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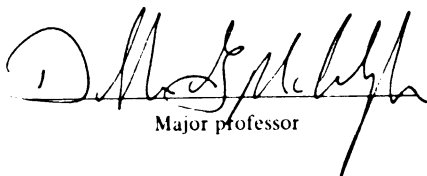
**ALTERNATIVE CONTROLS FOR TWO IMPORTANT
INSECT PESTS OF CHRISTMAS TREES**

presented by

Kirsten Marie Fondren

has been accepted towards fulfillment
of the requirements for

M.S. degree in Entomology


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**ALTERNATIVE CONTROLS FOR TWO IMPORTANT INSECT PESTS OF
CHRISTMAS TREES**

By

Kirsten Marie Fondren

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

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ABSTRACT

ALTERNATIVE CONTROLS FOR TWO IMPORTANT INSECT PESTS OF CHRISTMAS TREES

By

Kirsten Marie Fondren

Balsam twig aphid (*Mindarus abietinus* Koch) and pine needle scale (*Chionaspis heterophyllae* (Cooley)) are two important pests of Christmas trees that are typically controlled with broad-spectrum insecticides. Our objectives were to evaluate potential alternative control methods for each insect and describe their biology on Michigan Christmas tree plantations. The phenology of both insects was related to accumulated degree-days base 50°F. Balsam twig aphid began to hatch at approximately 70 DD₅₀, and produced the second generation at approximately 150 DD₅₀. Flushing time of the host plant had a significant effect on aphid damage levels. Larvae of *Chrysoperla rufilabris* Burmeister reduced the population of *M. abietinus* when applied in the field. Economically, *M. abietinus* damage did not affect retail or wholesale value until it reached approximately 30%. The second generation of pine needle scale hatched at approximately 1280 DD₅₀ and continued for three weeks. The coccinellids *Chilocorus stigma* (Say) and *Microweisia misella* (LeConte) were important scale predators. We tested horticultural spray oil and found it worked as well as an organophosphate insecticide when applied at the peak of the second instar, or approximately 1500 DD₅₀.

To Deb

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TABLE OF CONTENTS

LIST OF TABLES.....	ix
LIST OF FIGURES	xi
 CHAPTER 1	
PHENOLOGY AND IMPACT OF THE BALSAM TWIG APHID (<i>MINDARUS ABIETINUS</i> KOCH) (HOMOPTERA: APHIDIDAE) ON FIR CHRISTMAS TREE PLANTATIONS	
Abstract	1
Introduction.....	2
Methods.....	5
Study sites	5
Balsam twig aphid phenology.....	6
Tree phenology	9
Balsam twig aphid damage	12
Economic impact	12
Statistical Analysis.....	14
Results.....	15
Balsam twig aphid phenology.....	15
Tree phenology	18
Balsam twig aphid damage	22
Economic impact	24
Discussion	26
Literature Cited	31
Tables	35
Figures.....	39
 CHAPTER 2	
POTENTIAL FOR AUGMENTATIVE BIOLOGICAL CONTROL OF THE BALSAM TWIG APHID (<i>MINDARUS ABIETINUS</i> KOCH) (HOMOPTERA: APHIDIDAE) IN MICHIGAN CHRISTMAS TREE PLANTATIONS.....	
Abstract	50
Introduction.....	51
Methods.....	54
Study sites	54
Objective 1. Natural enemy complex.....	54
Objective 2. Evaluation of <i>C. rufilabris</i> in lab and field cages.....	57
Objective 3. Field test of <i>C. rufilabris</i>	59
Statistical Analysis.....	61
Results.....	61
Objective 1. Natural enemy complex.....	61
Objective 2. Evaluation of <i>C. rufilabris</i> in lab and field cages.....	64

Objective 3. Field test of <i>C. rufilabris</i>	65
Discussion	67
Objective 1. Natural enemy complex.....	67
Objective 2. Effectiveness of <i>C. rufilabris</i> in lab and field cages	68
Objective 3. Open field release of <i>C. rufilabris</i>	70
Practical applications	71
References Cited	73
Tables	78
Figures.....	82

CHAPTER 3

PHENOLOGY AND NATURAL ENEMIES OF THE PINE NEEDLE SCALE (*CHIONASPIS PINIFOLIAE* (FITCH))(HOMOPTERA: DIASPIDIDAE) IN CHRISTMAS TREE FIELDS

Abstract	90
Introduction.....	91
Materials and Methods.....	95
Field sites	95
Objective 1. Phenology of the second generation.....	96
Objective 2. Natural enemies.....	97
Objective 3. Rates of predation and parasitism	98
Statistical Analysis.....	98
Results.....	99
Objective 1. Phenology of the second generation.....	99
Objective 2. Natural enemies.....	101
Objective3. Rates of predation and parasitism	102
Discussion	103
References Cited	107
Tables	111
Figures.....	121

CHAPTER 4

POTENTIAL EFFICACY OF HORTICULTURAL OIL FOR CONTROL OF PINE NEEDLE SCALE (*CHIONASPIS PINIFOLIAE* (FITCH)) (HOMOPTERA: DIASPIDIDAE) IN CHRISTMAS TREE FIELDS

Abstract	123
Introduction.....	124
Materials and Methods.....	128
Study sites	128
Objective 1. Phenology of the second generation.....	129
Objective 2. Efficacy of horticultural oil	130
Objective 3. Effectiveness of commercial application	132
Statistical Analyses	133
Results.....	133
Objective 1. Phenology of second generation scales	133
Objective 2. Efficacy of horticultural oil	134

Objective 3. Effectiveness of commercial application of horticultural oil	135
Discussion	136
References Cited	141
Tables	145
Figures.....	149
APPENDIX 1. RECORD OF DEPOSITION OF VOUCHER SPECIMENS	155

LIST OF TABLES

Chapter 1.

Table 1. Correlations of density of <i>Mindarus abietinus</i> fundatrices with density of sexuparae in subsequent weeks.....	35
Table 2. Comparisons between early and late budbreaking trees in Ingham County. Aphid numbers found on each group were compared using pooled t tests or Welch tests. Amount of aphid damage (percentage of shoots damaged) sustained by each group of trees was compared using a Welch test.....	37
Table 3. Results of chi-square tests of customer preference, 1999 surveys	38

Chapter 2.

Table 1. Potential predators of <i>M. abietinus</i> collected in the field in 1999 by hand or beat samples, from 14 April to 30 June	78
Table 2. Adult predators found on the sticky traps in each field, 2000	79
Table 3. Species of Coccinellidae found on sticky traps in each field, 2000	81

Chapter 3.

Table 1. Study sites used in 1999, 2000 and 2001 for pine needle scale.....	111
Table 2. Number of eggs present under female scale armor during oviposition and hatching period.....	112
Table 3. Pine needle scale phenology, Van Buren County, 1999 (means $\pm$ 1 SE where applicable)	115
Table 4. Pine needle scale phenology, Montcalm County, 1999 (means $\pm$ 1 SE where applicable)	116
Table 5. Pine needle scale phenology 2000 and 2001	117
Table 6. Parasitoids recovered from scale mummies in 2000	118

Table 7. Percent predation of pine needle scale in each life stage, Van Buren County 1999. See Table 3 for the percentage of the population that was in each life stage on each sample date	119
Table 8. Percent predation of pine needle scale at each life stage, Montcalm County, 1999. See Table 4 for the percentage of the population that was in each stage on each sample date	120

Chapter 4.

Table 1. Varieties, ages and numbers of trees used in each trial	145
Table 2. Number of eggs present under female scale armor during oviposition and hatching period.....	146

LIST OF FIGURES

Chapter 1.

- Figure 1. Percentage of *M. abietinus* eggs hatched in Ingham County in March 2000 (number hatched/100 eggs counted). Cumulative degree days base 50 degrees F (10 degrees C) are indicated for 21 March, 23 March, and 30 March.....39
- Figure 2. Phenology of the first and second morphs of *M. abietinus* in 2a) Ingham and 2b) Grand Traverse Counties, 1999.....40
- Figure 3. Phenology of the first and second morphs of *M. abietinus* in 3a) Ingham, 3b) Grand Traverse, and 3c) Antrim Counties, 2000.....41
- Figure 4. Phenology of the first and second morphs of *M. abietinus* in 4a) Ingham, 4b) Grand Traverse, and 4c) Antrim Counties, 2001.....42
- Figure 5. Percentage of damaged shoots vs. approximate date of budbreak, Ingham Co., 2000 (means +/- SE).....43
- Figure 6. Percentage of shoot expansion in early spring for paired early and late budbreaking trees in Ingham County (n = 12 trees). Pearson's product-moment correlation analysis: $p < 0.0001$, $r = 0.92$44
- Figure 7. Percentage of shoot expansion in 1999 and 2000 for ten randomly selected trees in Ingham County. Pearson's product-moment correlation analysis: $p < 0.0001$, $r = 0.97$45
- Figure 8. Percentage of shoot expansion in early spring for the aphid sample trees in Ingham County (n = 30 trees measured in both years). Seven trees were excluded because of missing values. Spearman's rank correlation analysis: $p = 0.12$, $r_s = 0.33$46
- Figure 9. Results of 1999 retail customer surveys. The y-axis indicates the number of customers who stated that they would consider buying each tree. Within each height group, damage was a significant factor (see Table 3) unless marked with n.s.....47
- Figure 10. Results of 2000 customer surveys (n = 50 customers). Trees were grouped into three categories based on the percentage of damaged shoots/ 300 shoots examined per tree. The percentage of damaged shoots is presented as the actual value. Damage categories are

indicated by 'Light' (n = 3 trees), 'Medium' (n = 2 trees), and 'Heavy' (n = 3 trees). The percentage of change in perceived value is presented as mean $\pm$ 1 SE.....48

Figure 11. Wholesale grades assigned to trees varying in aphid damage used in 1999 survey (n = 18). Percentage of damaged shoots is presented as mean $\pm$ SE. No significant differences were found ($F = 1.38$; $df = 2,15$; $p = 0.28$).49

Chapter 2.

Figure 1. *Mindarus abietinus* and syrphid larvae counts in clipped branch tip samples, 199983

Figure 2. Numbers of coccinellids, lacewings, and syrphids found in beat and visual examinations of sample trees, 1999. In Antrim Co., no survey was done on 2 June because of rain. On 15 June, a survey was done but no predators were observed.....84

Figure 3. Predators found in beat samples, 2000. Data are presented as the number of each taxon collected per sample date.....85

Figure 4. Number of *M. abietinus* eggs per cm of current-year shoot growth (mean $\pm$ 1 SE) on branch tips from field cages in 2000. A) Heavily infested branches (n = 26). B) Moderately infested branches (n = 23)....86

Figure 5. Mean ($\pm$ 1 SE) number of aphid eggs/cm² of current-year foliage in 2001 field cages. Letters above bars indicate significant differences. A) 'closed' cages were kept closed before lacewings were added; B) 'open' cages had one end left open enabling access by predators until lacewings were added.....87

Figure 6. Mean ($\pm$ 1 SE) aphid counts before and after the lacewing applications in the open field releases in 2001. Data within a column marked with different letters are significantly different ($p = < 0.05$).....88

Figure 7. Mean ($\pm$ 1 SE) density of *M. abietinus* eggs on new foliage in open releases of *C. rufilabris* larvae in 200189

Chapter 3.

Figure 1. Mean ($\pm$ SE) number of eggs present beneath the female scale armor in 2000 in the Van Buren and Ingham County fields on each sample date...121

Figure 2. Number and stage of *Chilocorus stigma* and *Microweisla misella*

observed in our 1999 field study. The total number of each observed on ten trees in Van Buren County and six trees in Montcalm County.....122

Chapter 4.

- Figure 1. Percentage of scale population in the 2nd instar, or hyaline stage, in each field. A) Montcalm and Van Buren Counties, 1999. B) Ingham and Van Buren Counties, 2000 and 2001. Dotted lines indicate approximately 1500-1600 degree days. Data were not available for every sample date149
- Figure 2. Mean ($\pm$ SE) pine needle scale mortality in the Van Buren County field in 2000. Treatments were applied on 25 July after pretreatment samples were taken. Significant differences among treatment groups on each date are indicated by different letters. N = 10 trees per treatment.....150
- Figure 3. Mean ($\pm$ SE) pine needle scale mortality in the Ingham County field in 2000. Treatments were applied on 31 July 2000 after pretreatment samples were taken. There were no significant differences among treatment groups on any date. N = 10 trees per treatment.....151
- Figure 4. Pine needle scale mortality in Van Buren County, 2001. Treatments were applied on 6 August 2001. Means were separated with Fisher's LSD where the global ANOVA was significant ($p < 0.05$). Means with different letters are significantly different. Data are presented as mean $\pm$ 1 SE152
- Figure 5. Percentage of mortality (mean $\pm$ 1 SE) in the Montcalm County field in 2001. Treatments were applied on 17 May and repeated on 29 May 2001. There were no significant differences among treatment groups (Kruskal-Wallis; $n = 4$) except on 4 June. Significant differences on 4 June are marked with different letters.153

Chapter 1

Phenology and Impact of the Balsam Twig Aphid (*Mindarus abietinus* Koch) (Homoptera: Aphididae) on Fir Christmas Tree Plantations

Abstract. The balsam twig aphid (*Mindarus abietinus* Koch) is a major insect pest of balsam and Fraser firs grown for Christmas trees. In this study, our objectives were to: 1) monitor the phenology of *M. abietinus* in fir plantations; 2) assess relationships among aphid density, tree phenology, and damage to tree foliage; and 3) develop an aesthetic injury level for *M. abietinus* on Christmas trees. We monitored the phenology of *M. abietinus* and fir trees on three commercial Christmas tree plantations in central and northern Lower Michigan for three years (1999-2001). Phenology of *M. abietinus* was strongly correlated with accumulated degree-days base 50°F (10°C). The first generation matured at approximately 150 DD₅₀ and the second generation began to occur at approximately 150-200 DD₅₀. In each year, trees that broke bud approximately one week later than most other trees in the same field escaped aphid damage. The rate of shoot expansion in early spring was often positively correlated with the amount of aphid damage. We surveyed retail customers at a choose-and-cut plantation in two years to determine the level of aphid damage that affected the retail value of trees. Customers did not consistently differentiate between trees with light or moderate damage. Very heavy damage (mean of 50% damaged shoots) did affect customer perception. Wholesale grades were assigned to specific trees with varying levels of *M. abietinus* damage. Light

to moderate aphid damage (less than 50% of shoots with affected needles) was not a critical factor in customer choice or wholesale grade.

