Proposal for a
Thesis in the Field of
Sustainability
In Partial Fulfillment of the Requirements
For a Master of Liberal Arts (ALM) degree in extension studies

Tentative Thesis Title
Synchrony of winter moth (*Operophtera brumata*) larval eclosion with different tree species phenology and its effect on defoliation damage in New England

Summary

Forests pests are particularly responsive to disruptions from climate change, rapidly influencing shifts in herbivory intensities, changing their distribution and outbreak frequency, altering their relationships with natural enemies, and generally reducing biodiversity. It is not uncommon for spring defoliating insects to cause the death of host trees or to cause a devastating loss for fruit crops due to the flowers being consumed. One spring feeding insect, winter moth, defoliates newly emerged spring leaves and flowers in a wide variety of deciduous tree species in New England. It has been noted that phenological synchrony between budburst and larval eclosion is critical for the fitness of many spring-feeding insect herbivores, yet it is currently not well understood if the synchrony of winter moth eclosion with host tree bud break influences the feeding habits of the winter moth caterpillar.
To further knowledge about this synchrony, this study will monitor egg hatch timing with different host tree species and categorize the resulting defoliation damage. The main objective is to evaluate how the synchrony of winter moth hatching with tree leaf out affects defoliation damage inflicted upon different host tree species in New England, particularly in the Arnold Arboretum’s woody collection. To accomplish these objectives four hypothesis will be tested: winter moth selects host tree species based upon the synchrony of larval hatching with bud break; delayed hatching of winter moth caterpillars will result in less defoliation damage; eggs laid on rough dark colored bark will hatch earlier then eggs laid on smooth light colored bark; and winter moth caterpillars that have fed on oak leaves will have larger pupae then those who have fed on other tree species.

Refining the measure of egg hatch date variation by taking into account bark type and the directional aspect of the tree trunk (north or south) is a critical analysis needed for other pieces of this study. The analysis of winter moth caterpillars feeding behaviors on host trees in relation to the stage of budburst will be accomplished by observations of tree buds to record budburst stage, number of caterpillars, and percent defoliation throughout the spring season. The quality of food source, determined by leaf maturity and tree species, would significantly affect the winter moth population fluctuations from year to year. This will be evaluated by controlling egg hatch timing and by comparing the weights of pupa that have fed exclusively on particular tree species. The expected data from this study will determine if climate factors that influence egg hatch and bud break will effect which host trees species receive damage, and potentially if the winter moth’s range distribution is likely to change.
Introduction

Research Significance & Objectives

With the changing climate the intensity and severity of insect pests is becoming more of a concern. Insects that were previously not known to cause significant damage to their hosts are becoming more worrisome because they are expanding their area of distribution, escalating herbivory intensities, increasing outbreak frequencies, and modifying their relationship with natural enemies.

The larvae of one such insect, winter moth (*Operophtera brumata*), defoliates newly emerged spring leaves and flowers in a wide variety of deciduous tree species in New England. Examining how winter moth synchrony with bud break affects the extent of defoliation damage will contribute to filling some gaps in knowledge needed to predict economic and ecological influencing factors of winter moth. Monitoring egg hatch synchrony with different host tree species and categorizing the resulting defoliation damage will allow better understanding of how this synchrony influences which tree species receive more damage. Climate change strongly influences tree phenology and therefore, may also affect the extent of damage by winter moth defoliation including possible range of distribution of the moth and which tree species receive the most defoliation damage.

