F_1-sterile eggs for experimental field applications. Predicting hatch and insect viability has not been consistent, due primarily to unknown effects of storage conditions. Use of the F_1-sterile egg technique in applied programs will necessitate increased stockpiling, manipulation, and integration of egg masses produced over a longer period of time, optimization of egg and neonate survival (insect fitness), and a model that will allow more efficient and accurate stockpiling for climatologically diverse, site-specific application.

Delivery and Application. Once a target population is selected, an estimate for hatch in the target areas is obtained from local entomologists; timing for budbreak of primary hosts also is requested. These dates are then used to select the F_1-sterile eggs that will be used at this site; generally 6-10 production dates will be selected for a site. Approximately 2-3 weeks before the predicted hatch of the target population, F_1 eggs are removed from the 5-7°C walk-in refrigerators, placed in large styrofoam coolers with freezer-pacs, and driven or flown to the release site. Generally, delivery requires fewer than 12 hours.

The delivery system described above has been dictated primarily by the relatively late selection of target populations. Generally, when eggs are removed from chill (7°C) they already are in the post-diapause phase so that temperatures above 4.5°C will promote metabolic activity (Mithat 1933). Depending on age and time in chill, precocious hatch may occur, and does. Delays, overheating during transport, metabolic activity of closely packed F_1-sterile eggs all contribute to hatch and neonate viability.

Early identification of target populations and holding F_1-sterile eggs on or near the target site for 1-5 months before to predicted hatch may enhance the ability to synchronize with the target population and improve egg viability (Lynch and Hoy 1978, Lance et al. 1982, ODell and Pupedis, unpublished). Recent experiments indicate that holding F_1-sterile eggs for 2-3 months at ambient or high humidity before chill will increase the ability to stockpile eggs over longer periods of time (Tanner unpublished). While we know improvements can be made in the delivery system, we do not have the specific data to develop a model for predicting egg hatch, given storage and delivery specifications. Nor do we have the information to predict the fitness of the neonate given those same conditions.

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POST DIAPAUSE IN GYPSY MOTH: A REVIEW AND DISCUSSION

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ABSTRACT

Physiological processes that affect gypsy moth development during post diapause are reviewed and discussed in the context of diapause in other insects. These processes include neurosecretory release, loss of hardiness to cold, increase in respiration, yolk consumption, and eclosion. Studies to elucidate temperature and other environmental effects on these post-diapause physiological processes and their interaction are recommended for improving our capability for predicting hatch phenology and neonate fitness.

INTRODUCTION

The egg stage of gypsy moth has been described variously as having three phases: prediapause (embryonation), diapause (diapause development), and post diapause (incubation). Recent investigations indicate that the prediapause phase is a distinct physiological process that can be quantitatively and qualitatively defined (Bell 1989; Gray et al. 1991). Diapause and post diapause have not been similarly defined; in fact, the literature suggests that there may not be a distinct transition between them (Goldschmidt 1932; Pantyukhov 1964; Bell 1989; Tauber et al. 1990). Nevertheless, diapause termination and the subsequent post-diapause events leading up to hatch must be described realistically in order to meet the goal of a geographically robust model of diapause and egg hatch. To accelerate progress in developing such information, this paper reviews knowledge of gypsy moth post diapause, discusses this information in the context of diapause in other insects, and develops recommendations for research.

For the purposes of this review, the post-diapause phase begins 130 days after oviposition, including 30 days of embryonation (see Bell 1989), and 100 days of chill at 5-10°C. The latter is a composite optimum of many investigations (Giese and Cittadino 1977; Bell 1989; Tauber et al. 1990; ODell, 1992). Under natural conditions, gypsy moth eggs are exposed to a prechill, post-embryonation period that may vary considerably in length and environmental conditions. While the effect of prechill is not discussed here, its importance to egg viability and hatch phenology should be investigated.

The post-diapause phase is characterized in the literature as having several physiological processes, each of which may be affected differentially by temperature. These are neurosecretory release (Loeb and Hayes 1980), loss of cold hardiness (Pantyukhov 1962), increase in respiration (Bell 1989), yoke consumption (Masaki 1956; Capinera et al. 1977), and eclosion. A discussion of these processes follows.

Neurosecretory Release

Many lepidoptera exhibit a pattern of neurosecretory accumulation and release synonymous with diapause development during winter chilling and subsequent post diapause (incubation) during spring warming (see Loeb and Hayes 1980; Turnock et al. 1986). Generally, warming of insects after a discrete chilling period elicits movement of most stainable material out of brain neurosecretory cells, after which the insects resume development. A similar sequence of neurosecretory activity has been demonstrated for pharate first instar gypsy moth (Mitsuhashi 1963; Loeb and Hayes 1980).

Loeb and Hayes (1980) describe a general pattern of brain neurosecretion commencing with diapause and chilling, followed by apparent accumulation of storage material for 120 days, and culminating with complete release (disappearance) of that material after 120 days at 4°C and 7 days of incubation at 24°C. Hatch of siblings occurred 1-3 days later; on the day of hatch, neurosecretory activity in mobile larvae was limited to two newly visible giant cells in each larval cerebral hemisphere.