Introduction

Christmas tree production is a major agricultural industry in Michigan. Roughly 21,853 ha (54,000 acres) in Michigan are in commercial Christmas tree production, and approximately 3.2 million trees are sold annually, with sales totaling \$42.5 million in 1999 (MI Ag. Stat. Serv. 2000). Managing and preventing insect damage is critical for maintaining the aesthetic and economic value of Christmas trees. Currently, insect pest management in Christmas tree plantations is based primarily on use of broad-spectrum insecticides (McCullough and Fondren 1998) and relatively little research has addressed alternative controls. Changes in pesticide availability resulting from the federal Food Quality Protection Act of 1996 may have major impacts on the availability of broad-spectrum insecticides for minor-use commodities such as Christmas trees (DiFonzo and McCullough 1998).

Production of fir Christmas trees such as balsam fir (*Abies balsamea* (L.) Mill.) and Fraser fir (*A. fraseri* (Pursh) Poir.) has increased dramatically in recent years. The area of Fraser fir planted in Michigan has increased by 92% since 1994 (MI Ag. Stat. Serv. 1998). The high economic value of firs (US \$34-45 retail, \$13-19 wholesale) compared to more traditional species such as Scotch pine (US \$21 retail, \$8 wholesale) is increasing the interest in growing firs and, correspondingly, in pest management in fir

plantations (MI Ag. Stat. Serv. 2000). Fraser fir is one of the most profitable Christmas tree species produced in Michigan (Jones et al. 1999).

The balsam twig aphid is a major pest affecting balsam and Fraser firs, especially when grown as Christmas trees (Kleintjes 1997a, 1997b, Nettleton and Hain 1982, Bradbury and Osgood 1986). Fraser and balsam fir Christmas trees have relatively few insect pests other than *M. abietinus* (Räther and Mills 1989, McCullough et al. 1998). In a 1998 survey of Michigan Christmas tree growers, more insecticide sprays were used on firs for control of balsam twig aphid (*Mindarus abietinus* Koch) than for any other pest (McCullough and Fondren 1998).

The life cycle of *M. abietinus* typically begins before budbreak in early spring, when stem mothers hatch from the overwintering eggs. They do relatively little feeding and cause little if any damage to the tree (Varty 1966). At maturity they viviparously produce the second generation (sexuparae), which form colonies that feed on sap in newly expanding needles (Varty 1966, Bradbury and Osgood 1986, Kleintjes 1997a). Feeding by the sexuparae causes most of the needle damage. The second generation typically matures into alate (winged) females, which disperse and produce the final generation of males and oviparous females. A small percentage (6% in Varty 1966) of the second generation does not develop wings and produces more sexuparae, adding another generation of parthenogenic females that will become alates (Varty 1966, Nettleton and Hain 1982). In either case, the cycle is completed by mid-June to mid-July (Räther and Mills 1989).

High populations of *M. abietinus* sexuparae cause current-year needles to become curled and distorted, reducing needle biomass and consequently tree growth (Saunders

1969, Bradbury and Osgood 1986, DeHayes 1981, Berthiaume et al. 2000). However, trees can outgrow up to 55% of the aphid damage by the end of the season, and typical shearing practices remove some of the damage (Nettleton and Hain 1982). Failure to account for these factors can lead growers to overestimate the amount of aphid damage that will be present at harvest time.

Insecticide sprays are often applied only after damaged shoots are observed (Kleintjes 1997a, 1997b). Once the second generation (sexuparae) appears, however, it is too late to apply control measures, because the aphids are protected within the new growth and damage has already occurred (Kleintjes 1997a, Berthiaume et al. 2001). Application of insecticides at this time may kill some aphids and their natural enemies, but does not reduce needle damage (Kleintjes et al. 1999).

Host plant resistance to *M. abietinus* has been suggested on the basis of budbreak date (Desrosiers 1998, Carter and Nichols 1985). Most observers suggest that if the trees have not yet broken bud when the sexuparae begin to feed, they will not have new nutrient-rich growth to feed on and may not survive. The role of genetic control in the date of budbreak, or initiation of shoot growth in early spring, may be used to selectively propagate resistant cultivars. Other factors that may indicate potential host plant resistance include monoterpene levels (DeHayes 1981) and the provenance of the seed source (DeHayes 1981, Mattson et al. 1989).

Several authors have reported that *M. abietinus* reduces the economic value of Christmas trees, but data supporting this observation is scarce (Saunders 1969, Bradbury and Osgood 1986, Kleintjes 1997a, Kleintjes et al. 1999). The exact amount of *M. abietinus* damage that results in economic loss has not been determined, although other

workers have estimated it (Kleintjes et al. 1999). To identify an economic or aesthetic injury level, the relationships among *M. abietinus* numbers, the amount of tree damage, and the resulting loss of economic value need to be defined (Raupp et al. 1988, Pedigo et al. 1986). Developing an action threshold would be an important addition to improving *M. abietinus* management.

Our objectives in this study were to: 1) monitor the phenology of *M. abietinus* in fir plantations; 2) assess relationships among aphid density, tree phenology, and damage to tree foliage; and 3) develop an aesthetic injury level for *M. abietinus* on Christmas trees.

Methods

Study sites. We monitored the phenology of *M. abietinus* and associated host trees in three commercial fields in northern and central Lower Michigan in 1999-2001. Fields were located in Ingham County (42°44'N, 84° 33'W), Grand Traverse County (44°32'N, 85°31'W), and Antrim County (44°59' N, 85°06'W), and consisted of either balsam fir or a mixture of balsam and Fraser fir. In 1999, the trees in each field were approximately 8-9 years old. No insecticides, fertilizers or irrigation were used in any field during our study. All fields were planted to a standard 1.83 m by 1.83 m (6 ft by 6 ft) spacing. The Ingham County field vegetation was dominated by herbaceous plants including crabgrass (*Digitaria* spp.) and poison ivy (*Rhus radicans*). The Grand Traverse County field was located on sandy soil with ground cover such as mosses and herbaceous

plants including *Fragaria virginiana* and *Rubus* spp. In Antrim County, the plantation had a thick ground cover composed primarily of crabgrass (*Digitaria* spp.) and other grasses (*Setaria* spp., *Bromus* spp., *Echinochloa* spp., etc.).

Balsam twig aphid phenology. Our initial goal was to monitor the phenology of *M. abietinus* development weekly in each field and year, beginning with egg hatch in March and continuing until the end of oviposition in late June or early July. However, logistics made complete observations impossible for every field and year, so we focused on the aspects of *M. abietinus* phenology that have the most bearing on timing control measures, i.e. the timing of egg hatch, the duration of the first generation (stem mothers) and the beginning of the second generation (sexuparae).

Egg hatch. In 1999, we began to monitor the aphid population after hatching had already occurred, so the exact duration of egg hatch could not be recorded. However, the aphids were still in the first generation (stem mother) stage when monitoring began, so we could estimate the date and degree-day accumulation where egg hatch had been completed. In the Ingham County field in 2000, we were able to monitor egg hatch daily from 21 March (74 DD₅₀) to 30 March (96 DD₅₀) by counting the percentage of egg hatch (hatched eggs/100 eggs) on branch tips clipped at random from the midcrown level of ten trees throughout the field. Egg hatch could be determined in the laboratory by examining the branch tips under a microscope and looking for flattened or torn chorions. Unhatched eggs were turgid and would leak when poked with a minuten pin. In Grand Traverse County and Antrim County in 2000, we determined the percentage of egg hatch on 28 March only (50 DD₅₀). Egg hatch was not monitored in 2001.

First and second generations. In 1999, we selected 40 trees in each of the Ingham, Grand Traverse, and Antrim County fields in early spring (13 April in Grand Traverse County, 15 April in Ingham County, 20 April in Antrim County) on the basis of aphid damage from the previous season, to use for monitoring the phenology of the aphid population. These 40 trees were tagged and used throughout the three years of the study for aphid sampling. In 1999, ten of these trees were randomly selected each week and two branch tips were clipped on either side at midcrown level. The midcrown level was chosen because *M. abietinus* tends to colonize the midcrown level most consistently (Nettleton and Hain 1982, Varty 1966). Clipped branch tips were transported to the lab in coolers and placed in 70% ETOH. All branch tips were dissected under a microscope in the laboratory to remove all aphids (per Varty 1966, 1968). Although this method allowed us to get a close approximation of the actual numbers of aphids on a given branch tip, it was time-consuming (aphid numbers could exceed 600 per branch tip), destructive, and could be problematic in active Christmas tree plantations where shape and fullness are critical. Therefore, we decided to use an additional sample method that could be used each week on the same trees without being destructive.

In May 1999, we developed a non-destructive method (the ‘beat method’, also used by Kleintjes et al. 1999) to sample aphids from a random sample of ten trees in each field, separate from the 40 trees we had already selected. We used a separate sample population so branch tips would not be clipped off during the season. For this sample method, we randomly selected ten trees in each field by walking in diagonal transects across the field and marking a tree at 20 m intervals. These trees were tagged and given a unique identification number to be used in all three years of the study. On these ten trees,

aphids were sampled weekly. On the north and south sides of each tree at mid-canopy level, we rapped the foliage three times with a dowel and counted aphids falling onto black cloth held in an embroidery hoop 22.9 cm in diameter. In 1999, these ten trees were also used to monitor the rate of shoot expansion (see tree phenology section). In 2000 and 2001, we expanded this sample method from ten trees to include all 40 sample trees in each field. For all data analyses, the sum of the aphid numbers found on the north and south sides was used as the variable representing aphid numbers per tree.

In 2000, aphid density was sampled weekly on all trees ($n = 50$ per field) using the 'beat method'. All aphids collected in the field were placed using a fine camelhair brush into microfuge tubes filled with 70% ETOH. During the winter, aphids were identified in the laboratory to instar (after Varty 1968).

In 2001, we focused on monitoring aphid phenology intensively only through the beginning of the second generation, which was most closely tied to our objective of determining an action threshold. Using Taylor's Power Law (Hayek and Buzas 1997), we determined that sampling approximately 30 trees would provide adequate information. To end up with a sample size of 30, we selected 20 trees at random from our original sample population, and also used the ten trees that had been used for 'beat samples' in 1999. Trees were sampled weekly in the same manner as in 2000. Because collecting all of the aphids on each sample was time consuming, we collected aphids from only the first 20 trees in the field, or until ≥ 100 aphids had been collected. In the laboratory, aphids were examined under a microscope and identified to instar to determine phenology. Representative samples of *Mindarus* were identified by D. Voegtlin at the Illinois Natural History Survey. Voucher specimens were deposited in the

A.J. Cook Arthropod Research Collection at Michigan State University, voucher no. 2002-01.

Cumulative degree-days from the nearest weather station to each study site were obtained weekly from the Michigan State University Agricultural Extension website. Lansing was used for the Ingham County field, Kalkaska and Lake City were used for the Grand Traverse County field, and Kalkaska was used for the Antrim County field. Cumulative degree-days expressed as base 50°F (10°C) were used because degrees Fahrenheit are more accessible and familiar than degrees Celsius to growers and extension personnel in the United States (Mussey and Potter 1997, Herms 1990, Pruess 1983). Degree-days are abbreviated here as DD₅₀.

Tree phenology. Our goal in monitoring tree phenology was to determine whether the timing of bud break affected susceptibility to aphid damage. Two methods to measure tree phenology were used: date of budbreak and rate of shoot expansion. However, the exact date of budbreak could not be monitored for all fields each year because it would have required us to examine each tree daily. Our fields were separated by a three-hour drive, which made that impractical. Also, since the buds on a given tree break over a period of several days, an exact 'date of budbreak' is a relatively subjective measure. To represent the date of budbreak, we used the sample date when, on average, 50% of the buds had broken on each tree.

In the Ingham County field, ten pairs of early and late budbreaking trees were selected in early May 1999, when it was apparent that some trees had not yet broken bud while others had completed budbreak and new shoots were beginning to expand. The trees in each pair were adjacent to each other, to ensure that they were subject to similar

soil temperatures, microclimate, and other factors. These trees were monitored in each year to determine if the relative order of budbreak and shoot expansion remained similar between years.

In Grand Traverse County, we identified ten trees in the field that had not yet broken bud on 26 May 1999. In 2000 and 2001, these trees were observed for approximate date of budbreak, to determine if they were consistently later than the surrounding trees.

In 2000 in the Ingham County field, we monitored budbreak every few days on each marked tree ($n = 70$). On 23 April, 27 April, 30 April, 3 May, 7 May, and 9 May, we counted the percentage of buds that had broken in each of 12 sectors per tree. Sectors were designated as the top, middle, and bottom thirds of the tree facing each cardinal direction. Budbreak was defined as the stage when the cap no longer covered the tip of the bud, and the new needles were $\geq 10\%$ visible ('Stage 2' per Osawa et al. 1983). We defined the approximate date of budbreak as the date when on average 50% of the buds had broken. This method was thorough, but was extremely time consuming.

In Grand Traverse County in 2000, we were able to monitor the approximate status of budbreak for all sample trees in this field only on 2 May, 4 May, and 10 May. All of these trees had completely broken bud by 10 May. Therefore, we divided the trees into three categories: trees that had begun to break bud on or before 2 May were categorized as early, trees that had begun on 4 May were categorized as middle, and trees that broke bud after 4 May were categorized as late budbreakers.

Rates of shoot expansion. The rate of shoot expansion was used in all three years as a relative indicator of tree phenology and a surrogate for date of budbreak. Since

shoot growth and expansion cannot begin until the buds have broken, it seemed reasonable to assume that trees which broke bud earlier would also begin shoot growth earlier. This method of calculating the percentage of the final shoot length attained on each sample date was easily quantifiable, did not require daily visits to the field, and was objective so different observers could work simultaneously (per Mingo and Dimond 1979).

In 1999, a separate, randomly selected sample population of ten trees per field was designated for the purpose of measuring the rate of shoot expansion and its relationship with aphid density, and also for measuring aphid density with the 'beat method'. These ten trees were tagged permanently and were used each year throughout the three-year study. To measure shoot expansion in 1999, 12 shoots on each of the ten trees were marked before or shortly after budbreak in early spring and the length from the base of the bud collar to the tip of the shoot was measured weekly. Each shoot was located in one of twelve sectors of the tree, which was divided vertically in thirds and horizontally by the four cardinal directions. In 2000, we used this method to monitor the rate of shoot expansion on all of the sample trees in each field ($n = 50$). In 2001, we selected 20 of the sample trees at random, tagged four shoots on the upper half of the south side of each tree, and measured them once in early spring and once at the end of the growing season to determine the percentage of shoot expansion at an early point in the aphid's life cycle.

We calculated the percentage of shoot expansion of each tree on each sample date by dividing the length of each shoot by its final length and averaging the results for each tree. The final shoot length was defined as the length when the weekly measurements did

not increase beyond the margin of experimental error (± 5 mm), usually in late June (after Mingo and Dimond, 1979).