My objective is to evaluate how the synchrony of winter moth hatching with tree leaf out affects defoliation damage inflicted upon different host tree species in New England, particularly in the Arnold Arboretum’s woody collection.
Background

There is increasing worry about the changing climate and apprehension about how it is affecting the environment around us. Invasive species are a major concern particularly when they have devastating effects on the local flora and fauna (Lovett et al., 2006; Logan et al., 2003). There are growing numbers of exotic pests that have created significant negative influences upon eastern North American forests including chestnut blight (*Endothia parasitica*), which has eliminated mature chestnuts (*Castanea dentata*) from American forests; gypsy moth (*Lymantria dispar*), which has caused major defoliation on oak trees in eastern forests; emerald ash borer (*Agrilus planipennis*), which is spreading across the North east destroying ash (*Fraxinus* spp.) trees within a couple of years from infestation; and hemlock woolly adelgid (*Adelges tsugae* Annand), which is spreading across the north eastern forests eliminating Canadian hemlocks (Alalouni et al., 2013; Lovett et al., 2006; Churchill et al., 1964).

Tree defoliating insects (such as gypsy moth) that exhibit severe outbreaks cause short-term effects to forests including reduced productivity of leaf area and seed crop, increased light penetration to the forest floor, and reduced transpiration which consequently will increase the water depletion from forests (Alalouni et al., 2013; Lovett et al., 2006). Repeated defoliations in a forest can considerably raise stream water nitrogen concentrations collected from insect feces, unconsumed green foliage, dead caterpillars, and increased leaching of nitrogen from damaged foliage (Lovett et al., 2006). Many hardwood trees that have been defoliated can regrow their leaves which will allow them to survive several years of spring defoliation without dying (Lovett et al.,
2006; Tikkanen & Julkunen-Titto, 2003). However, if these trees are already stressed from other factors such as drought or other pests they may not be able to survive a single year of defoliation (Lovett et al., 2006).

Climate Change Effects on Insect Pests

Forests pests are particularly vulnerable to disruption from climate change (Logan et al., 2003). Climate change is and will have a variety of influences on insects such as shifts in herbivory intensities, changing distribution and outbreak frequency, changing relationships with natural enemies, and a general decrease in biodiversity (Jepsen et al., 2008; Logan et al., 2003). Plant populations cannot expand their ranges as quickly as insects and therefore, changes in appropriately timed critical life stage events, or phenological synchrony, between herbivores and their host plants may be a significant pathway for climate change to influence insect ecology (Watt and MacFarlane, 2002). Predicting the potential impacts that climate change can have on exotic species is fundamentally more difficult to forecast than for native host-pest interactions (Logan et al., 2003). Unlike agriculture systems where management responses can change host plant assemblages, forest ecosystems fixed in place are far more vulnerable (Logan et al., 2003).

Winter moth (Operophtera brumata) is a prime example of a pest that is being significantly influenced by climate change. In its native Europe it has been expanding its range of distribution and has also been reported to be adding to its list of preferred host species (Jepsen et al., 2008). Historically winter moth has been temperature limited; however, it is now increasing its easterly and northerly ranges in Europe (Jepsen et al.,
In northern Fennoscandia prolonged outbreaks of winter moth have caused forest death over large areas (Tenow & Bylund, 2000).

Winter Moth Introduced to North America

In recent years New England has experienced a series of invading pests such as the Asian Long-horned Beetle, Hemlock Wooly Adelgid, Emerald Ash Borer, and Winter Moth. For more than a decade the invasive European winter moth, *Operophtera brumata* L. has defoliated forests in eastern Massachusetts (Simmons et al., 2014). Winter moth is a relatively recent addition to the list of invading pests of New England and knowledge of its ecological and environmental effects on the forests of this area is sparse (Simmons et al., 2014). Winter moth was first discovered in Massachusetts in 2003 but is presumed to have been present since the 1990s because of corresponding forest defoliation (Elkinton et al., 2010). Winter moth has also been found in Rhode Island, Connecticut, eastern Long Island, New York, New Hampshire, and Maine (Elkinton et al., 2010).

This is not the first time that winter moth has been found in North America as it was discovered in Nova Scotia, Canada in the 1930s and in areas on the west coast in the 1970s (Simmons et al., 2014; Elkinton et al 2015). Nova Scotia suffered major impacts to hardwood forests, British Columbia experienced defoliation to urban shade trees, and Oregon battled the winter moth effects on their orchards (Roland & Embree, 1995). In its native habitat of Europe, this insect is not as destructive as it is in North America where it operates on a cyclic basis resulting in only occasional outbreaks (Simmons et al., 2014).