Loeb and Hayes (1980) indicate that the pattern of neurosecretory activity in the embryo gypsy moth brain described above is very similar to events that take place in brains of other lepidoptera that diapause at more mature stages. This is a significant observation. Turnock et al. (1986) demonstrated that, following diapause, temperature has a differential effect on the subsequent metamorphic development of the bertha armyworm, *Mamestra configurata*, which overwinters as a diapausing pupa in the soil. They exposed pupae to brief warm periods at the beginning of post-diapause pupal-adult metamorphosis and then incubated at lower temperatures. Pupae exposed to brief warm periods eclosed earlier than those exposed to the constant incubating temperatures. The differences were greater than expected by the additional thermal units, suggesting that the early warm period effect was not on the rate of metamorphic development but on an earlier process concerned with neuroendocrine control. The release of neurosecretory material (NSM) from the gypsy moth brain (Loeb and Hayes 1980) and subsequent increase in metabolic respiration (Bell 1989) may very well be synonymous with the armyworm pupal "initiation of development" and subsequent metamorphic development. If so, eggs exposed to warm winter period(s) following completion of diapause may be affected differentially by temperature than those not similarly exposed.

Extreme fluctuations in temperature commonly occur in late winter-early spring when eggs of gypsy moth have completed diapause. In addition, the location of the oviposition site (under bark *vs.* exposed on tree) and the material on which the eggs are laid (rock *vs.* bark) differentially may affect exposure and susceptibility to early warming. Thus, depending on their location, eggs may be exposed to temperatures that could initiate the release of NSM, followed by periods of lower temperature in which subsequent metabolic activity takes place. Eggs so exposed may develop more rapidly than those not exposed to such a "warm temperature trigger"

(Turnock et al. 1986). The magnitude of the difference in eclosion time between the groups of armyworm pupae in laboratory experiments described by Turnock et al. (1986) suggests that the differential effects of temperature on the initiation of development (neurosecretory release) and subsequent development are of biological significance and should be considered important in construction of models of insect development under natural conditions. It is suggested here that experiments be carried out to determine if such effects will enhance the predictability of gypsy moth hatch.

Cold-hardiness

The state of diapause does not, in itself, confer cold-hardiness. In most species, a prolonged exposure to relatively low (but above freezing) temperatures is required to induce the ability to survive subfreezing temperatures (Beck 1983). Very little information has been reported on the cold-hardiness of gypsy moth eggs. Pantyukhov (1964) studied certain physiological factors in gypsy moth egg resistance to cold. Using the iodine number (ratio of saturated to unsaturated fatty acids) as a measure of cold-hardiness, he found that the number of eggs increased from late summer to late winter and then decreased rapidly to prediapause levels. His studies also indicated an intraspecific physiological differentiation, giving improved resistance to eggs overwintering in those parts of their distribution where the climate is most severe. Goldschmidt (1932) indicated that a certain temperature sum is necessary for the eclosion reaction to occur, which within a certain race is constant. He also indicated that there is perhaps a critical cold point below which the eclosion reaction slowly occurs, and this also may differ by race. Geographic and/or strain differences in diapause or post-diapause characteristics also have been reported for other species (Kimberling and Miller 1988; Morris and Fulton 1970) but, like Goldschmidt (1932), cold-hardiness was not addressed.

Generally, studies conducted on the mechanisms by which insects withstand subzero temperatures have been concerned with the role played by polyhydroxol alcohols. Glycerol, in particular, has been implicated in the survival of both freeze-tolerant and susceptible insects (Duman 1979). Hanec (1966) found that seasonal changes in glycerol content in eggs of the forest tent caterpillar followed a similar pattern reported for other species; the glycerol content is low during late summer, reaches a peak in early winter, and does not decrease until post-diapause or hatching development begins in late spring. The seasonal pattern of rise and fall of the iodine number reported by Pantyukhov (1964) is strikingly similar, suggesting that polyhydroxol alcohols are involved in the cold-hardiness of gypsy moth eggs.

In our laboratory, wild egg masses are collected each January for use in studies over the next 8 months. These are stored at 3°C and 90-100 percent RH. Generally, percent hatch does not begin to decline until mid-August. We consider these eggs to be cold hardy and able to survive temperatures <3°C. Generally, egg masses collected in Connecticut after March 1 can no longer survive at 3°C for more than several days. Doane (1969) found that chilling of gypsy moth eggs collected in early spring (March) induced about an approximate eight-fold increase in the susceptibility of larvae to nuclearpolyhedrosis virus (NPV). It is likely that this increase in susceptibility to NPV was indicative of a decline in fitness or vigor caused by chilling after the loss to cold hardiness. Generally, susceptibility to chilling temperatures probably follows biochemical changes, which might include fatty acids (Pantyukhov 1964), polyhydroxol alcohols (Duman 1979), and thermal hysteresis proteins (Duman 1979), as well as other unknown factors.

Investigations of diapause in the eggs of gypsy moth suggest that there is a progressive increase in the thermal sensitivity of the insect, with increase in the chill period such that fewer heat units above a development threshold are required for hatch as length of chill increases (Goldschmidt 1932; Masaki 1956; Tauber et al. 1990). Similar patterns of post-diapause thermal response have been reported for eggs of the winter moth, *Operophtea brumata* (L.) (Kimberling and Miller 1988); larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Régnière 1990); and adults of the fall webworm, *Hyphantrea cunea* Drury (Morris and Fulton 1970). Each of these species also is subjected to naturally occurring subfreezing temperatures and thus are likely to be affected by similar physiological factors that induce cold-hardiness. It is proposed here that changes in cold-hardiness factors during post diapause are directly linked to changes in thermal response (i.e., temperature sensitivity). For example, Hanec (1966) has shown that decline of glycerol in eggs of the forest tent caterpillar is initiated early in post diapause and that the concentration of glycerol and the major cooling point during this period varies inversely with temperature. Thus, over a 60-day post-diapause chill period of 4.5°C, glycerol content steadily declined from 22.6 percent (mean dry weight) to 19.8 percent, 17.3 percent, 16.9 percent, and 12.5 percent measured at 7, 15, 30, and 60 days, respectively. This process may correspond to the gradual termination of diapause, characterized by a slow return of the thermal responses to non-diapause levels, suggested by Tauber et al. (1990). If so, then measurement of factors affecting cold-hardiness could be used to enhance hatch prediction models.