Balsam twig aphid damage. In each year, all trees that were used for aphid sampling and shoot expansion were monitored for aphid damage. In 1999, the percentage of shoots per tree that exhibited aphid damage was quantified after aphid oviposition in June, when damage is most apparent, and again at the end of the growing season in August or September. Aphid damage was defined as obvious needle curling on current-year foliage. Each tree was divided into 12 sectors for sampling: vertically by thirds and horizontally by the four cardinal directions. In 1999, two shoots in each sector were tagged in June, recorded as damaged or undamaged, and observed again in September. The percentage of damaged shoots for each tree was calculated as the total number of damaged shoots/24 sampled shoots per tree. This method did not always account for the patchy nature of aphid damage, so we made a more thorough measurement the next year. In 2000, 25 randomly chosen shoots in each sector were inspected and recorded as damaged or undamaged. The percentage of damaged shoots for each tree was calculated as the total number of damaged shoots/300 shoots per tree. This method was time consuming, so we improved our efficiency in 2001 by having one observer visually estimate the percentage of shoot damage in each sector of each tree. To obtain a measure of damage for each tree, we averaged the results from all 12 sectors for each tree.

Economic impact. In 1999 and 2000, we conducted surveys of retail customers on a choose-and-cut farm in Ingham County to determine the impact of aphid damage on retail tree value. In early December of each year, randomly chosen customers who were

planning to buy a fir tree were surveyed to determine their opinion of a select group of trees with differing levels of aphid damage. Insect damage, insects, or entomology were not mentioned to the customer until after the survey was completed.

In 1999, two groups of nine live, uncut trees (18 total) were selected on the basis of height and level of insect damage and designated Group I and Group II. All trees were located near one end of a section of trees, close to a lane frequented by customers, and close enough to each other that each customer could complete the survey of nine trees in less than 15 minutes. Within each group, there were three trees in each of three height categories. Categories were defined as short (approx. 1.8 m, 6 ft), medium (2.1 m, 7 ft) or tall (2.4 m, 8 ft). Within each height category, one tree had little or no aphid damage (0-10% of shoots damaged), one had medium damage (21-56% of shoots damaged) and one had heavy damage (>56% of shoots damaged). Damage was measured by randomly selecting a branch tip in each of eight sectors per tree (top and middle thirds by four cardinal directions) and counting the number of damaged and undamaged shoots (needles curled). The percentage of damaged shoots in each sector was calculated and averaged per tree. Trees were free of other obvious defects and relatively uniform in shape. Fifty customers each were randomly assigned to either Group I or Group II (total of 100 customers), to help eliminate bias due to unavoidable subjective differences in the individual trees. Customers were asked to fill out a form for each tree, with questions addressing the height, shape and color of the tree and whether or not they would purchase such a tree.

Methods were revised slightly in 2000. Nine trees of approximately medium (2.1-2.4 m, 7-8 ft) height and similar taper and a range of aphid damage (3% to 64% damaged

shoots) were selected, cut and placed in a row in front of the field in random order. Fifty customers were shown an undamaged 'model' tree first and asked to compare the others to it. To quantify the customers' response, they were asked what they would pay for the 'model' tree (between US \$15 and US \$50) and whether they would pay more or less for each of the other trees, in US \$5.00 increments. The percentage of change in their price, relative to what they would pay for the model tree, was used to indicate the customer's preference for each tree (the 'apparent' or 'perceived' value of each tree).

To estimate effects of *M. abietinus* damage on wholesale value, a grower experienced with the USDA wholesale grading standards for Christmas trees graded the 18 trees used in the December 1999 retail customer survey. Grades assigned were 1, 2, or cull (USDA 1997). The 'premium' grade was not used in the study because it is primarily a show grade, used for competitive tree shows, and not normally used for wholesale sales; wholesale trees are graded as '1 or better', '2' and 'cull'. (*Melvin R. Koelling, Michigan State University, pers. comm.*)

Statistical Analysis. Data were tested for normality with the Shapiro-Wilk test (PROC UNIVARIATE, SAS v8). Because aphid numbers, percentage of shoot expansion and the percentage of damaged shoots per tree were all random variables, Pearson's correlation analysis was used to assess the linear relationships between aphid numbers, tree phenology, and damage to the tree. When the data did not fit a normal distribution, Spearman's nonparametric correlation analysis was used (Sokal and Rohlf 1995). In all years, whenever the variable 'aphid numbers' was used, it represented the sum of the beat samples on the north and south sides of the tree.

Data from the ten pairs of early-late budbreaking trees in Ingham County were tested for homogeneity of variance (PROC TTEST, SAS v8). We compared aphid numbers and the percentage of damaged shoots between the ‘early’ and ‘late’ trees with a pooled t test if the variances were homogeneous, or the Welch test if variances were not homogeneous. If the Welch test was used, degrees of freedom were adjusted using Satterthwaite’s procedure (Kuehl 1994, Satterthwaite 1946). To test the categorical budbreak data from Grand Traverse County, we used the Kappa measure of agreement (Siegel and Castellan 1988).

The 1999 customer survey data (acceptance or rejection of each tree) was assessed using chi-square contingency analysis (Siegel and Castellan 1988). For the 2000 customer survey data, the strength of the association between the percentage of change in the customer’s price and the percentage of shoot damage was assessed using Spearman’s correlation coefficient (Sokal and Rohlf 1995). All tests were conducted at a significance level of $\alpha = 0.05$. Data were analyzed using SAS v. 8 (SAS Institute, 1999).

Results

Balsam twig aphid phenology. *Egg hatch.* In Ingham County in 1999, hatching was complete by our first field visit on 16 April, or 95 cumulative degree-days base 50°F (10°C) (hereafter DD₅₀). In Grand Traverse County in 1999, hatching had already begun by our first field visit on 13 April (46 DD₅₀). In Antrim County in 1999, hatching began

before 20 April (54 DD₅₀). We took this as evidence of a very early start to the aphid's life cycle in early spring.

In 2000, we recorded the phenology of egg hatch more precisely in Ingham County, and the percentage of egg hatch on one early field visit to the fields in Grand Traverse and Antrim Counties. In the Ingham County field, egg hatch began before our first field visit on 21 March (74 DD₅₀), and continued to 30 March (96 DD₅₀) (Figure 1). In Grand Traverse County on 28 March (50 DD₅₀), eggs were 41% hatched (41 hatched/100 eggs examined). In Antrim County, 76% of eggs had hatched on 28 March (50 DD₅₀)(35 hatched/46 eggs examined).

In 2001, we did not record the duration of egg hatch. Field visits began after egg hatch had been completed, on 29 April in Ingham County (130 DD₅₀) and 5 May in Grand Traverse and Antrim Counties (140-150 DD₅₀).

Fundatrices. Newly hatched aphids, or stem mothers, were extremely small and difficult to see in the field. As they began to mature, they became more conspicuous, especially by the third and fourth instars. I observed the new stem mothers feeding on the previous year's foliage or through the bud scales. They completed four instars in approximately four weeks.

In each field and year, stem mothers reached reproductive maturity between 100-150 DD₅₀, although calendar dates varied (Figures 2-4). Under the microscope, reproductively mature stem mothers could be identified by observing fully formed embryos of the sexuparae inside her abdomen. As the stem mothers began to reproduce, at approximately 150-200 DD₅₀, their number decreased and the second generation,

sexuparae, quickly replaced them. The sexuparae could be distinguished from the stem mothers under the microscope by the greater number of eye facets (per Varty 1968).

The second generation (sexuparae). In each field, the second generation was first observed between 150-200 DD₅₀. In 1999, the second generation was first observed in Ingham County on 2 May or 160 DD₅₀ (Figure 2a). In Grand Traverse County, the second generation was first observed on 6 May or approximately 149 DD₅₀ (Figure 2b). The first sample date where the sexuparae had almost completely replaced the stem mothers was 10 May (229 DD₅₀) in Ingham County and 18 May (247 DD₅₀) in Grand Traverse County.

In 2000 in Ingham County, the second generation was first observed on 27 April (154 DD₅₀) (Figure 3a). By 3 May (189 DD₅₀), the second generation comprised approximately 75% of the population. On the next sample date in Ingham County (11 May, 327 DD₅₀), the second generation had completely replaced the stem mothers. In 2000 in Grand Traverse County, the second generation was not observed on 2 May (110 DD₅₀), but by the next sample date on 10 May (228 DD₅₀), sexuparae made up 90% of the aphid population (Figure 3b). In Antrim County, sexuparae were first observed on 2 May (110 DD₅₀) (Figure 3c). By 10 May (228 DD₅₀), sexuparae comprised 50% of the aphid population in Antrim County.

In 2001 in Ingham County, the second generation was first observed on 30 April (139 DD₅₀), and had completely replaced the stem mothers by 13 May (320 DD₅₀) (Figure 4a). In Grand Traverse and Antrim Counties, most aphids were mature stem mothers on the first sample date, 5 May (150 DD₅₀) (Figure 4b,c). By the second sample

date on 14 May, Antrim and Grand Traverse Counties had reached roughly 200 DD₅₀, and the sexuparae had replaced the stem mothers. Grand Traverse and Antrim Counties did not reach 300 DD₅₀ until 30 May, more than two weeks after Ingham County had reached 320 DD₅₀ (on 13 May). This illustrated the lag in cumulative degree-day accumulation between the more northern counties and Ingham County, which were separated by approximately 2 degrees latitude, or roughly 288 km (180 miles).

Predicting aphid numbers. The numbers of stem mothers observed in our samples in early spring were often positively correlated with aphid numbers in the following weeks (Table 1). This correlation is important if the stem mothers are to be used as an indicator of future aphid numbers and potential damage. In 1999, we did not sample early enough to determine if stem mother numbers could predict later aphid numbers. In 2000, with an earlier start to aphid sampling, we found significant correlations between numbers of stem mothers (before 27 April in Ingham County, and before 10 May in Grand Traverse and Antrim Counties) and subsequent density of the second generation in each field. In 2001, we found significant correlations in Ingham County, but not in Grand Traverse County or Antrim County.

Tree phenology. The timing of budbreak for most of the trees in Ingham County corresponded with 140-189 DD₅₀ (22 April 2000-3 May 2000). Trees with budbreak on or after 7 May 2000 sustained no aphid damage, while trees which broke bud before 7 May had 10% to 25% damaged shoots (Figure 5).

In general, trees which broke bud approximately 7-10 days later than most of the surrounding trees tended to exhibit little or no aphid damage, regardless of the number of aphids found on the trees early in the season. Comparisons of the number of aphids per

tree and the percentage of damaged shoots were significant between the ten pairs of early- and late- budbreaking trees in Ingham County (Table 2). Of the late-budbreak trees in the ten selected pairs, all had less than 1% damage. The amount of damage ranged from 0% to 70% throughout the field. Among the entire sample population in Ingham County, trees that broke bud late (9 May or later) had significantly lower numbers of aphids than trees that broke bud before 9 May on all sample dates except 30 March (when aphids were newly hatched stem mothers) and 1 June (when most were sexuales).

Shoot expansion rate and damage. We also wanted to determine if the rate of shoot expansion was related to the amount of aphid damage. In 1999, the rate of shoot expansion of the ten 'beat sample' trees was not significantly correlated with aphid damage in either Ingham County or Grand Traverse County.

However, in 2000, with a higher sample size, the correlation between percentage of shoot expansion in early May and aphid damage was positive and highly significant in both Ingham County (10 May: $r_s = 0.52$, $n = 67$, $p < 0.0001$) and Grand Traverse County (13 May: $r_s = 0.60$, $n = 49$, $p < 0.0001$). In Antrim County, this relationship was not significant (13 May: $r_s = -0.02$, $n = 49$, $p = 0.87$).

In 2001, the rate of shoot expansion was significantly correlated with aphid damage only in Ingham County ($r_s = 0.40$, $n = 40$, $p = 0.01$). There was essentially no relationship between aphid damage and the percentage of shoot expansion on 14 May in Grand Traverse County ($r_s = 0.06$, $n = 23$, $p = 0.78$) or in Antrim County ($r_s = 0.30$, $n = 30$, $p = 0.11$). The variability in rates of shoot expansion was highest in Ingham County (range of 0-98%) than in either Grand Traverse or Antrim Counties (range approx. 7-

39%). Interestingly, the amount of shoot damage was not above 21% on any tree in Ingham County in 2001, but in Antrim and Grand Traverse Counties shoot damage ranged widely, from 0 to over 60%.

Shoot expansion rate and aphid numbers. In 1999, the percentage of shoot expansion was not significantly correlated with aphid numbers on any sample date, although in Ingham County on 17 May (when aphid numbers ranged widely; range of 0 to 228/tree; mean 68.6 ± 23), this relationship was marginally insignificant ($n = 10$, $p = 0.08$, $r_s = 0.58$). In Grand Traverse County, the percentage of shoot expansion was also marginally insignificantly correlated with aphid numbers on 18 May (range 3-39; mean 23.7 ± 3.5 aphids per tree) ($n = 10$, $p = 0.07$, $r_s = 0.60$), but not correlated on the other sample dates.

In 2000 and 2001, the relationship between rates of shoot expansion and aphid numbers was variable. In Ingham County in 2000, the percentage of shoot expansion on all trees (total $n = 70$ minus 3 missing values) was significantly and positively correlated with aphid numbers on 11 May (when the aphid population was 27% stem mothers and 73% sexuparae) ($n = 67$, $p < 0.0001$, $r_s = 0.56$) and 15 May (100% sexuparae) ($n = 67$, $p = 0.0002$, $r_s = 0.44$). In Grand Traverse County and Antrim County in 2000, this relationship was not significant on any sample date.

In 2001 in Ingham County, the percentage of shoot expansion was positively correlated with aphid numbers on nearly every sample date. For example, on 30 April the relationship was highly significant ($n = 41$, $p = 0.003$, $r_s = 0.45$). In Antrim County, the percentage of shoot expansion was positively correlated with aphid numbers only on 14 May ($n = 30$, $p = 0.022$, $r_s = 0.42$) when 92.4% of the aphids were sexuparae. No

significant correlations were observed on other sample dates in Antrim County nor on any date in Grand Traverse County.

Consistency in tree phenology. We tested the consistency of bud break timing and shoot expansion between years to determine whether the phenology of our sample trees was influenced more by genetic predisposition or environmental factors. If no correlation existed, then selection of trees with later budbreak tendencies would not be useful for breeding programs.

First, we looked at the relative order of budbreak for trees that we had observed in two or more years. In general, trees that broke bud 7-10 days later than the surrounding trees in 1999 continued this tendency. In Grand Traverse County, ten trees that were particularly late in breaking bud in 1999 were also up to two weeks later than other trees in the same field in both 2000 and 2001.

To test the relative order of budbreak in Grand Traverse County, we categorized 32 trees that had been observed in both 2000 and 2001 as early, mid, or late budbreakers. Contingency analysis showed a significant measure of agreement between the categories in 2000 and 2001 ($K = 0.29$, $p = 0.02$).