Factors Influencing Winter Moth Defoliation
In New England’s climate, the longer cold winter temperatures hold the winter moth eggs in dormancy, and then, the warming to spring temperatures allows eggs to hatch very quickly in the spring so caterpillars take advantage of the swelling leaf buds. Extensive damage to the tree occurs when caterpillars are feeding inside the buds prior to leaf expansion (Simmons et al., 2014). Combining this ability with the lack of natural parasitoid predators in New England, there is very little to halt the constant yearly outbreaks of winter moth. The severe defoliation from winter moth in New England can lead to a significant decrease in annual radial growth and also, the potential to change the dynamics of forest composition because of increased light levels where trees have been defoliated (Simmons et al, 2014).

Caterpillar host preferences and the extent of defoliation is closely related to the synchrony (simultaneous action) between egg hatch and budburst (Simmons et al., 2014). In Europe, and possibly in North America also, the variation in budburst (emergence of new leaves) can be as much as two weeks apart for oak species alone (Feeny, 1970). It is not uncommon to see higher caterpillar populations on the earlier bud bursting trees than the later ones, which can allow the later leaf-out trees to escape defoliation (Feeny, 1970). On the other hand, if the winter moth eggs hatch too early before bud break, the delicate first instar larvae will starve to death because they cannot penetrate closed buds (Feeny, 1970). The early egg hatch is likely required for caterpillars to complete their larval cycle before the oak leaves toughen and become a poor food source, before the leaves increase in tannins and decrease in nitrogen and protein (Visser & Holleman, 2000; Feeny, 1970).
Not all tree species break bud at the same time and in fact, within tree species there can be a large variation in phenology (Visser & Holleman, 2000). This synchrony mismatch may be the explanation why winter moth defoliation damage seems to be strongest on particular tree species and among those tree species the extent of damage may vary between different microclimates (Gwiazdowski et al, 2013; Visser & Holleman, 2000).

Climate Effects on Larval Eclosion

The life cycle of most insects, including winter moth, is very sensitive to temperature changes in order to time their life events to that of their preferred hosts (Curry and Feldman 1987). One of the ways to estimate the shifts in insects’ life events is through monitoring growing degree days (GDD). GDD is a calculation based on daily temperature extremes and is often used as a reference to determine when phenological events such as flower timing or insect emergence will occur (Hibbard & Elkinton, 2015). GDD reasonably accurately estimates the rate of development for various insects and there have been a number of studies on the use of GDDs for predicting the synchrony of larval emergence with host tree bud burst (Curry and Feldman 1987; Visser and Holleman, 2001). Winter moth population densities are greatly influenced by this synchrony (Embree, 1965; Jepsen et al. 2009).

Through exposing winter moth eggs to various chilling and warming treatments it has been concluded that the developmental threshold for winter moth is 4°C, eggs chilled below this temperature would hatch at a lower GDD (Kimberling & Miller, 1988). Eggs cultured at 14°C needed 470 (±47) growing degree days for 50% egg hatch but eggs
chilled for two weeks at 1°C only needed 382 (±33) growing degree days and eggs chilled for twelve weeks at 1°C needed even fewer, 156 (±12) growing degree days (Visser and Holleman, 2001; Kimberling & Miller, 1988). Additionally, the date of oviposit has no effect on timing of egg hatch and that there is little to no GDD accumulation effects on the eggs in November and December (Hibbard & Elkinton, 2015). There may be a difference in egg hatch on different tree species because of the thermal boundary layer effects of the bark (Hibbard & Elkinton, 2015). Further, winter moth hatch timing has desynchronized with oak bud burst due to global climate change causing earlier bud break relative to winter moth hatch (Visser & Holleman, 2001).