Respiratory Metabolism and Yolk Consumption

Bell (1989), using laboratory-reared eggs, reported that following a period of 60 chill days at 7°C, the rate of respiratory metabolism of eggs held at 25°C increased exponentially with additional increments of chilling; he suggested that the respiratory increase reflected an increasing synchrony of post-diapause development that reached a maximum at 150-180 days. Bell (1989) also noted that ongoing studies indicated that increased exposure of diapausing eggs to chilling resulted in an accelerated increase in metabolism. During incubation, this metabolic activity presumably utilizes stored yolk. The quantity of yolk does not change during diapause, but after the required diapause period the yolk within the gut is absorbed when temperature is elevated appropriately (Capinera et al. 1977). Masaki (1956) noted that histological observations made by Tuleschkov (1935) indicated that yolk in the gut must be digested and absorbed before hatching. Loeb and Hayes (1980) reported that after 120 days at 4°C, laboratory-reared gypsy moth eggs hatched in 7-10 days when incubated at 24°C. During this period, brain neurosecretory material almost disappeared (Loeb and Hayes 1980) and, presumably, respiratory metabolism and yolk absorption increased rapidly until hatch. This is close to the minimum hatch pattern of eggs stored at 3°C. As noted previously, routinely we collect and hold wild egg masses at 3°C from January to October. The time for 50 percent hatch of these eggs, when incubated at 25°C, declines from approximately 10-14 days in March (approximately 120 ambient chill days) to a minimum of 5 days from July through October. Our experience with wild egg masses stored during the same time period, but at 4-5°C, indicates that 50 percent hatch occurs in a minimum of 2-3 days when eggs are incubated in June, but that egg mortality increases rapidly thereafter. Bell (1989) found that laboratory reared eggs stored at 7°C for 180 days take only 1.5 days for 50 percent hatch. The observations described above suggest that the threshold temperature for metabolic respiration and yolk consumption is approximately 4°C, so that at temperatures above this, physiological development leading to readiness to hatch will occur. Studies to describe quantitatively and qualitatively the effect of temperature and other environmental factors on this pre-readiness-to-hatch process are likely to increase our capability of predicting hatch and provide baseline qualitative information for predicting neonate fitness.

Eclosion

Under natural conditions, gypsy moth egg masses are subjected to variable ambient conditions mediated by both the location and medium of oviposition (see earlier discussion). All of these variables affect the thermal condition of the egg when the gypsy moth is ready to hatch. The hatch response to any particular thermal condition will be modified by superimposed photoperiodic and perhaps, thermoperiodic responses.

Studies to determine a temperature threshold for gypsy moth hatch have been almost exclusively concerned with the effects of constant temperatures, although Giese and Cittadino (1977), in their review of variable temperature relationships, state that studies strongly indicate that gypsy moth diapause may be terminated earlier under natural conditions. Goldschmidt (1932) suggests 6°C as a critical temperature for gypsy moth larvae to activate muscles and chew their way out of the egg. Mithat (1933) calculated that 5.5°C at 100 percent RH was the threshold for development (hatch) of first instar gypsy moth, but observed that hatch began at a temperature over 8°C. Maksimovic (1958) confirmed that the threshold for development and hatch was between 5 and 9°C. Our observations indicate that some laboratory-reared eggs stored at 7°C may eventually hatch, but eggs stored at 5°C will not; egg viability at both temperatures declines after 180 days in chill. This suggests that egg food reserves (yolk) could be depleted at 5-7°C, reducing the vigor of hatching larvae and, thus, affecting the timing of hatch. Capinera et al. (1977) state, "We suspect that high temperature may convert large, yolk-rich eggs to the equivalent of small, yolk-deficient eggs." While this statement referred to conditions during embryonation, a similar statement might be made for eggs during post diapause; i.e., eggs exposed for long periods at temperatures conducive to metabolic activity but not hatch (ca. 4-7°C) may reduce the vigor or fitness of the larvae. This could have dramatic effects on the development and behavior of larvae. Rossiter (1991) reported that when gypsy moth individuals came from larger eggs, both sexes hatched earlier, and related egg size and insect fitness to maternal effects. Here, it is suggested that the thermal conditions eggs are exposed to during the post-diapause stage, particularly close to hatch, could affect insect fitness (read vigor) prior and subsequent to hatch. For example, eggs laid on rocks may be less affected, metabolically, than eggs laid on the bark of trees, because rocks remain relatively cool in the spring.

Photoperiodism in gypsy moth has been reported as affecting adult eclosion (ODell 1978;

Ma et al. 1982), male flight activity (Cardé et al. 1974; ODell and Mastro 1980), larval migration (ODell and Godwin 1979), and sperm movement (Giebultowicz et al. 1988). McManus and ODell (unpublished) observed that egg hatch under controlled laboratory conditions occurred almost exclusively during photophase and was synchronized with lights on. Field and laboratory observations also suggest that temperature mediates eclosion affecting the daily pattern of hatch and the proportion of eggs that hatch each day. Thus, for any particular day, the timing of larval dispersal, feeding, and establishment will depend on the effect of temperature and photoperiodic cues on eclosion.