We also correlated the percentage of shoot expansion for the same trees between years wherever possible. In Ingham County, the ten pairs of trees that had been selected for differences in budbreak date in 1999 showed a significant positive correlation in early rates of shoot expansion between years 2000 and 2001 ($n = 15$ because of missing values; $p < 0.0001$, $r = 0.92$) (Figure 6). The ten trees that had been selected at random for 'beat samples' in 1999 in Ingham County also exhibited a significant and positive correlation in rate of shoot expansion between 1999 and 2000 ($n = 10$, $p < 0.0001$, $r = 0.97$) (Figure

7). The 23 trees selected solely on the basis of aphid damage in Ingham County had neither particularly late or early budbreak (Figure 8). The percent shoot expansion in early spring for these trees was not significantly correlated between years ($n = 23$, $p = 0.12$, $r_s = 0.33$), perhaps due to the lack of variability. In the Grand Traverse County field, we observed a similar pattern. In Antrim County, 19 trees selected on the basis of aphid damage in 1999 did show a significant positive correlation between rates of shoot expansion in 2000 and 2001 ($n = 19$, $p = 0.01$, $r_s = 0.56$).

Balsam twig aphid damage. Establishing a relationship between aphid numbers and the resulting amount of damage to the tree is essential to our objective of establishing an aesthetic injury level or action threshold. Such a threshold would only be possible if aphid numbers early in the season were positively correlated with a quantifiable amount of aesthetic damage (Pedigo et al. 1986). In 1999 in Ingham County, aphid numbers sampled using the beat method on 17 May, at the peak of the second generation (mean of 68 aphids/tree), were not significantly correlated with aphid damage ($r_s = 0.62$, $n = 10$, $p = 0.06$), but were strongly correlated on 24 May ($r_s = 0.89$, $n = 10$, $p = 0.0006$) when sexuparae had reached the fourth and last adult instar (mean = 99.6 aphids/tree).

In Grand Traverse County in 1999, aphid numbers on 18 May, at the beginning of the second generation, were not related to aphid damage ($r_s = 0.02$, $n = 10$, $p = 0.95$). This relationship was stronger but still not significant on 26 May, when the second generation was at its height ($r_s = 0.42$, $n = 10$, $p = 0.22$).

In 2000, when a much larger sample size was used for sampling aphids with the beat method, aphid numbers were significantly correlated with tree damage in almost

every case. In Ingham County, aphid numbers were significantly correlated with tree damage as early as 6 April ($r_s = 0.49$, $n = 65$, $p < 0.0001$), when the population was still entirely stem mothers, and this relationship remained highly significant on every sample date thereafter.

In 2000 in Grand Traverse County, aphid numbers were significantly correlated with tree damage on every sample date. On 2 May, at 110 DD₅₀ and just before the appearance of the second generation, aphid numbers were significantly correlated with tree damage ($r_s = 0.35$, $n = 49$, $p = 0.01$). This relationship remained significant on 10 May, 19 May, and 26 May.

In 2000 in Antrim County, where aphid numbers were relatively low, the relationship was still highly significant on most sample dates. Even on 28 March, at the start of aphid sampling and only 50 DD₅₀, aphid numbers were positively correlated with tree damage ($r_s = 0.34$, $n = 50$, $p = 0.02$). This correlation was not significant on 18 April or 25 April, but on 2 May, just before the second generation appeared, it was highly significant ($r_s = 0.45$, $n = 50$, $p = 0.001$).

In 2001 in Ingham County, aphid numbers were significantly correlated with the percentage of damaged shoots on almost every sample date: 30 April, 7 May, 12 May, and 21 May. In Grand Traverse County in 2001, the relationship between aphid numbers and damaged shoots was not significant on any sample date. Aphid numbers in Grand Traverse County in 2001 tended to be low (highest value of 28.5 ± 4 per tree on 6 June), but damage ranged widely, from 1% to 62% damaged shoots. In Antrim County, aphid numbers were positively correlated with damage on 14 May only. On 14 May in Antrim County, aphid numbers averaged 31.7 ± 4.5 per tree, and the sexuparae had almost

completely replaced the stem mothers. Damage in Antrim County also ranged widely, from 0 to 76% damaged shoots per tree, although all except three of the trees had from 0 to 42% damage.

Economic impact. The two replicates in the 1999 customer survey presented varying results. We analyzed the two groups separately, since the patterns of customer choice were different between the two. For example, the Group I customers preferred the tall trees the most (56% of the positive responses from customers were for tall trees), but the Group II customers preferred the medium height trees (54% of positive responses were for medium height trees) (Figure 9).

The 1999 Group 1 surveys showed that both height and *M. abietinus* feeding damage were significant factors in a customer's decision to accept or reject a particular tree (height: $X^2 = 58.9$, $df = 2$, $p < 0.0001$; damage: $X^2 = 10.76$, $df = 2$, $p = 0.0046$). However, when the trees were analyzed separately according to height class, damage was not a significant factor in their decision to accept the medium or tall trees (Table 3). Damage was a significant factor for the short trees, but the data were heavily skewed in that only one customer chose the short tree with no damage (Figure 9). In both groups, the short trees were the least preferred: 16% and 20% of customers in Group I and Group II, respectively, accepted short trees, regardless of damage.

The Group II surveys also showed that both height and damage were significant factors at $p < 0.0001$, but when heights were considered separately, damage was still a significant factor at each level (Table 3). Trees in this group with medium amounts of aphid damage (21-56%) were apparently preferred over trees with little or even heavy damage (Figure 9).

In the 2000 customer surveys (Figure 10), the percentage of damaged shoots was a significant factor in customer preference if the trees were divided into damage categories (light = 3-6% of shoots affected; medium = 9-14%, heavy = 23-64%). Analysis of variance indicated that damage was a significant factor affecting customers' perceived value ($F = 23.5$; $df = 2, 397$; $p < 0.0001$). The light and medium damage trees did not significantly differ from each other in apparent value (Fisher's LSD, $p < 0.05$), but both were significantly different from the heavily damaged trees.

These data indicated that the effect of aphid damage on apparent retail value is not a linear relationship. When the percentage of change in value was regressed on the actual percentage of damaged shoots, the regression explained only 6% of the variability. Using the Spearman rank correlation analysis, the relationship between apparent value and percentage of shoots with damage was significant but not strong ($r_s = -0.26$, $p < 0.0001$).

In terms of wholesale grades, trees with up to 40% damaged shoots were still considered a Grade 1, while trees in Grade 2 ranged from 32 to 62% damage (Figure 11). Most likely due to this high variability, no significant differences were found in aphid damage among the wholesale grades ($F = 1.38$; $df = 2, 15$; $p = 0.28$). This would indicate that the wholesale value of the tree did not necessarily drop because of aphid damage. The cull trees (unsaleable) consisted of one tree with 42% damaged shoots, and one with nearly 100% damage. The tree with 42% damage was noted as being 'not full enough'; it was likely more sparse in appearance than other trees with the same amount of aphid damage.

Discussion

Our study showed that *M. abietinus* phenology is more closely tied to degree-day accumulation than to calendar date, implying that for this aphid, accumulated degree-days, which reflect temperature, are an important cue for development. The temporal separation of aphid development by about one week between Ingham County and the more northern counties confirmed this observation. Varty (1966, 1968) also showed that temperature is an important controlling factor in *M. abietinus* phenology, and suggested that degree-days might be useful for predicting development rates in the field.

Scouting during the first generation of *M. abietinus* (the stem mother generation), can indicate the potential size of future *M. abietinus* populations in upcoming weeks. Even using our relative sampling method of rapping foliage over an embroidery hoop, stem mother numbers were often significantly correlated with later aphid sample numbers (Table 1). Although the fecundity of the stem mothers ranges widely, from 3 to 60 offspring (Varty 1966), the average fecundity is approximately 22 to 41 offspring per female (Varty 1968). Hence, applying control measures before the stem mothers mature and reproduce would likely have a strong effect on subsequent generations.

The relationship between aphid numbers and tree phenology was significant for the ten pairs of early- and late-budbreaking trees in Ingham County. In the other fields when tree phenology was measured by percentage of shoot expansion, results were variable. It is relatively unlikely that *M. abietinus* is capable of deliberately selecting trees with earlier flushing tendencies, since the winged adult *M. abietinus* is a weak flier and is likely to be dispersed by air currents within a field (Amman 1963). This indicates

that aphid eggs may be equally likely to be present on trees with earlier or later flushing times, and the differences in aphid numbers we observed may be the result of differential survival rates between earlier and later flushing trees. As an example, the ten pairs of early and late budbreaking trees in Ingham County consistently differed in aphid numbers and damage in all three years, although the initial number of aphids was not always significantly different. In addition, the strong positive correlation between the rates of shoot expansion and aphid damage on all sample trees in Ingham and Grand Traverse Counties in 2000 indicated that trees which broke bud and began to expand new shoots later in the season escaped at least some aphid damage.

The extent of genetic control of budbreak has been studied for several tree species (e.g. Li and Adams 1993, Murray et al. 1994). Although the timing of budbreak is influenced by local factors, especially temperature (e.g. Partanen et al. 1998, Worrall and Little 1986, Lowe et al. 1977), the tendency of balsam fir to break bud at a given time is determined in part by genetic factors (Lester et al. 1976, Lowe et al. 1977).

Our data indicated that budbreak and early rates of shoot expansion in balsam and Fraser fir are at least partly controlled by genetic factors, since trees measured in two or three consecutive years were almost always consistent in their relative order of budbreak and early shoot expansion. Although we did not do provenance testing, other workers have found strong correlation between the origin of the seed source and the date of bud break for several conifers (Li and Adams 1993, Murray et al. 1994, Beuker 1994, Lester et al. 1976). Future research could further explore this, perhaps by selecting and breeding trees with later flushing tendencies. If host plant resistance can be used in selection or

breeding programs, the need for insecticide applications in fir fields could be substantially reduced.

The economic impact of *M. abietinus* appears to be relatively low. Most of the retail choose-and-cut customers we questioned after they had completed the surveys stated they would not have noticed light to moderate aphid damage if we had not pointed it out. This agrees with the findings of Kleintjes et al. (1999), who found that choose-and-cut retail customers still selected trees with up to 50% of the shoots damaged. In our 1999 customer surveys, *M. abietinus* damage was not a consistent factor in customer's choice of tree, but height was important. Kleintjes et al. (1999) also found that tree height and shape were significant factors affecting customer choice, but that aphid damage levels did not significantly differ between selected and unselected trees. Our Group II surveys in 1999 appeared to show that trees with medium damage were preferred, which further suggests that a medium level of aphid damage (e.g. 21-56% damaged shoots) does not have a detrimental effect on perceived tree value. In our 2000 surveys, damage was not a significant factor in consumer perception of tree value, which confirms Kleintjes' findings that retail choose-and-cut customers do not consider *M. abietinus* damage to be an important factor. In our study, the change in perceived value varied widely in the light and medium damage levels, indicating that other factors such as height, shape, fullness, or taper may be equally or more important than the amount of aphid damage, especially at light or medium levels of damage.

The effect of *M. abietinus* damage on wholesale grade is also less than might be expected. The level of damage that caused a tree to drop from a Grade 1 to a Grade 2 rating (representing economic loss) is relatively high, and strongly dependent on its

within-tree distribution. The USDA standards for grading wholesale Christmas trees include 12 characteristics, only one of which is insect damage (USDA 1997). For a tree to receive a Grade 1 rating, one face may have one noticeable defect (such as moderate to heavy aphid damage), and each face is allowed one minor defect (such as light aphid damage). Wholesale trees are unlikely to be reduced from a '1 or better' grade to a '2' grade because of aphid damage unless the damage is moderately heavy on two or more sides of the tree (USDA 1997). The term 'moderately heavy' is subject to interpretation and is not defined by the USDA, but 'abnormal curling of needles' is mentioned, with 'slightly abnormal', 'moderately abnormal', and 'severely abnormal' as categories. Only trees with 'severely abnormal curling of needles' are classified as culls. Although a few trees in a given field may exhibit such severe damage, the majority of trees in the fields we studied did not reach that level. In 2001, 67-100% of the trees used in our study had damage levels of 25% or less. In 2000, 74-94% of trees used for our study had less than 25% damage. Some other studies on balsam twig aphid have found similar results. In the study by Nettleton and Hain (1982), trees averaged only 23% damage, with a range of 11-59% damaged shoots. In the study by Kleintjes et al. (1999), 30 trees selected by retail customers on a choose-and-cut farm sustained 20-50% damaged shoots or less.

The number of stem mothers that result in trees with an economic level of damage varies depending on the acceptable economic injury level. If 25% of damaged shoots per tree is chosen as a conservative economic injury level, the likelihood of a tree sustaining that much damage can be calculated based on the number of stem mothers sampled. In the Ingham County field in 2000, five or more stem mothers per tree ($n = 32$ trees), resulted in 12 trees with greater than 25% damage, or a 38 percent chance that tree value

would be economically affected. Trees with two or more stem mothers ($n = 45$) had a 33 percent chance of sustaining 25% or more damage. These probabilities indicate that the risk of economic damage is relatively low. Although Kleintjes et al. (1999) found that approximately two or more stem mothers on a given tree would result in about 50% of damaged shoots, we found that this did not hold true 100% of the time. The action threshold for balsam twig aphid will vary depending on the economic injury level and the level of risk the grower is willing to accept.

An interesting possibility for management of *M. abietinus* could include developing a method to forecast outbreak years, and planning ahead. In Canada, *M. abietinus* has been reported to occur in outbreak cycles of 4-6 years (Varty 1968, Mattson et al. 1989, Räther and Mills 1989). These outbreaks are reported to occur simultaneously over large areas (Mattson et al. 1989, Räther and Mills 1989). Further research may determine whether *M. abietinus* follows this pattern in Christmas tree plantations.

The cost of applying control measures such as insecticide sprays for *M. abietinus* may not always be economically justified. Taken together, our results indicate that careful scouting, selecting trees with later budbreak tendencies, and avoiding control measures that are not economically justified will help reduce pest control costs, increasing marginal profits for growers and the overall competitiveness of the Michigan Christmas tree industry.

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Table 1. Correlations of density of *Mindarus abietinus* fundatrices with density of sexuparae in subsequent weeks.

Field	Year	Dates stem mother sampled	Date sexuparae sampled	No. trees	Mean no. fundatrices	Mean no. sexuparae	<i>p</i>	<i>r_s</i> = Spearman's rank correlation
Ingham	2000	26 March	6 April	37	12.5 ± 2.1	6.6 ± 0.74	0.0012	0.51
		6 April	13 April	64	6.6 ± 0.74	16.8 ± 1.5	<0.0001	0.74
		6 April	27 April	65	6.6 ± 0.74	4.3 ± 0.65	<0.0001	0.84
		22 April	11 May	66	11.4 ± 1.4	15.5 ± 2.6	<0.0001	0.63
		22 April	17 May	66	11.4 ± 1.4	52.7 ± 5.5	<0.0001	0.68
Grand Traverse	2000	2 May	10 May	49	8.7 ± 1.1	19.7 ± 2.6	0.01	0.36
		2 May	19 May	49	8.7 ± 1.1	11.7 ± 1.7	0.0001	0.46
		2 May	26 May	49	8.7 ± 1.1	29.7 ± 5.2	0.05	0.29
Antrim	2000	28 March	18 April	50	0.9 ± 0.14	3.36 ± 0.45	0.0001	0.52
		28 March	25 April	50	0.9 ± 0.14	1.9 ± 0.27	0.37	0.13
		28 March	2 May	50	0.9 ± 0.14	2.6 ± 0.42	0.0007	0.46

Table 1 cont'd.