Winter Moth at the Arnold Arboretum

This study is observing how the synchrony of larval eclosion with host tree bud break will affect which tree species are fed upon and secondly, if this synchrony has any influence on the extent of damage done to these trees species. In turn, this information will help understand if the changes in climate causing desynchronization will affect which tree species that the winter moth defoliates and also how severe this pest will become. The ideal location to perform this study is a site that contains both a collection of different tree species and a large number of same tree species gathered from varying locations to represent the wide range of genetic diversity within a tree species. The Arnold Arboretum is one of these ideal locations as it holds an extensive collection of woody plant species that have been acquired from all over the world where the climate is similar.
The staff at the Arnold Arboretum has been battling the winter moth pest for the past eight years. They use bug barrier traps to monitor winter moth population levels and then use this information to plan spraying schedules in an attempt to minimize winter moth defoliation damage in the core woody plant collections. Although winter moth damage has affected a large variety of woody plant species at the Arnold Arboretum, the Arboretum’s research documentation has not yet adequately compared the extent of winter moth defoliation damage to individual species in relation to timing of bud break and to the correlation of winter moth caterpillar feeding. The probability for introduction of invading insect species, such as winter moth, into new areas of North America is strong enough to justify a substantial effort to fully understand the relationship between the synchronization of herbivore pest and host.

Research Question, Hypotheses and Specific Aims

The overall question to be answered is whether the degree of synchrony of egg hatch with bud break has any influence on the extent of larval feeding damage to host tree species. To evaluate this, four hypotheses will be tested.

- The primary hypothesis is: Winter moth selects host tree species based upon the synchrony of larval hatching with bud break. This test will determine if the tree species that receive the most damage are the same ones that broke bud as the caterpillars were hatching. Different tree species do not break bud at the same time. Even within the same tree species bud break can be separated by many days. In Europe, winter moth caterpillars have evolved to time their egg hatch to match the bud break of oak species to take advantage of fresh young leaves that are high in nitrogen and low in tannins. However, with climate change shifting tree phenology,
the winter moth also appears to be shifting its preferred host species. In New England, winter moths feed upon a large variety of deciduous tree species and it is important to understand how bud break timing influences the species choice that the winter moth feeds upon.

- The secondary hypothesis is: Delayed hatching of winter moth caterpillars will result in less defoliation damage. Since climate change is influencing plant phenology it is also likely that synchrony with winter moth larval eclosion is also affected. Determining if decreased defoliation damage occurs when larval eclosion is delayed is necessary to make informed speculations about the possibility of winter moth becoming more or less of a threat to tree species as climate factors change.

- The third hypothesis is: Eggs laid on rough, dark colored bark will hatch earlier than eggs laid on smooth, light colored bark. Ideally this would be separated into four categories (dark rough, dark smooth, light rough, and light smooth bark) however the trees available to study may limit this range. This hypothesis was developed to refine the measure of egg hatch date variation by taking into account other variables. There is a large variety in bark types in the assortment of tree species that the winter moth caterpillars feed upon. Some trees are covered with smooth light colored bark as in beech and birch species while others have rough dark bark as in oak and apple trees. Since there is a difference in larval eclosion on the south and north facing sides of oak trees by as many as three days it is possible that bark type also influences larval eclosion.

- Finally, the fourth hypothesis is: Winter moth caterpillars that have fed on oak leaves will have larger pupae than those who have fed on other tree species. This hypothesis
was developed to investigate the food source quality obtained from different tree species. Female fecundity (number of eggs produced per female) is correlated with female pupal size or weight (Roland 1995). This knowledge is useful to determine that if certain tree species are fed upon, will this stimulate an increase in population numbers of white moth in the next generation.

Specific Aims

1. Establish when winter moth egg hatch occurs in order to measure hatching synchrony. This can be accomplished through creating a box graph in order to discover the hatch days and exclude outliers.