Chippendale (1977) describes diapause as "a genetically controlled life phase for which preparatory biochemical adjustments, including the accumulation of nutrient reserves, and behavioral adaptations, often including the location of a protective site, occur in advance." The preparatory adjustments that influence physiological development of the gypsy moth during post diapause includes, in order of their occurrence, storage of egg nutrients, selection of an overwintering site, storage of neurosecretory material, and induction of cold hardiness. While all these adjustments affect post-diapause physiological processes, location of the oviposition site (including the composition of the oviposition medium) appears to have the most influence on eclosion and, perhaps, on other post-diapause processes. Studies that develop quantitative descriptions of naturally occurring egg mass environments, particularly development of temperature indices, must be carried out before information on eclosion and other post-diapause processes can be integrated into predictive models.

SUMMARY

This paper summarizes the knowledge of the physiological processes and activities that affect gypsy moth development during post diapause, and subsequently, the hatch and fitness of neonates. It is intended to generate ideas and studies that will lead to a better understanding of post diapause and provide quantitative information that can be used to enhance prediction of hatch, first instar establishment, and insect fitness, all of which directly affect survival.

Four physiological processes that occur during the post-diapause period of gypsy moth have been discussed: neurosecretory release, loss of cold-hardiness, respiratory metabolism-yolk consumption, and hatch. The sparse literature on these processes suggests that they occur sequentially and they may occur at a specific temperature-dependent rate (Holtzer et al. 1976). Studies to elucidate temperature and other environmental effects on these post-diapause physiological processes, and their interaction, are highly recommended for improving our capability for predicting hatch phenology and neonate fitness.

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GENETICS OF DIAPAUSE IN THE GYPSY MOTH: A REVIEW

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ABSTRACT

Literature on the inheritance of diapause characteristics in the gypsy moth is reviewed and discussed. The inheritance of three traits associated with diapause or post-diapause have been examined for the gypsy moth: (1) the number of days from egg laying to first hatch; (2) the number of days from the end of chill to the beginning of hatch (incubation time); and (3) duration of the hatch period in days. Days to first hatch appeared to be sex-linked, and have environment-specific expression when a North American non-diapause selected strain was used, but appeared to be polygenetically inherited using a Yugoslavian non-diapause selected strain Work with several geographical races suggest that incubation time and duration of hatch are both polygenetically inherited.

INTRODUCTION

The gypsy moth, *Lymantria dispar*, has become a major tree pest in the United States since its introduction from Europe in the mid-19th century. The gypsy moth is a univoltine species in the United States. It appears to undergo only one generation annually wherever it occurs in the world (Goldschmidt 1934). The gypsy moth has a period of arrested morphogenesis (diapause), during which it is a fairly mature embryonic larva (Leonard 1968). This diapause period permits survival during periods of cold temperatures and food shortage, and effectively helps synchronize hatch with the appearance of tender young foliage of preferred host plants. Some possible components of intra- and interpopulation variation which may have, or have been shown to have, a genetic component are listed in Table 1 by diapause phase. The inheritance of three traits associated with diapause or post diapause have been examined for the gypsy moth: (1) number of days from egg laying to first hatch, with and without a period of chill; (2) the number of days from the end of chill to the beginning of hatch (incubation period); and (3) duration of the hatch period in days.

Understanding inheritance of diapause-related traits will increase our ability to predict timing of hatch and its population consequences (survival, dispersal, mortality) resulting from crosses between different races that occur either intentionally (e.g., control programs) or unintentionally (e.g., accidental introduction). In addition, understanding the inheritance of diapause-related traits may aid in predicting population quality, increases in population size, and population adaptation.

Two gypsy moth nondiapause strains, with a reduced number of days from egg laying to first hatch, have been selected for one using a North American population (NA) (Hoy 1977) and the other using a population from Yugoslavia (Y) (Marovic and Vasic 1978). The North American strain was reared on artificial diet while the Yugoslavian strain was reared on foliage. In both studies the number of days from egg laying to first hatch decreased constantly with selection following the second generation and reached approximately 30 days in the eighth generation. Concomitant to the decrease in days from egg laying to first hatch was an increase in the percent hatch of selected egg masses without chill. These results indicate that genetic variability was present in the wild population for diapause termination without chill.

Table 1. Components of intra- and interpopulation variation that may have or have been shown to have genetic components

Pre-Diapause	Diapause	Post Diapause
Amount of yolk deposited in the egg	Time required for completion	Synchrony of hatch
Egg size	Temperature sensitivity - pre-chill - during chill	Duration of hatch
Time required for completion of embryogenesis	Humidity sensitivity	Initiation of hatch
Temperature sensitivity during embryogenesis		Time required for post-diapause development
Humidity sensitivity		Temperature sensitivity
		Humidity sensitivity

The inheritance of diapause was investigated by crossing nondiapause strains with near-wild laboratory strains, North American (WNA) and Yugoslavian (WY). The mode of inheritance of hatching times of North American populations appeared to differ, depending on whether the eggs had experienced a token chill or not (Lynch and Hoy 1978). Unchilled hybrid eggs began hatching at approximately the same time as unchilled near-wild eggs, while chilled hybrids were exactly intermediate in phenotype between the chilled selected line and chilled near-wild type (Fig. 1). These results suggest that diapause, as measured by time to first hatch, is dominant in the absence of chill but intermediate with chill. The percent hatch of embryonated eggs (Fig. 2) follows a similar pattern. The increased hatch and reduced time to first hatch observed after chill may be indicative of a shortened diapause requirement in the hybrid (heterozygous) condition. The token chill allows a larger percentage of the population to complete diapause in the heterozygote but is insufficient to change significantly the proportion hatching from wild eggs. As time in chill increases, differences between the proportion of heterozygote and wild eggs ready to hatch should decrease and reach a level similar to that observed for the unchilled selected strain.