Field	Year	Dates stem mother sampled	Date sexuparae sampled	No. trees	Mean no. fundatrices	Mean no. sexuparae	<i>p</i>	<i>r_s</i> = Spearman's rank correlation
		28 March	10 May	50	0.9 ± 0.14	8.3 ± 1.2	0.0155	0.34
		18 April	25 April	50	3.4 ± 0.45	1.9 ± 0.27	0.0009	0.46
		18 April	2 May	50	3.4 ± 0.45	2.6 ± 0.42	0.0009	0.46
		18 April	10 May	50	3.4 ± 0.45	8.3 ± 1.2	0.14	0.21
		25 April	2 May	50	1.9 ± 0.27	2.6 ± 0.42	0.0007	0.46
Ingham	2001	30 April	7 May	43	3.8 ± 0.43	4.0 ± 0.62	0.001	0.49
		30 April	12 May	43	3.8 ± 0.43	8.9 ± 1.4	0.0002	0.55
		30 April	21 May	43	3.8 ± 0.43	8.3 ± 0.93	0.03	0.45
		7 May	12 May	43	4.0 ± 0.62	8.9 ± 1.4	<0.0001	0.57
		7 May	21 May	43	4.0 ± 0.62	8.3 ± 0.93	<0.002	0.45
Antrim	2001	5 May	14 May	30	5.9 ± 0.76	31.7 ± 4.5	0.43	0.15
		5 May	23 May	30	5.9 ± 0.76	43.9 ± 5.2	0.25	0.22

Table 2. Comparisons between early and late budbreaking trees in Ingham County.

Aphid numbers found on each group were compared using pooled t-tests or Welch tests. The amount of aphid damage (percentage of shoots damaged) sustained by each group of trees was compared using a Welch test.

Sample date	Aphid numbers (mean $\pm$ 1 SE)		t	df	p =
	Late trees	Early trees			
30 March	6.0 $\pm$ 1.2	10.5 $\pm$ 2.3	-1.77	14	0.099
6 April	1.2 $\pm$ 0.4	9.1 $\pm$ 2.5	-3.06	8.4	0.015
22 April	1.9 $\pm$ 1.1	16.1 $\pm$ 5.5	-2.53	8.64	0.0331
27 April	0.1 $\pm$ 0.1	5.5 $\pm$ 2.0	-2.69	9.04	0.0248
3 May	0.75 $\pm$ 0.49	5.7 $\pm$ 1.8	-2.63	9.17	0.0269
11 May	0.1 $\pm$ 0.1	22.8 $\pm$ 8.6	-2.62	9.0	0.0276
17 May	5.6 $\pm$ 0.99	63.1 $\pm$ 22	-2.61	9.04	0.0284
24 May	3.2 $\pm$ 0.81	31.9 $\pm$ 8.5	-3.37	9.17	0.008
1 June	8.2 $\pm$ 2.5	14.2 $\pm$ 2.9	-1.54	17	0.1432
Damage	0.1 $\pm$ 0.1	15.0 $\pm$ 4.0	-3.71	9.01	0.0048

Table 3. Results of chi-square tests of customer preference, 1999 surveys

Group	Tree height	Chi-square	df	<i>p</i>
I	Short	26.4	2	<0.0001
	Medium	3.77	2	0.15
	Tall	4.14	2	0.13
II	Short	19.3	2	<0.0001
	Medium	11.2	2	0.0038
	Tall	7.75	2	0.021

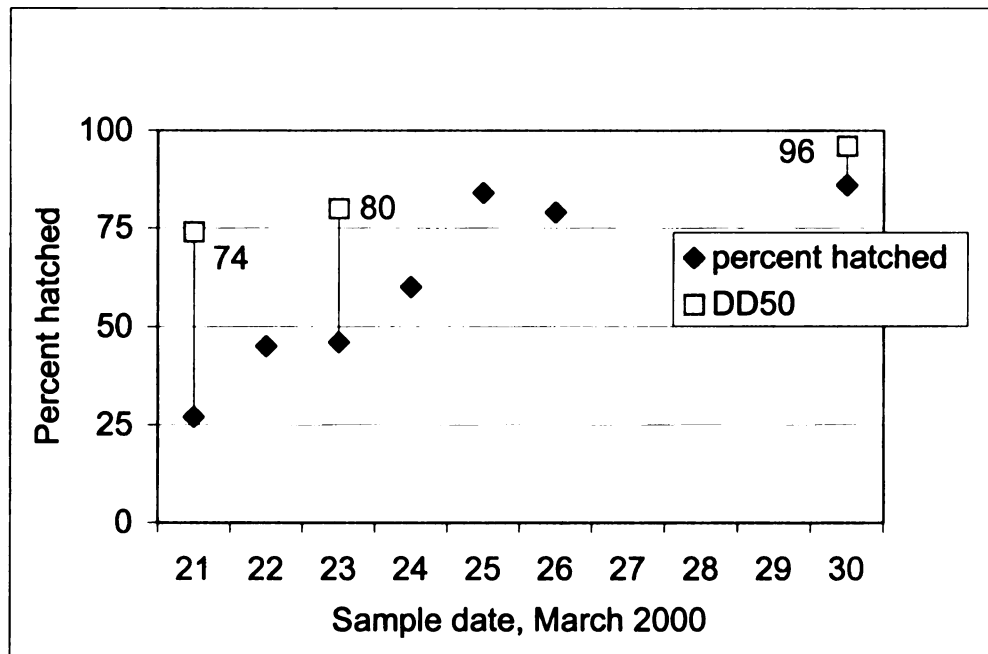


Figure 1. Percentage of *M. abietinus* eggs hatched in Ingham County in March 2000 (number hatched/100 eggs counted). Cumulative degree-days base 50 degrees F (10 degrees C) are indicated for 21 March, 23 March, and 30 March.

Fig. 2a

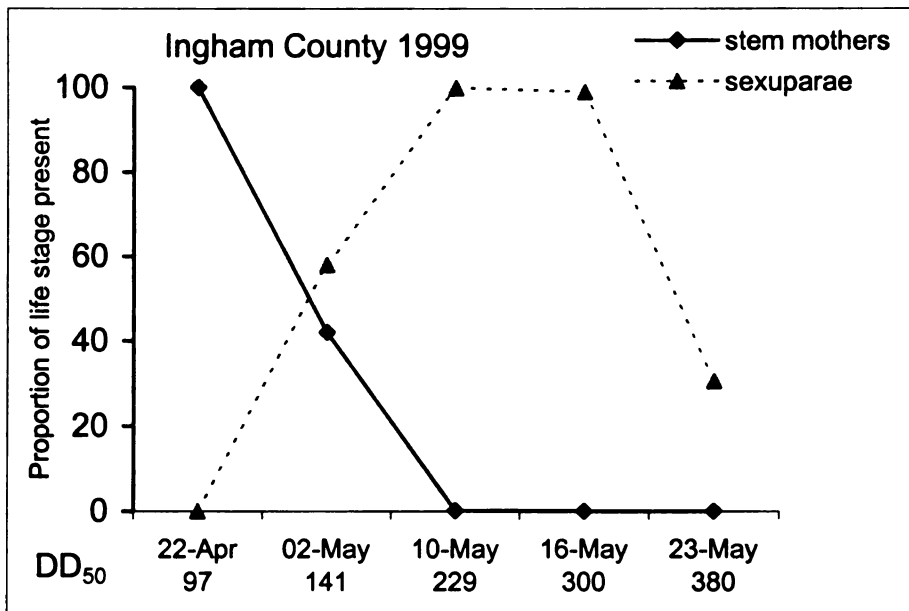


Fig. 2b

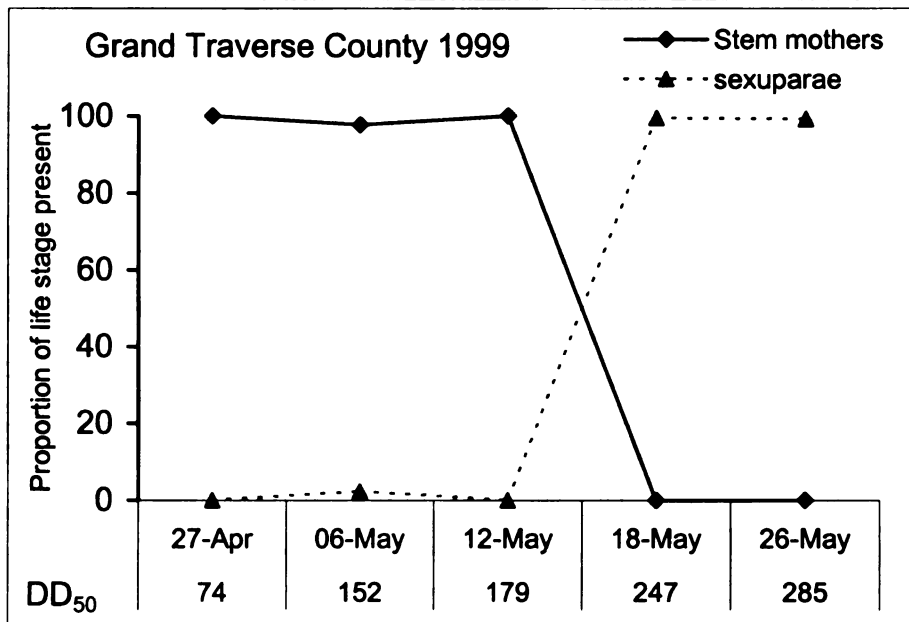


Figure 2. Phenology of the first and second morphs of *M. abietinus* in a) Ingham and b) Grand Traverse Counties, 1999.

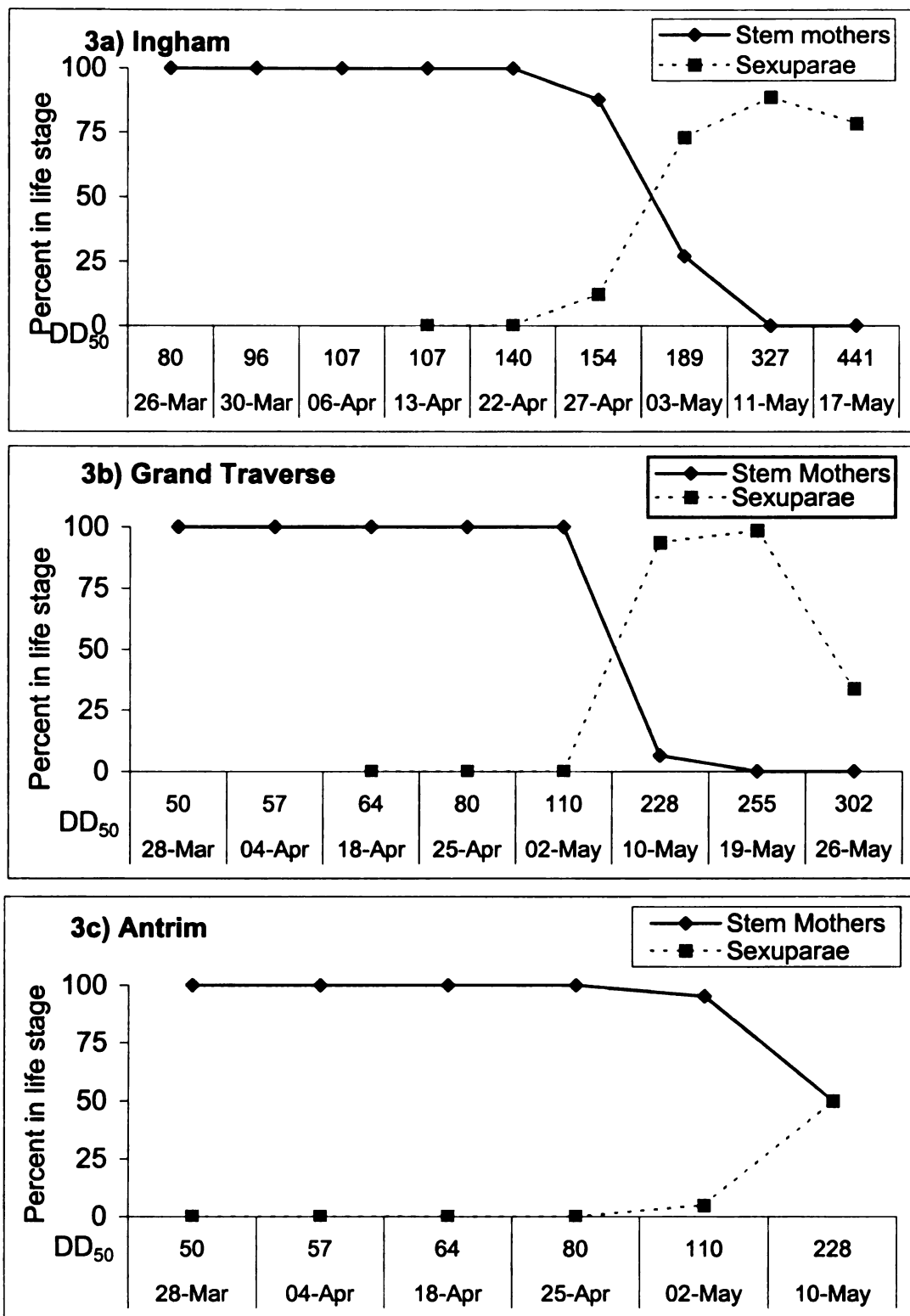


Figure 3. Phenology of the first and second morphs of *M. abietinus* in (3a) Ingham, (3b)Grand Traverse, and (3c) Antrim Counties, 2000.