2. Establish a standard for what bud break entails.

3. Establish a scale to quantify the rate of defoliation damage.

4. Select tree species to collect data from: it is necessary to select a variety of tree species in order to accurately monitor how the winter moth caterpillar will respond to the differences between tree species. The differences between tree species may include bud break timing, leaf thickness, leaf nutrition, size of leaf, and other morphology.

5. Develop a graph or multiple graphs to clearly illustrate tree preference based upon graphing tree bud break and winter moth egg hatch dates.

Methods

Monitoring when the winter moth eggs hatch this spring and quantifying the extent of defoliation will significantly help determine if winter moth egg hatch synchrony with
host tree leaf out is closely correlated to the degree of defoliation caused by the caterpillars. To accomplish this goal, a number of different deciduous tree species at the Arnold Arboretum must be observed throughout the spring season. The tree species selected will be throughout the Arboretum but avoiding collections that may have been exposed to winter moth control treatments. Most of the data collected will be focused on observations of when the eggs hatch and when different tree species break bud, along with periodic examinations of defoliation damage to tree species throughout the spring season. A scale from one to ten must be created to rate levels of damage to these tree species. Some manipulation will be needed to delay the egg hatch timing for a comparison of the extent of defoliation for early and late hatch dates.

Hatch Timing on Different Types of Tree Bark

The first step is to monitor egg hatch timing on selected species of trees with different bark color and texture to determine if bark type is influencing hatch dates. To accomplish this, fifty eggs on the north side and fifty eggs on the south side of a tree trunk will be marked off with tape and number of eggs hatched will be recorded, twice a week beginning mid-March, until all eggs change to a blue color then they will be monitored daily until the eggs have hatched. The tree species selected will be based upon which trees received a bud barrier the previous fall. Bud barriers encourage the female moths to lay eggs in a ring around the trunk before becoming trapped in the barrier. Tree species selected will be Betula pubescens (accession number 669-51-A), Carpinus laxiflora (accession number 908-85-A), and Fagus sylvatica ‘Pendula’ (accession number 14597-A) for their smooth light colored bark and compared to Malus ‘Marshall Oyama’
(accession number 163-52-A), *Quercus velutina* (accession number 329-2015-A), and *Acer saccharum* (accession number 331-2015-A) with their dark rough bark.

Defoliation Synchrony

The presence of winter moth caterpillars on these tree species in relation to the stage of leaf-out is the next set of observations needed. This will be accomplished by cutting three average representative buds off each tree, once a week beginning in late-March and continuing in June until all of the caterpillars have pupated. Ideally ten different tree species with three replications of each different tree species will be chosen giving a sample size of thirty trees. The ten tree species chosen will likely be two different species each of the following genera: *Acer, Carpinus, Fagus, Malus,* and *Quercus.*

Three terminal buds from each tree will be collected three times over a ten week period: when bud leaves are half way emerged, when bud leaves are fully emerged, and again in late May before caterpillars pupate. These buds will then be placed in ethanol to preserve them for analysis where each bud will be dissected in order to count the caterpillars present. The overall tree bud leaf-out stage will be recorded on a scale from one to ten based on the percent of leaf consumed (see diagram 1) and the number of caterpillars found per bud will also be documented. The bud break timing and egg hatch timing will be graphed with the defoliation damage to create a humpback curve that will illustrate the relationship between the tree species bud break and that amount of defoliation they received.
Additionally, in order to help minimize sampling variation that could occur when sampling small buds that may have large variance, a secondary quantification of caterpillar densities will be undertaken in May when the caterpillars are larger. A branch of similar size and same bud numbers from each tree will be shaken with a pole allowing the caterpillars to fall onto a tarp that was spread out below the branch. The number of caterpillars on the tarp will be recorded and used to create a second humpback graph.

Defoliation with Delayed Hatching

Monitoring egg hatch synchrony with host tree leaf out can be accomplished by securing a mesh bag over the end of a tree branch encompassing at least a foot in branch length immediately after 3 caterpillar eggs per bud yielding about 75 eggs per mesh bag will be placed on the branch tip. Eggs have been collected from tree bug barriers that
were placed on a variety of trees at the Arnold Arboretum last fall. A better collection method to adequately randomize the bud selection needs to be developed.