In addition to the environment-specific phenotype expression for days to hatch, percent hatch also was shown to be sex-linked (the gene is on the X chromosome) (Lynch and Hoy 1978). This sex-linked, environment-specific mode of inheritance is similar to that observed in the silk moth (Tazima 1964).

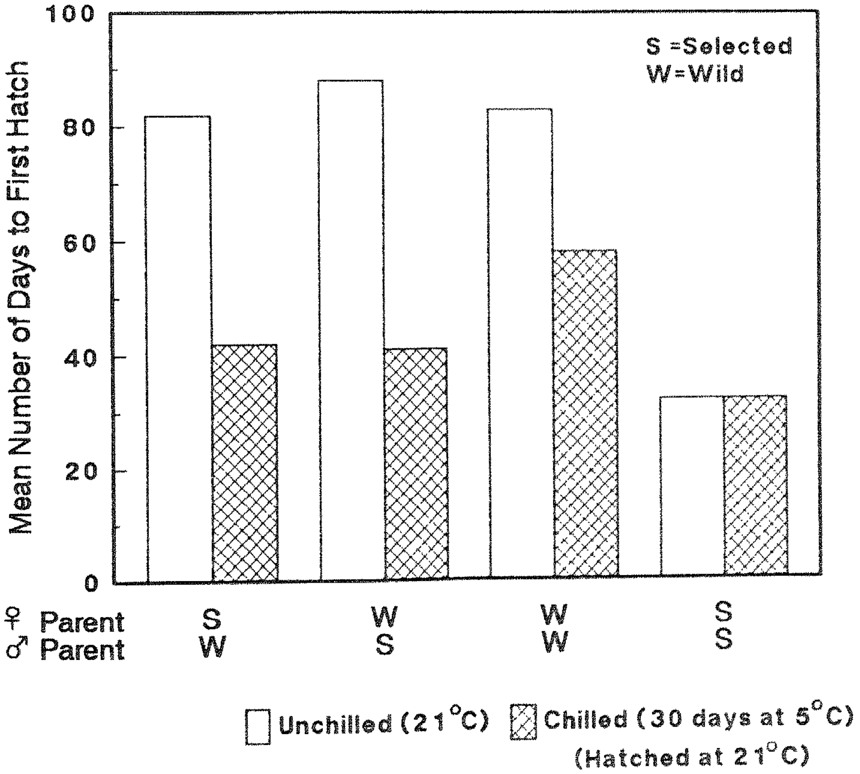

Figure 1.--Mean number of days until the first egg hatched at 21°C in each half egg mass of selected 'non-diapause,' wild-type, and reciprocal-hybrid North American gypsy moths that were either unchilled or exposed to 5°C for 1 month. The 30 days of chill are not included in the number of days to first hatch for chilled groups. Data from Lynch and Hoy 1978.

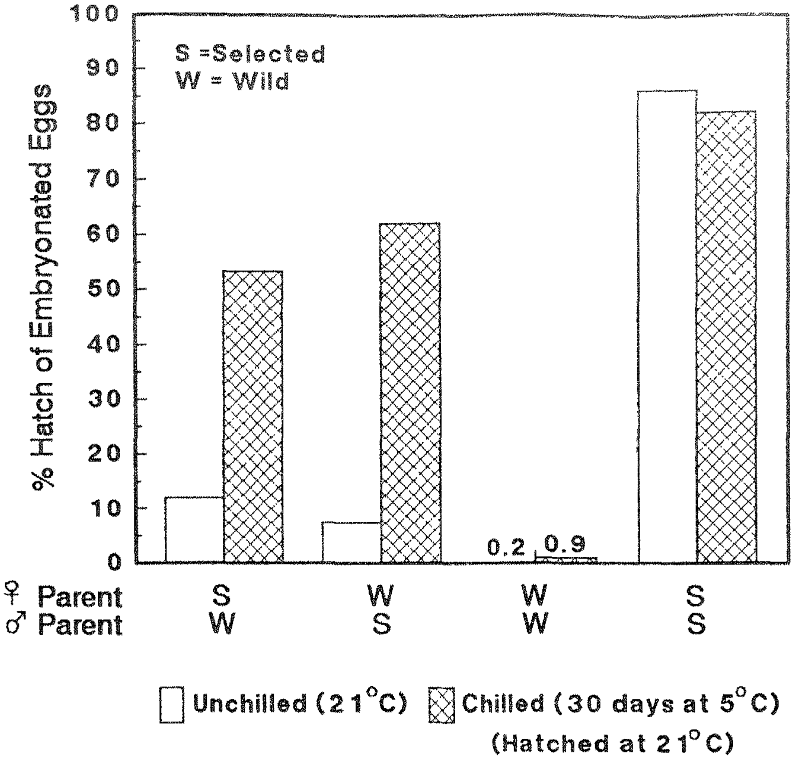

Figure 2.--Mean percentage hatch of embryonated eggs at 21°C of selected non-diapause, wild-type, and reciprocal-hybrid North American gypsy moths that either were unchilled or exposed to 5°C for 1 month. Data from Lynch and Hoy 1978.