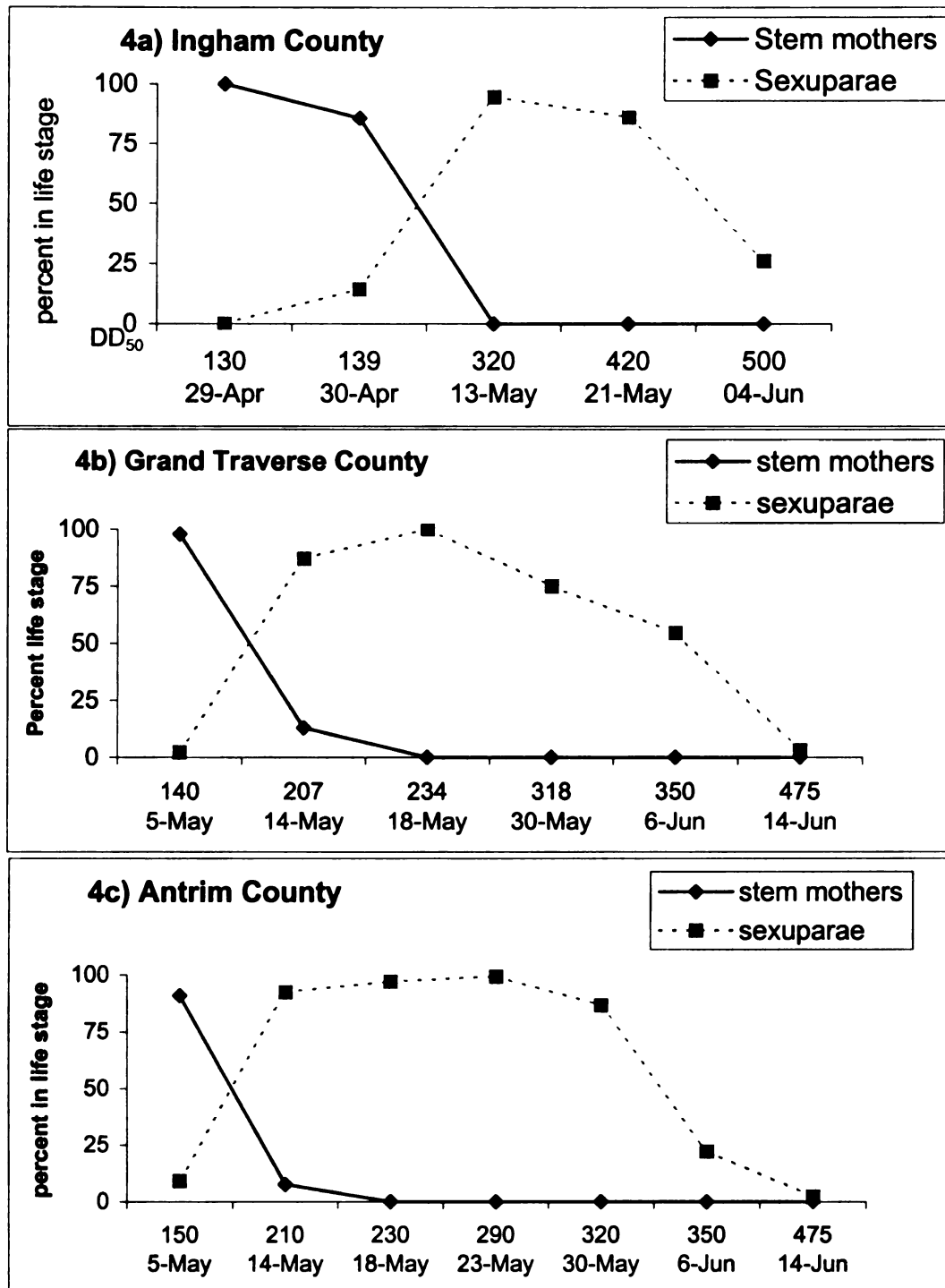


Figure 4. Phenology of the first and second morphs of *M. abietinus* in (4a) Ingham, (4b) Grand Traverse, and (4c) Antrim Counties, 2001.

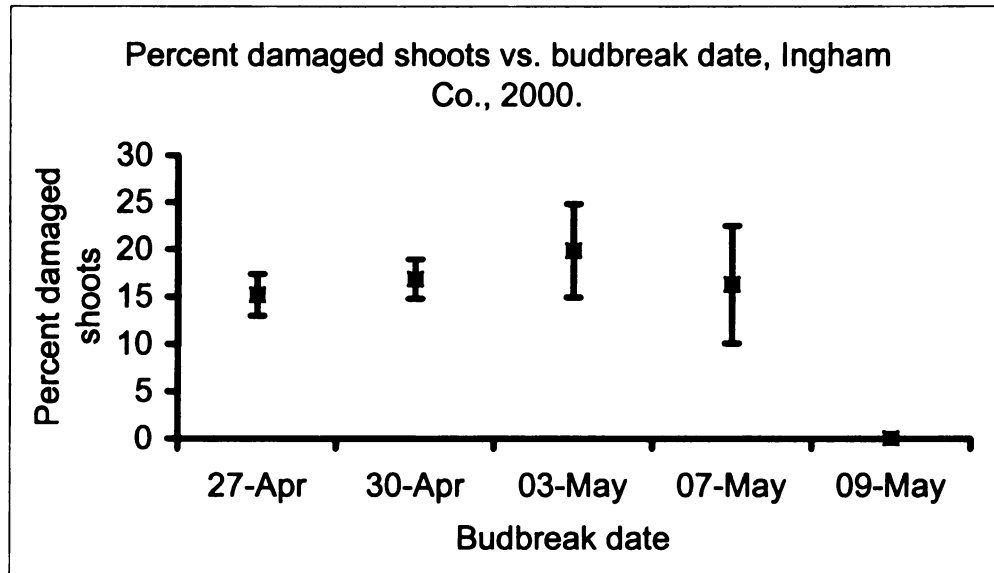


Figure 5. Percentage of damaged shoots vs. approximate date of budbreak, Ingham Co., 2000 (means $\pm$ SE).

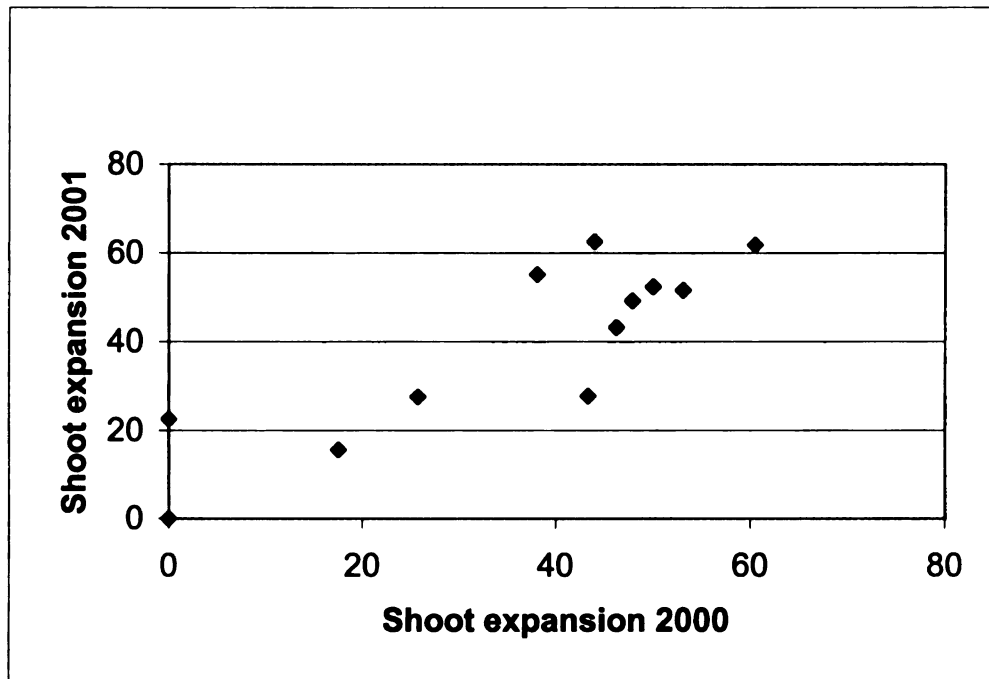


Figure 6. Percentage of shoot expansion in early spring for paired early and late budbreaking trees in Ingham County ($n = 12$ trees). Pearson's product-moment correlation analysis: $p < 0.0001$, $r = 0.92$.

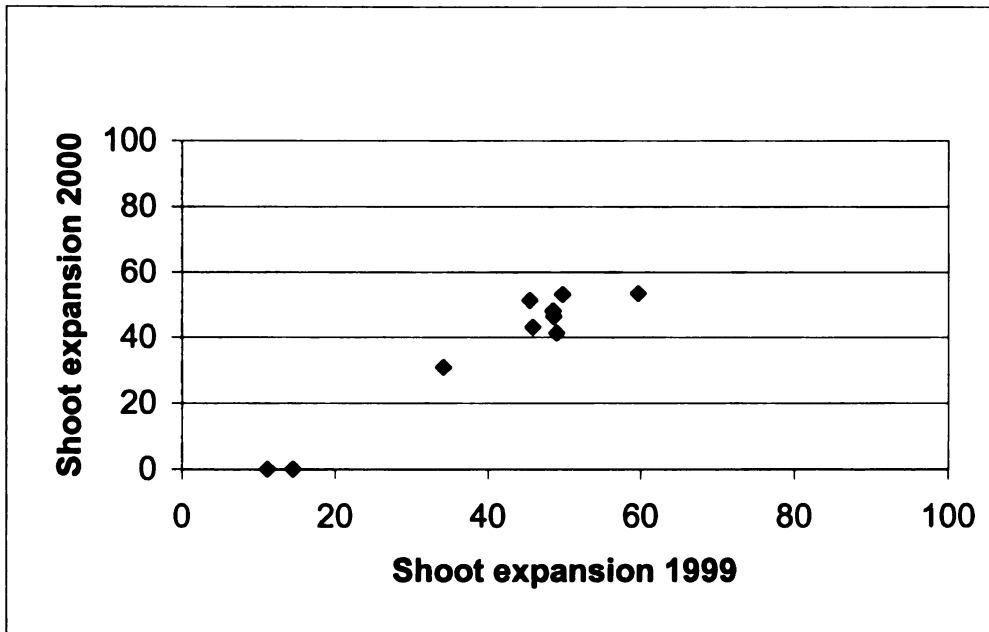


Figure 7. Percentage of shoot expansion in 1999 and 2000 for ten randomly selected trees in Ingham County. Pearson's product-moment correlation analysis: $p < 0.0001$, $r = 0.97$.

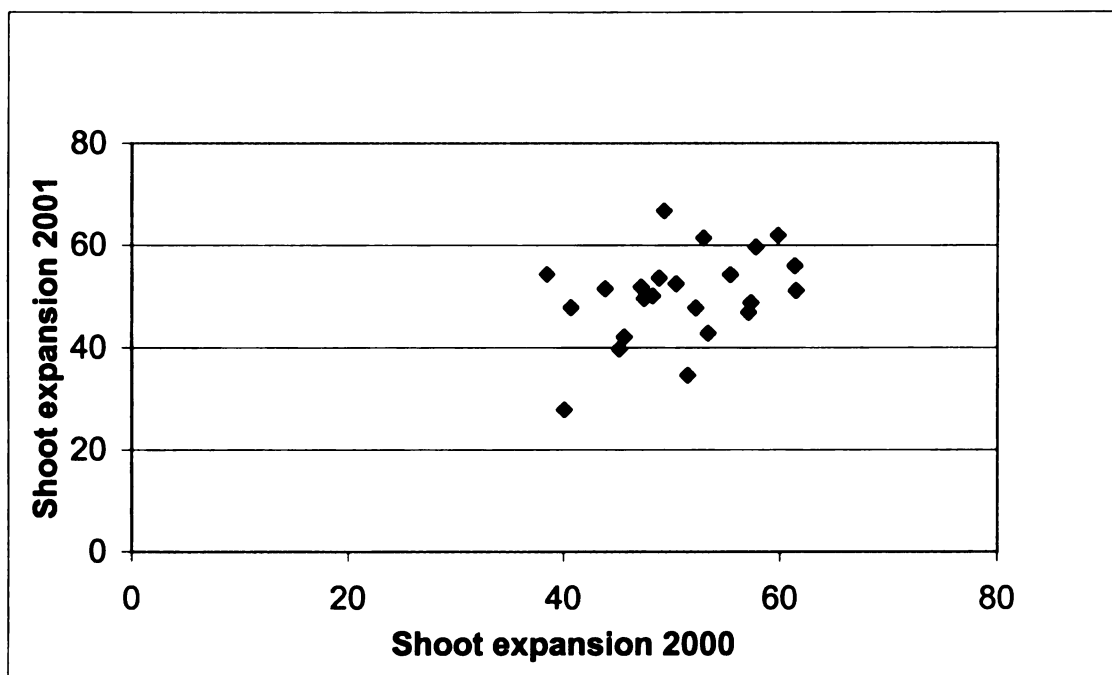


Figure 8. Percentage of shoot expansion in early spring for the aphid sample trees in Ingham County ($n = 30$ trees measured in both years). Seven trees were excluded because of missing values. Spearman's rank correlation analysis: $\rho = 0.12$, $r_s = 0.33$.

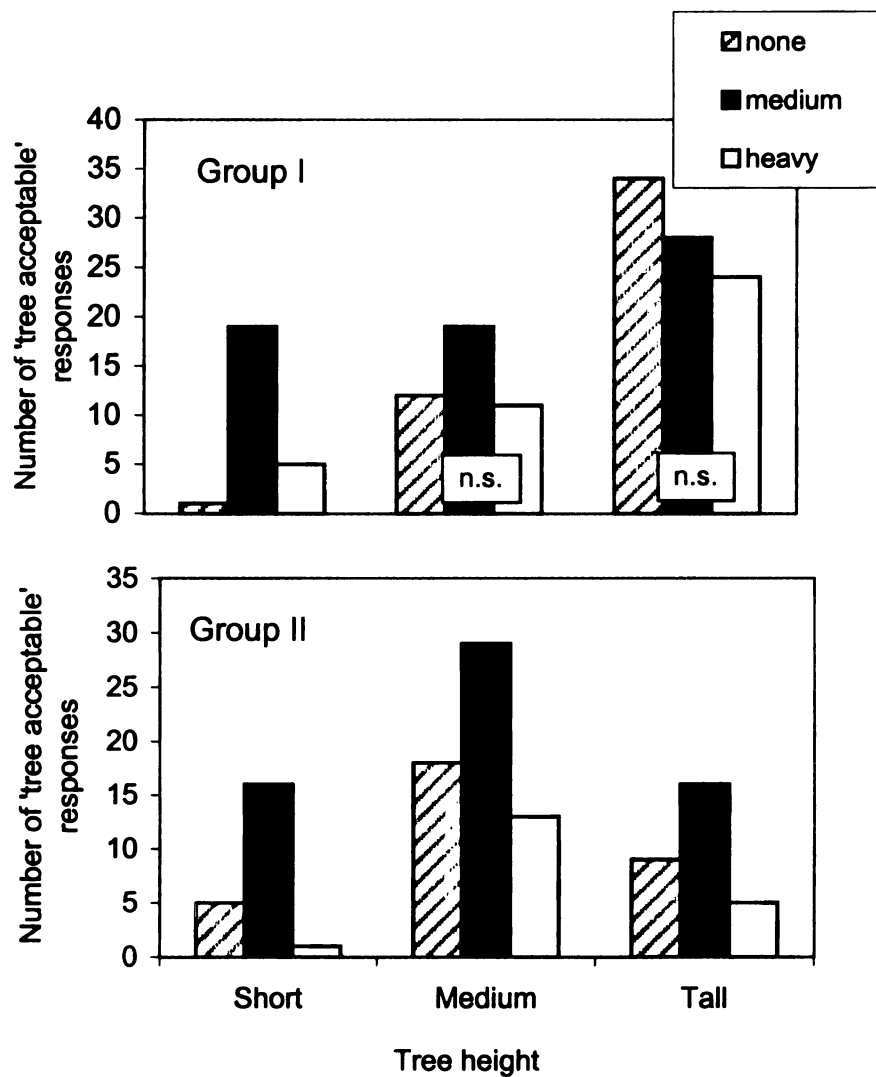


Figure 9. Results of 1999 retail customer surveys. The y-axis indicates the number of customers who stated that they would consider buying each tree. Within each height group, damage was a significant factor (see Table 3) unless marked with n.s.