After the buds break and the harvesting of buds changes to the harvesting of leaves, there must also be steps taken to harvest only the leaves that would have emerged from the terminal buds. However, the main concern or limitation for this study is the potential for large amounts of data variation between samples on the same tree species. To avoid this problem, the sample size may need to be more than the proposed three buds harvested per tree.

To carry out the defoliation with delayed egg hatching trial, an extremely fine mesh bag that is secured to the tree branch in such a way as to prevent any caterpillars from escaping particularly in the early instar stages. The mesh bags will be placed on the trees before bud burst to ensure that no wild winter moth larvae interfere with the study. Additionally, there may need to be more than two cohorts of caterpillar hatch dates to obtain meaningful data. More research needs to be completed on this topic. Also, more than two petri dishes of eggs to be hatched should be prepared as backups in case of unforeseen variables causing hatch failure. Consideration of adding additional trees species such as beech to the trial may provide interesting data as well. If possible it would be beneficial to conduct a pilot trial in late winter to ensure the fine mesh bag will not let the first instar larvae escape. This can be accomplished by placing winter moth eggs on cut branches in a greenhouse in early March to trigger an early hatch date.

It would be ideal if the selected branches for the delayed egg hatching trial have enough foliage for the caterpillars to complete their feeding process and pupate. However, if this is not possible those caterpillars will be collected immediately if they have consumed their food source and then placed in a five gallon bucket with an inch of
sphagnum moss and a mesh lid. Then the caterpillars can be continually provided with fresh leaves from the same tree they were collected from until they complete their caterpillar life stage. This will provide the caterpillars for the fecundity study.

Overall the trees selected for trials must be chosen carefully to eliminate risk of tampering from the public or risk of receiving insecticides that would alter results.

Expected Results

The first set of results are necessary to determine if the tree bark type has any influence over winter moth egg hatch timing since this may affect which host trees receive the initial feedings from the caterpillars. It is expected that eggs on dark bark facing south will be the first to hatch since they will receive more heat from the sun than eggs on north facing light colored bark trees. If the results do not support this then the bark color and exposure to sunlight does not need to be taken into account when looking at the synchrony of tree bud break with caterpillar hatch timing.

The observations of percent defoliation and on the population changes of winter moth on the different tree species throughout the spring season should help determine if the caterpillars can change their host tree species to optimize food source. It is expected that the tree species that break bud when the caterpillars are hatching will receive the most defoliation damage. It is also possible that trees that broke bud before the egg hatching will receive more damage. It is also possible that particular tree species receive extensive defoliation regardless of when they break bud. The extent of caterpillar feeding damage may be strongly influenced by tree species, bud break synchrony, or both.
These observations, along with the data collected on how the age and species of the caterpillar’s food source affects amount of defoliation and caterpillar fecundity, should help understand if there is a possibility of distribution shifts with climate change. If the caterpillars perform equally well on different tree species it could be theorized that tree species is not a limiting factor for their distribution. If the winter moth caterpillars perform significantly poorer (lower defoliation rates and lower pupal weights) when their synchrony of egg hatch is after bud break then it could be theorized that the winter moth performance is closely related to this synchrony and with climate change influencing the phenology of trees it is possible that winter moth distribution will also be influenced.

Glossary

Defoliation - To strip, destroy, or cause loss of leaves from plants.

Eclosion - The emergence of an insect larva from an egg.

Fecundity - Number of eggs laid per female.

Instar - a phase between periods of molting in the development of an insect larva.

Phenology - The study of periodic plant and animal life cycle events and how they are influenced by seasonal and inter-annual variations in climate as well as habitat factors.

Synchrony - The functioning or developing of something according to the same time scale as something else.

Bibliography


**Timeline**

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