Results of crosses between the Y and WY strains indicated a slightly different mode of inheritance than those observed for the North American strains (Marovic 1981). Unchilled hybrids were intermediate in phenotype between the unchilled selected line and unchilled near-wild type, and there were differences between the reciprocal crosses (Fig. 3). No token chill treatment was done, but holding and hatching the eggs of hybrids and near-wild types in an outdoor insectary, where normal overwintering temperatures were experienced, resulted in the hybrid eggs hatching before the near-wild eggs. These results indicate that a long number of days to hatching for unchilled eggs is incompletely dominant and that the male parent transfers this property to a larger extent than the female (probably X-linked). In addition, there is no apparent environment-specific phenotype interaction. The percent hatch of unchilled embryonated eggs appears to be highly sex-linked (Fig. 4) (Marovic 1981). Unchilled hybrid eggs whose male parent comes from the selected line will have a similar percent hatch to those of the unchilled selected line, while those with near-wild-type male parents have a percent hatch similar to the unchilled near-wild strain. When held in an outdoor insectary these differences disappeared.

The unchilled Yugoslavia hybrids were found to have longer durations of hatch than that of the unchilled near-wild and unchilled selected strains (Fig. 5) (Marovic 1981). In addition, hybrid eggs from matings using males from the selected strain had a longer duration of hatch than eggs with a near-wild male parent. The hatch from hybrid egg masses began earlier than that from wild egg masses (Fig. 3) but continued well into the period when near-wild egg masses were hatching (Figs. 3 and 5). This would suggest that more than one genotype was present in the hybrids. The presence of more than one genotype could result from a polygenic mode of inheritance or variability in the diapause required for the near-wild strain. The near-wild strains used in both the North American and Yugoslavian reciprocal crosses were of unknown purity with respect to diapause traits, so heterozygous individuals may have been present, which would confound the results of reciprocal crosses and increase the variability between egg masses.

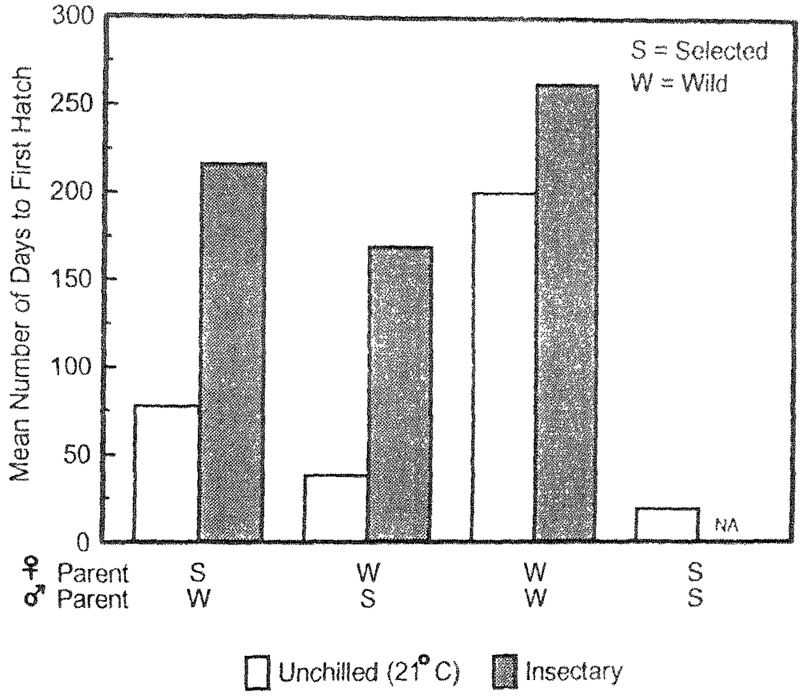

Figure 3.--Mean number of days until the first egg hatched in each egg mass of selected non-diapause, wild-type, and reciprocal-hybrid Yugoslavian gypsy moths that either were unchilled and incubated at 21 °C or held over winter in an outdoor insectary. Data from Marovic 1981.

If the near-wild strain used in the North American studies was not homozygous for diapause traits (which is highly possible), this impurity could have resulted in the environment-specific phenotype expression observed. The very low hatch of near-wild eggs may be evidence of a few heterozygous individuals in the population that would tend to begin hatching in the reciprocal crosses. After the token chill, some of the other genotypes (assuming multiple genes) present in the near-wild strain would have completed diapause but would have required a little longer to hatch, thus appearing to change the dominance of the trait. Similar purity problems with the Yugoslavian near-wild strain may have resulted in invalid conclusions about the inheritance of diapause traits. To clarify this issue, the experiment should be repeated using the nondiapause strain and a strain selected for consistent long diapause.