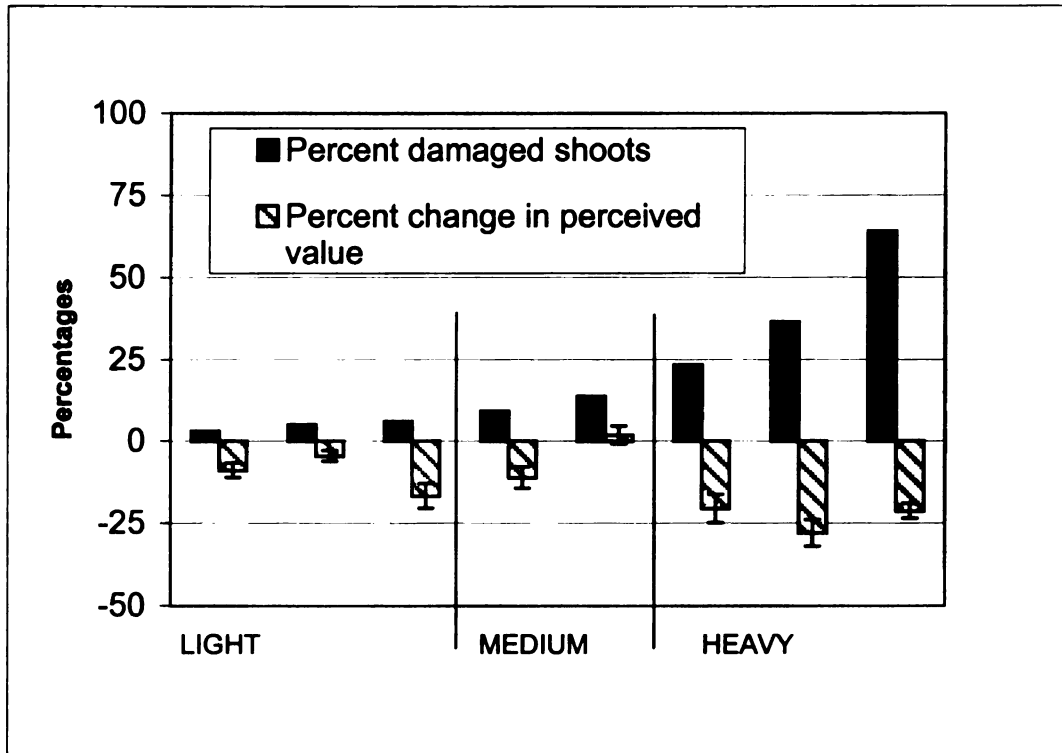


Figure 10. Results of 2000 customer surveys (n = 50 customers). Trees were grouped into three categories based on the percentage of damaged shoots/ 300 shoots examined per tree. The percentage of damaged shoots is presented as the actual value. Damage categories are indicated by 'Light' (n=3 trees), 'Medium' (n=2 trees), and 'Heavy' (n=3 trees). The percentage of change in perceived value is presented as mean $\pm$ 1 SE.

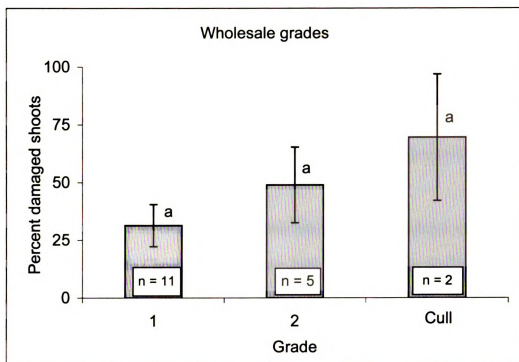


Figure 11. Wholesale grades assigned to trees varying in aphid damage used in 1999 survey (n=18). Percentage of damaged shoots is presented as mean $\pm$ SE. Percentage of shoots with aphid damage did not differ significantly among grades ($F = 1.38$; $df = 2, 15$; $p = 0.28$).

Chapter 2

Potential for augmentative biological control of the balsam twig aphid (*Mindarus abietinus* Koch) (Homoptera: Aphididae) in Michigan Christmas tree plantations.

Abstract. We investigated the effectiveness of natural enemies and augmentative biological control with *Chrysoperla rufilabris* Burmeister larvae in fir Christmas tree plantations to control *Mindarus abietinus* Koch. Our objectives in this study were to: 1) describe the natural enemy complex of *M. abietinus* present in unsprayed fir Christmas tree fields; 2) assess the effectiveness of *C. rufilabris* larvae as predators of *M. abietinus* in the laboratory and in field cages; and 3) evaluate the potential effectiveness of *C. rufilabris* in an open field release, as might be done on a commercial Christmas tree plantation. Results showed that *M. abietinus* can support a diverse complex of predators in the field. In the laboratory, *C. rufilabris* proved to be a voracious predator of *M. abietinus*. In field cages, the presence of a *C. rufilabris* larva consistently reduced the mean number of *M. abietinus* in each cage, as evidenced by a reduced mean number of *M. abietinus* eggs present, although the differences were not significant. In open field releases, *C. rufilabris* reduced the *M. abietinus* population significantly in two out of three fields.

Introduction

The balsam twig aphid (*Mindarus abietinus* Koch) is a serious pest affecting balsam (*Abies balsamea* (L.) Mill.) and Fraser fir (*A. fraseri* (Pursh) Poir.) Christmas trees (Kleintjes 1997a, Bradbury and Osgood 1986, Nettleton and Hain 1982). *Mindarus abietinus* is native to boreal forests, but has not been a target of large-scale biological control efforts like the more destructive balsam woolly adelgid (*Adelges piceae* (Ratz.)) (McGugan and Coppel 1962). Balsam twig aphid does not usually cause tree mortality, although it may cause chronic changes in tree properties and affect tree growth (Mattson et al. 1989, Berthiaume et al. 2000). High populations of *M. abietinus* cause needles to become curled and distorted, reducing needle biomass and consequently tree growth (Berthiaume et al. 2000, Carter and Nichols 1985). When damage is heavy, the current year's needles on the new shoots are tightly curled together and sticky with honeydew, forming a 'pseudogall' around the aphids that can protect them from contact insecticides as well as some predators (Nettleton and Hain 1982, Berthiaume et al. 2001).

The life cycle of balsam twig aphid typically begins before budbreak in early spring, when stem mothers (fundatrices) hatch from the overwintering eggs. At maturity they produce the second generation (sexuparae), which form colonies that feed on sap in newly expanding needles. The second generation typically matures into alate (winged) females, which disperse and produce the last generation of males and oviparous females (oviparae). Occasionally a portion of the second generation does not develop wings and produces more sexuparae, adding another generation of parthenogenic females that will

become alates. In either case, the last generation (males and oviparae) mates, the females lay eggs (usually one egg per female) and the cycle is completed by mid to late June.

Currently, broad-spectrum insecticides are the most commonly used control method for *M. abietinus* (McCullough and Fondren 1998, Bradbury and Osgood 1986). However, insecticide sprays to control *M. abietinus* in Christmas tree fields are often applied only after damaged shoots are detected (Kleintjes 1997a). This may kill some aphids and their natural enemies, but does not prevent or reduce damage to current year foliage (Kleintjes et al. 1999). Development of alternative control methods for insect pests in Christmas tree plantations has been relatively poorly studied, although interest in alternative methods using integrated pest management, selective insecticides, and biological control is increasing (Fondren and McCullough 2001, McCullough 1999, Kleintjes 1997b). The availability of alternative control options may become essential for the Christmas tree industry as pesticides are reviewed under the Food Quality Protection Act. Minor-use commodities such as Christmas trees are at particular risk of losing registrations for commonly used pesticides (DiFonzo and McCullough 1998).

Christmas tree plantations are capable of supporting a complex of natural enemies due to their year-to-year stability and relative lack of disturbance compared to annual agricultural crops (McCullough 1999, Raupp et al. 1992). This makes conservation biological control a feasible option in tree plantations, and several conservation methods have been suggested (McCullough 1999, Räther and Mills 1989). However, augmentative biological control has not been evaluated and is rarely used in Christmas tree plantations.

The natural enemies of the balsam twig aphid tend to appear well after the fundatrices hatch in the early spring, allowing them to mature with relatively little predation pressure until the second generation is already feeding on the new foliage. This makes predation alone unlikely to prevent shoot damage (Kleintjes 1997b, Räther and Mills 1989, Fondren and McCullough, unpubl. data). The most common natural enemies observed preying on *M. abietinus* include syrphid fly larvae (Diptera: Syrphidae), several coccinellids (Coleoptera: Coccinellidae), chrysopids (Neuroptera: Chrysopidae), and hemerobiids (Neuroptera: Hemerobiidae) (Berthiaume 1998, Kleintjes 1997b, Nettleton and Hain 1982, Varty 1966, Saunders 1969). No parasitoids are known to attack *M. abietinus* in North America (Räther and Mills 1989).

We were interested in evaluating an augmentative release of a commercially available predator for control of *M. abietinus*. Of the natural enemies associated with *M. abietinus* in the field, only chrysopids and coccinellids were commercially available. We decided to experiment with *Chrysoperla rufilabris* Burmeister larvae. This generalist species is commercially available, and *C. rufilabris* larvae have successfully controlled a closely related aphid (*Mindarus kinseyi* Voegtlin) on white fir seedlings (*Abies concolor* [Gord. & Glend.] Lindl.) in California (Nordlund and Morrison 1990, Ehler and Kinsey 1995). Eggs of *C. rufilabris* are also commercially available, but have often been shown to provide inadequate control (Dreistadt et al. 1986, Ehler and Kinsey 1995).

Our objectives in this study were to: 1) describe the natural enemy complex of *M. abietinus* present in unsprayed fir Christmas tree fields; 2) assess the effectiveness of *Chrysoperla rufilabris* larvae as predators of *M. abietinus* in the laboratory and in field

cages; and 3) evaluate the potential effectiveness of *C. rufilabris* in an open field release, as might be done on a commercial Christmas tree plantation.

Methods

Study sites. This research was conducted in 1999, 2000 and 2001 on three commercial Christmas tree farms in Lower Michigan. Fields were located in Antrim County (44°59'N, 85°06'W), Grand Traverse County (44°32'N, 85°29'W), and Ingham County (42°44'N, 84°33'W), and consisted of either balsam fir or a mixture of balsam and Fraser fir. Trees in each field were approximately 7-8 years old in 1999. No insecticides, fertilizers or irrigation were used in any field during our study. All fields were planted at the standard 1.83m by 1.83m (6 by 6 ft) spacing. Preliminary studies in 1998 and 1999 (Fondren and McCullough, unpubl. data) indicated that a *M. abietinus* population was present in each field.

Objective 1. Natural enemy complex. We began to monitor natural enemy activity in each field early in the spring, before aphid eggs hatched. We visited each field weekly throughout the aphid's life cycle, from late March or early April through late June or early July, to survey predators and monitor the development of the aphid population.

In April 1999, we selected 40 trees in each field based on the presence of needle curling damage on the year-old shoots. From these 40 trees, we randomly selected ten trees each week during the aphid life cycle and clipped branch tips at midcrown level in two randomly selected cardinal directions on the tree. These clipped branch tips were

immediately placed in sealed plastic bags, taken to the laboratory in a cooler and filled with 70% ETOH. They were stored in the laboratory until they could be examined (per Varty 1966, Ehler and Kinsey 1985). When time permitted, each branch tip was dissected under the microscope, and *M. abietinus* and natural enemies were removed with a fine brush. The number and stage of aphids and natural enemies were recorded. Although this method allowed us to get a close approximation of the actual numbers of aphids and predators on a given branch tip, it favored the discovery of small predators such as the larvae of syrphid flies, chrysopids, hemerobiids and coccinellids. Adult predators that could take flight as shoots were clipped were not well represented. Also, the clipped branch method was destructive, and could have eventually affected the appearance and value of the trees. Therefore, we decided to use an additional method for sampling aphids and natural enemies that was not destructive.

In 1999, we used a non-destructive method ('the beat method')(Kleintjes et al. 1999) to sample aphids and natural enemies from a random sample of ten trees in each field, in addition to the 40 labeled trees. The ten trees were selected at 20 m intervals along diagonal transects through each field, regardless of aphid damage. Natural enemies and aphids were sampled weekly on the ten trees by rapping the midcrown foliage on two aspects three times with a dowel, and counting the number of aphids and predators that fell onto black cotton cloth in a 22.9 cm diameter embroidery hoop. The midcrown level was chosen because *M. abietinus* tends to colonize the midcrown level most consistently (Nettleton and Hain 1982). To add another aspect to our survey of natural enemies in 1999, we also inspected each face (cardinal direction) of 20 trees per field each week

from 17 May to 21 June, and recorded the number of adult coccinellids, larval coccinellids, lacewing larvae, and syrphid larvae that were visible on each face.

The 'beat method' was more efficient for aphid and natural enemy sampling than the clipped branch method, and was adopted for all sample trees in 2000 and 2001. In 2000, we sampled all labeled trees (total of $n = 50$) in each field weekly using this method. However, this sample size was time consuming. We plotted the mean and variance of the number of aphids sampled per tree and determined that a sample size of 30 trees in 2001 would give us adequate information. Therefore, in 2001, we randomly selected 20 trees from among the 40 trees with aphid damage that we had originally marked in 1999, and continued weekly sampling of the other ten trees using the beat method.

In 2000 and 2001, we also set unbaited yellow sticky traps (22.9 cm x 28.0 cm) (Pherocon AM unbaited, Trécé Inc., Salinas, CA) in each field each week, to trap adult flying predators. Yellow sticky traps have been previously used to collect adult chrysopids, coccinellids and syrphid flies (Neuenschwander 1984, Ricci 1986, Bowie et al. 1999). Four to five traps, widely spaced throughout the field, were tied to an open tree branch at the top or midcrown level so that the trap could hang freely. Traps were set out and collected weekly in 2000 and biweekly in 2001, from early April to mid July. Traps were soaked in Histo-Clear II (National Diagnostics, Atlanta, GA) to free the insects. All of the insects present on the traps were removed in the lab, and adult chrysopids, hemerobiids, coccinellids and syrphids were counted and identified to species when possible.