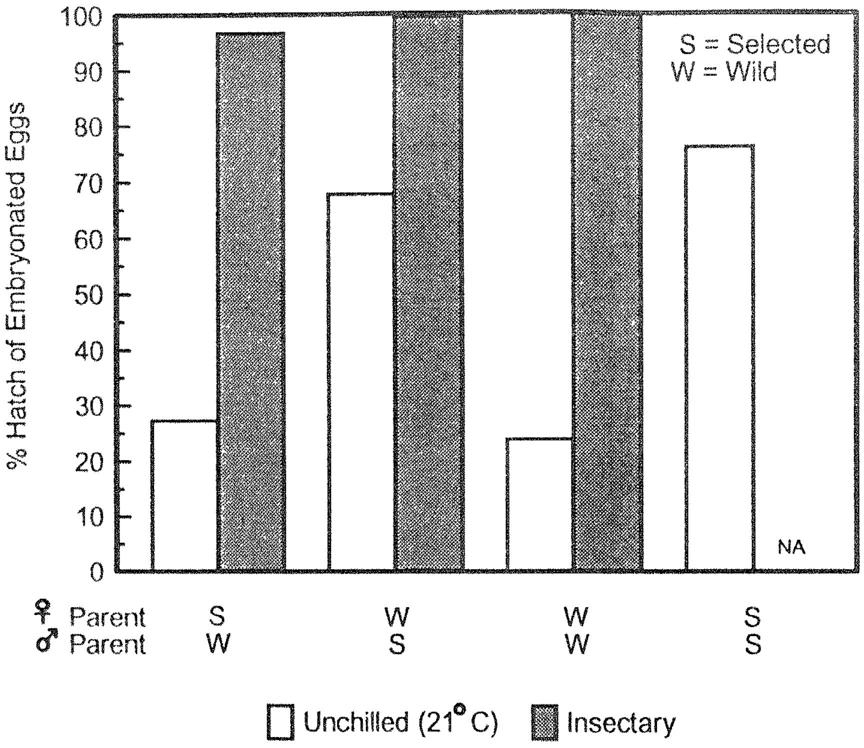

Figure 4.--Mean percentage hatch of eggs of selected non-diapause, wild-type, and reciprocal-hybrid Yugoslavian gypsy moths that either were unchilled and incubated at 21°C or held over winter in an outdoor insectary. Data from Marovic 1981.

Goldschmidt (1934) examined the whole process of diapause and hatch for several geographical races of gypsy moth and made the following observations. First, a constant heat sum was needed for hatching, and this constant varied with race. Second, there is a critical low temperature below which hatching is infinitely slow, and this may differ with race. Third, there is a temperature-independent process that he called "readiness to hatch," which increases with time and can vary with race. Fourth, the temperature sum required during the incubation period decreases with time, which may also vary with race. Goldschmidt (1934) then expressed these observations in a formula for the incidence of hatching time, based upon a temperature-sum concept:

$$\left(v - \frac{k}{S_t}\right) K_R\, t = \text{const.} \qquad (1)$$

v = experimental temperature
k = critical low temperature
t = time of hatching reaction
S_t = readiness to hatch
K_R = a racial constant

Goldschmidt (1934) hypothesized that the racial differences, with regard to days of incubation, were adaptational to the natural environments where the races were found; i.e., that site differences adapt the length of the diapause to the seasonal cycle of the region. Thus, gypsy moth that experience a long winter, a quick onset of spring, and a short period of vegetation would require a comparatively low temperature sum during the incubation period before hatch is initiated, while gypsy moths that experience a mild winter require a higher temperature sum.

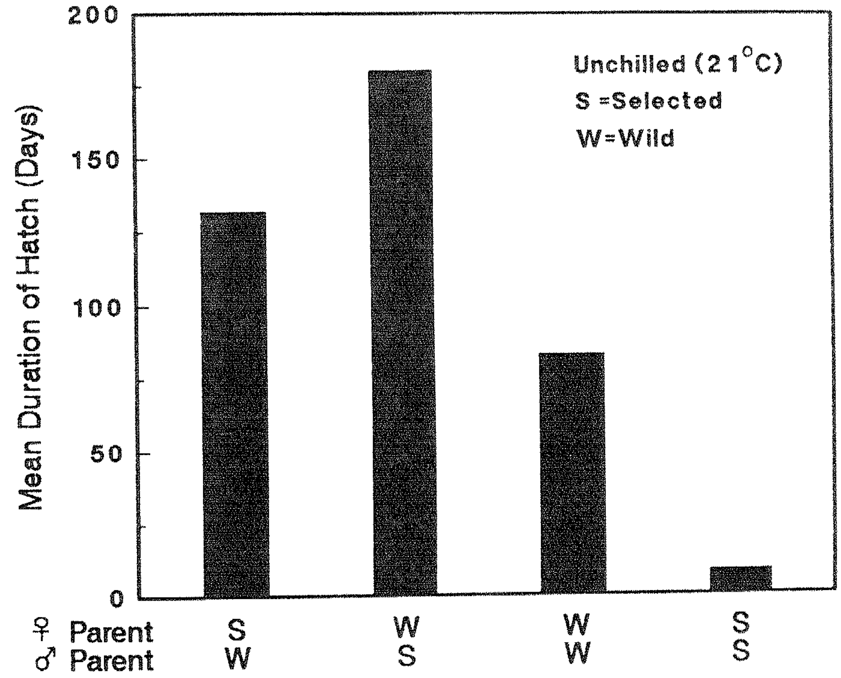

Figure 5.--Mean duration of hatch in days of selected non-diapause, wild-type, and reciprocal-hybrid Yugoslavian gypsy moths that were unchilled and incubated at 21°C. Data from Marovic 1981.

Goldschmidt (1932, 1933) examined crosses between geographical races in the F_1 and F_2 generations and made the following conclusions concerning the inheritance of incubation time: (1) the longer incubation time is incompletely dominant; (2) there is evidence of maternal effects for some crosses; (3) the F_2 distribution was a typical curve for a multiple factor segregation; and (4) that the great similarity of curves in different crosses and their form point to a segregation with few genes, possibly one main gene with multiple alleles combined with some modifiers. An example of the results from one of Goldschmidt's (1934) racial crosses is reproduced in Figure 6.

The New Jersey Standard Strain (NJSS) of gypsy moths produced for research, etc., has been reported to have a less intense diapause than a Japanese strain (Bell 1989; also see Keena and ODell, 1994). Keena (unpublished) reared NJSS and several North

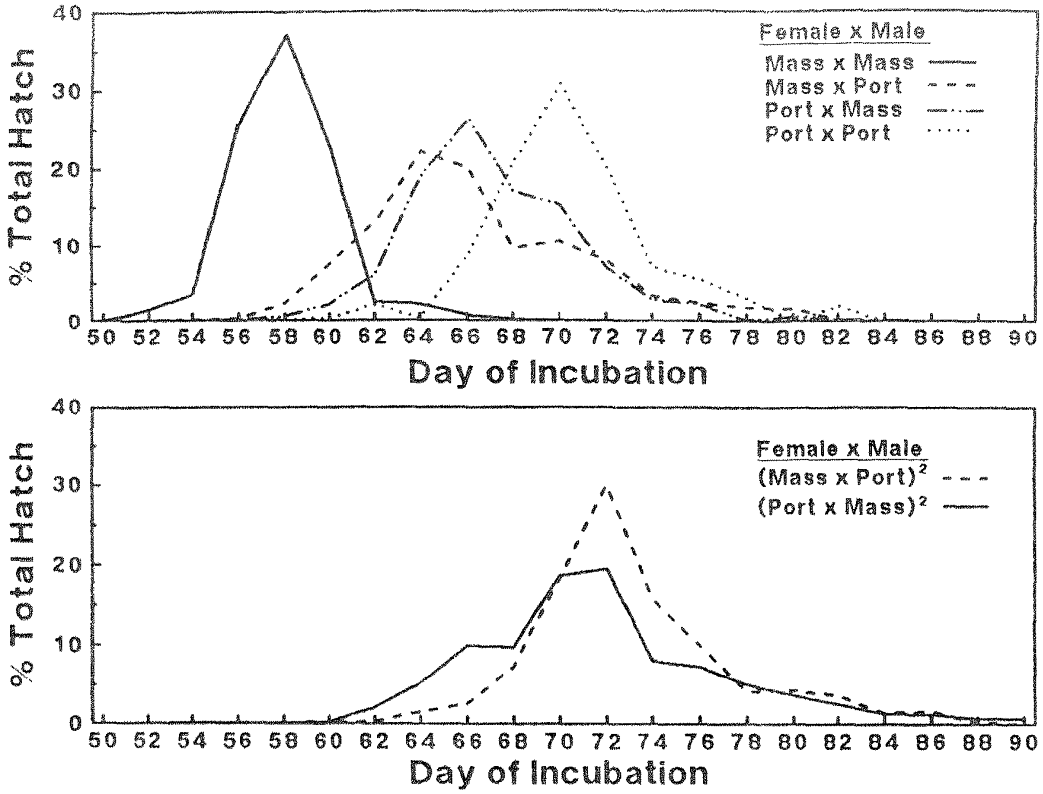

Figure 6.--Inheritance of incubation-time. Curves of incubation time, in days, at 11°C early in winter for the races at Massachusetts, USA, and Portici, Italy, reciprocal F_1, and reciprocal F_2. Data from Goldschmidt 1934.

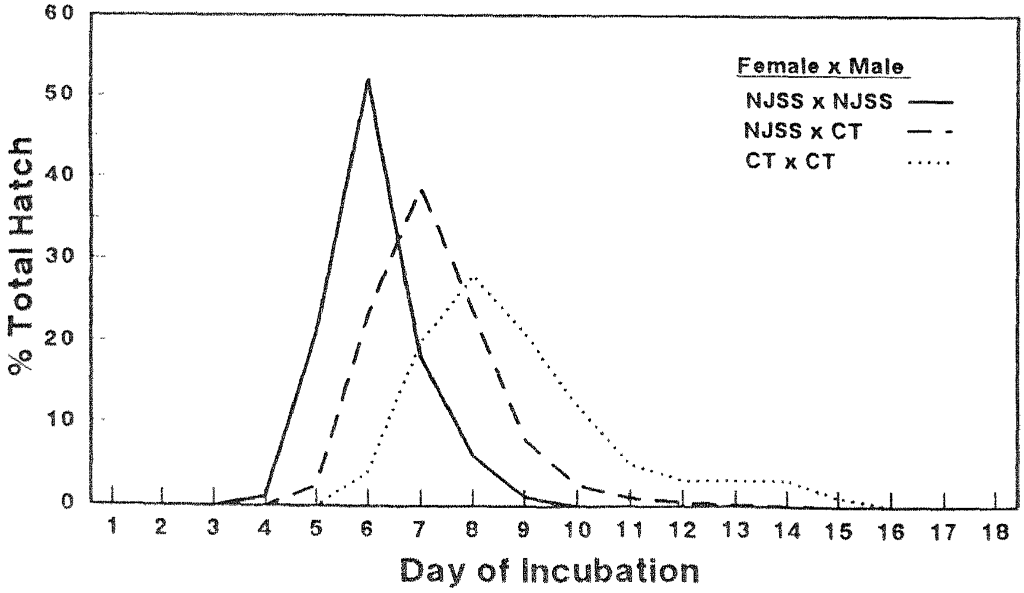

Figure 7.--Inheritance of incubation-time, in days, at 23°C for 16 hours light, and 25°C for 8 hours dark. Egg masses were chilled at 5°C for 150 days prior to incubation. Keena, unpublished data.

American wild strains, and found that NJSS has either a shorter diapause or a lower temperature sum required for hatching as evidenced by a shorter number of incubation days and more synchronous hatch (Fig. 7). When the NJSS is crossed with a North American strain (a strain from Connecticut, for example), the F_1 progeny have an intermediate number of days required for incubation and duration of hatch (Fig. 7) (Keena, unpublished). These results indicate that the incubation time, as determined by diapause traits, either involves several genes or is intermediate (no dominance) for these strains. In general, these results agree with the conclusions of Goldschmidt.

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