We identified adult Chrysopidae to genus using Brooks and Barnard (1990), and *Chrysoperla* species were identified using Brooks (1994). Adult Hemerobiidae were identified to genus using Oswald (1993), and *Hemerobius* species were determined with Klimaszewski and Kevan (1985). Coccinellids were identified to species using Gordon (1985). Adult syrphid flies were identified with Vockeroth (1969). Voucher specimens were deposited in the A.J. Cook Arthropod Research Collection, Michigan State University, voucher number 2002-01.

Objective 2: Evaluation of *C. rufilabris* in lab and field cages. *Chrysoperla rufilabris* larvae were ordered from Beneficial Insectary, Oak Run, CA. First instar larvae were shipped overnight in plastic bottles of 1,000 larvae mixed with rice hulls. The bottles were packed in a box with small ice packs to keep them cool.

Laboratory trials. In 2000, we tested *C. rufilabris* in the laboratory to determine if it would readily consume *M. abietinus*. This was important because even generalist chrysopids may dislike some prey (New 1975, Tauber et al. 2000). *Mindarus abietinus* are relatively small aphids and are covered with a white waxy substance, perhaps dissuading some potential predators.

The number of *M. abietinus* that *C. rufilabris* larvae would consume was determined by placing an uninfested branch tip of *Abies balsamea* in a water pic set upright in a 30 x 30 x 30 cm mesh cage. One second-instar *C. rufilabris* was placed on the branch tip and provided with 5, 10, 20, or 35 *M. abietinus* per day. Aphids were collected from infested fir trees in the Ingham County field and were usually fourth-instar sexuparae, the most abundant stage and largest morph (Varty 1968). *Chrysoperla rufilabris* larvae were observed daily and the number of days to the third stadium and

pupation were recorded. Live adult *C. rufilabris* were killed immediately with carbon dioxide and weighed. Only nine adult *C. rufilabris* were found alive; the others could not be weighed accurately because dessication begins very rapidly after death.

Field cage trials. On 22/23 May 2000, we selected 30 trees with a heavily infested branch and 30 trees with a moderately infested branch in the Ingham County field (60 trees total). At that time, the aphids were still 25% sexuparae, with the rest in the sexuales stage (males and females). Heavily infested branches were defined as having more than 65% of the current-year shoots heavily damaged by *M. abietinus* (30% or more of the needles curled). Moderately infested branches had less than 50% of the shoots damaged (30% or more of the needles curled). These branches were caged with grey nylon mesh sleeve cages. Predators observed on the branches were removed before the cages were closed by tying a string around the end of the cage. Each moderately and heavily infested branch was randomly assigned to one of three treatments: zero, one, or five *C. rufilabris* larvae, for a total of 10 trees of each treatment in each of the two groups. Lacewing larvae were added to the cages on 23 May, by gently placing them on the foliage of the branches with a camelhair brush. When five larvae were added, they were placed in different spots rather than all in one place. The cages were checked occasionally to make sure other predators did not get in. After aphid oviposition, we clipped the branch tips with the cages and returned them to the laboratory.

In 2001, methods were modified slightly. First, we chose 30 infested (≥ 1 stem mother present in a beat sample) trees in the Ingham County field just after budbreak on 1-2 May. Three infested branches were selected on each tree. To make sure a branch tip was infested, we observed at least one stem mother on it. Of these three branches, one

was tagged but not caged, one was caged but the end was left open (per Grasswitz and Burts 1995), and one was caged, predators were removed, and the cage was closed (any remaining predators were removed on 8 May). Our results in 2000 had indicated no significant differences between one and five lacewings per cage, so on 15 May, when aphids were all sexuparae, only one lacewing was added to 15 of the 30 closed cages and to 15 of the 30 open cages. The open cages that received a lacewing were cleared of other predators and then the cage was closed.

In both 2000 and 2001, branch tips from each of the cages each year were clipped and returned to the laboratory following aphid oviposition in mid-summer. The density of aphid eggs per cm of current-year foliage was used to estimate aphid density. The total number of eggs found in each sample was divided by the total length of the current-year shoots found in each sample.

Objective 3: Field test of *C. rufilabris*. In 2001, we conducted open releases of *C. rufilabris* in all three fields. In each field, we selected 20 infested trees at opposite ends of the section of trees, approximately 100 m apart. The initial aphid density at each end of the field was similar. Lacewings were released at one end of the field while the trees at the other end served as controls. In each field, the rows ran from north to south. Lacewings were released on the trees in the south end on a calm day to reduce the likelihood of drift. We used first instar *C. rufilabris* larvae shipped from Beneficial Insectary (Oak Run, CA) in bottles of 1,000 each, mixed with rice hulls. We released approximately 25 larvae (1/20 of a bottle) onto each tree in the lacewing treatment group by shaking a premeasured amount of the lacewing and rice hull mixture onto the upper canopy of the tree. The possibility of larvae dispersing among trees was limited because

trees were spaced widely and there was no contact between foliage on adjacent trees. In addition, chrysopid larvae tend to have a negative geotactic response (New 1975), which likely further limits dispersal to neighboring trees.

Samples of *M. abietinus* were taken in each field weekly using the beat sample method, a few days before treatment and for two weeks afterwards. In Ingham County, we took pretreatment aphid samples on 10 May 2001. In the other two fields, pretreatment samples were taken on 14 May. All aphids were in the second generation on the release dates. In Ingham County, the release took place on 16 May 2001. In Grand Traverse and Antrim Counties, the first lacewing release was on 18 May.

In Antrim and Grand Traverse Counties, we released additional *C. rufilabris* larvae on 30 May, because we had observed very high populations of *M. abietinus* on 23 May, especially in Antrim County. We replicated this second release in Grand Traverse County because aphid phenology and degree day accumulation were similar to Antrim County. The second release allowed us to see if a larger number of *C. rufilabris* would be necessary to effect a change in the aphid population. We added approximately 400 lacewing larvae (1/2 bottle) to ten trees randomly selected from the 20 trees that received the first lacewing release.

In addition to sampling the aphid population using the beat method, we counted aphid eggs after oviposition had occurred. We clipped branch tips from the midcrown level of each of the trees used in the open release experiment and examined them under a microscope. Number of aphid eggs and the length of all current-year shoots was recorded. Aphid egg density was expressed as the total number of eggs per cm of

current-year foliage. Length and width of each sample branch tip was also recorded, so egg density could also be expressed as aphid eggs per cm².

Statistical Analysis. The number of eggs/cm in the field cages in 2000 was square root transformed to meet the assumptions of ANOVA. In 2001, the number of eggs per cm² of new foliage in the field cages was transformed by taking the natural logarithm of the square roots. When the global ANOVA was significant, means were separated using Fisher's Least Significant Difference. For t tests, variances were tested for homogeneity; if equal, pooled t tests were used. If variances were not homogeneous, the Welch test was used (Welch 1938). Differences between one and five lacewing larvae per cage in 2000 were tested with pooled t tests ((PROC TTEST, SAS v8)(SAS Institute, 1999)).

The number of eggs/cm of new foliage in the open field lacewing releases was tested for normality using the Shapiro-Wilk test (PROC UNIVARIATE, SAS v8). Given normality of the treatment groups, analysis of variance or t-tests were used to detect treatment differences. In Ingham County, the Kruskal-Wallis test was used to determine differences in the egg density on trees treated with lacewing larvae (Sokal and Rohlf 1995). All tests were conducted at a significance level of $\alpha = 0.05$.

Results

Objective 1. Natural enemy complex. Syrphid larvae were rarely observed until shoots were dissected. Many syrphid larvae were found in the 1999 branch tip

samples and abundance of syrphid larvae fluctuated over time in synchrony with the number of aphids in the samples (Figure 1). Although syrphid larvae could not be identified to species, we were able to rear some to the adult stage in the laboratory. We also collected adult syrphid flies hovering near or on fir trees whenever possible. Genera of adult syrphids found or reared out included *Eupeodes* spp., *Syrphus* spp., *Sphaerophoria* spp., *Allograpta obliqua*, and *Toxomerus* spp. Each of the genera found had also been previously reported in association with *M. abietinus* or the closely related *M. kinseyi* Voegtlin (e.g. Ehler and Kinsey 1995).

In 1999, we combined the number of predators found in the beat samples (n = 10 trees per field) with visual observations of the number of predators visible on each tree. The results were variable among fields (Figure 2). In Ingham County, the abundance of larval coccinellids observed was 200% and 300% higher than other predators on 30 May and 7 June, respectively. In Grand Traverse County, the number of larval coccinellids was also high--130 on 8 June and 87 on 15 June, compared to a total of 6-18 other predators. The number of adult coccinellids observed in Grand Traverse County was higher than in Ingham County (a high of 91 in Grand Traverse Co. vs. a high of 5 in Ingham Co.). In Antrim County, the number of predators observed in visual counts was low, but lacewing larvae were most frequently observed, especially in beat samples.

We observed seven species of coccinellids in association with *M. abietinus* infestations, including *Harmonia axyridis* (Pallas), *Anatis mali* (Say), *A. labiculata* (Say), *Mulsantina picta* (Randall), *M. hudsonica* (Casey), *Coccinella trifasciata perplexa* Mulsant, and *C. transversoguttata richardsoni* Brown. Table 1 shows the first and last date of collection for each of these species in each field. The most abundant coccinellid,

in general, was *Harmonia axyridis*, an introduced coccinellid. The native coccinellid *Anatis mali* was relatively common, but far outnumbered by *H. axyridis*. *Harmonia axyridis* is well known to overwinter as adults, and in the field we observed adult *H. axyridis* as soon as weather warmed in the spring.

In the beat samples in 2000, lacewing larvae (both Chrysopidae and Hemerobiidae) were some of the most common predators present (Figure 3). Syrphid larvae were also relatively common. Notably, most predators did not appear in the beat samples in 2000 until approximately three weeks after the stem mothers had hatched. Hemerobiid adults were usually among the first aphid predators to be observed in the beat samples, although their numbers remained low, probably because the sampling method favored less mobile insects. We identified the hemerobiid adults as *Hemerobius stigma* Stephens, a common species known to prefer arboreal habitats and conifers in particular (Throne 1971, Stelzl and Devetak 1999). *Hemerobius stigma* overwinters as adults (Klimaszewski and Kevan 1985).

In 2000, the sticky traps caught several of the same predators. Adult hemerobiids were among the first aphid predators to appear, particularly in Ingham and Antrim Counties (Table 2). As in the beat samples, the most abundant hemerobiid was *H. stigma*. Adult chrysopids were also present in each field, although they were usually caught on the sticky traps later in the season. The most abundant species was *Chrysopa oculata*, which is predaceous as an adult as well as a larva (most green lacewings are not predaceous as adults). Syrphid adults found ovipositing or hovering near aphid colonies, or collected on sticky traps, were identified to species where possible. All were members of the subfamily Syrphinae, which are homopteran predators in the larval stage (Sadeghi

and Gilbert 2000). The most common genus found was *Syrphus* spp., followed by *Eupeodes* spp., *Allograpta obliqua* (Say), and *Sphaerophoria* spp. The adult coccinellids that could be identified from the 2000 sticky traps included several species present in more than one field (Table 3)

Bumble bees (*Bombus* spp.) were observed foraging on honeydew in 1999, 2000, and 2001, especially in Grand Traverse County. We did not take any samples or detailed observations, but we mention them here because it is highly unusual to observe large numbers of bumblebees foraging on aphid honeydew (Batra 1993). Future researchers may wish to address this phenomenon in more detail.

Objective 2: Evaluation of *C. rufilabris* in lab and field cages. *Chrysoperla rufilabris* larvae readily consumed *M. abietinus* in laboratory trials. To complete development on a diet of only *M. abietinus*, *C. rufilabris* larvae required at least 10 aphids per day. Larvae in the cages consumed the maximum of 35 aphids per day that we provided. Lacewings fed five aphids per day survived to the third instar but did not pupate. Only ten adult lacewings were weighed successfully, but the average adult weight increased with the number of aphids consumed. On ten aphids per day, the average weight of the four adults was $4.42 \text{ mg} \pm 0.59$. The average weight of the two adults reared on 20 aphids per day was $4.90 \text{ mg} \pm 2.27$. On 35 aphids per day, the average adult weight of four adults was $6.39 \text{ mg} \pm 1.08$.

2000 field cages. In field cages in 2000, results differed between moderate and heavily infested branches. On the heavily infested branches, the mean egg density in cages with no lacewing was nearly 50% higher than mean egg density in the cages with a lacewing, but the difference was not statistically significant ($F = 1.31$, $df = 2,23$; $p =$

0.29) (Figure 4). This may be due to the very high variability of the egg densities. On the moderately infested branches, mean egg density was significantly lower in the presence of a *C. rufilabris* larva ($F = 3.75$, $df = 2,20$; $p = 0.04$). There was no significant difference in the number of eggs/cm between one and five lacewings per cage at either the heavy density ($t = -0.57$, $df = 16$, $p = 0.58$) or the moderate density ($t = 1.15$, $df = 13$, $p = 0.27$).

2001 field cages. In 2001, the presence of one lacewing larva in a cage did not significantly decrease egg density in either type of cage ('closed' or 'open'). In the cages that had been open and accessible to predators before lacewings were added, no significant difference was observed in egg density between cages with and without a lacewing ($F = 0.11$; $df = 1,27$; $p = 0.74$) (Figure 5). However, the standard errors, especially in the 'no lacewing' treatment, were relatively high. Similarly, the difference between egg density in the closed cages with or without a lacewing was not significant ($F = 0.35$; $df = 1,28$; $p = 0.56$). The variability in the closed cages, as indicated by the standard errors, was extremely high.

Objective 3: Field test of *C. rufilabris*. Pre-treatment aphid density in Ingham County was relatively high (mean of 35 ± 2.4 aphids sampled per tree) compared to Grand Traverse County (Figure 6). In Ingham County, there was no significant difference in aphid density between the two treatment groups on 10 May ($t = -1.35$, $df = 38$, $p = 0.19$) (Figure 6). A week after treatment, on 21 May, no significant difference was apparent between the two treatment groups ($t = 0.16$, $df = 38$, $p = 0.87$). On 4 June, however, aphid numbers on the lacewing-release trees were 36% lower than aphid numbers on the control trees ($t = -2.45$, $df = 38$, $p = 0.02$). In Ingham County, *M.*

abietinus egg density was also significantly lower on the trees treated with *C. rufilabris* (Kruskal-Wallis $H = 38.9$, $df = 1$; $p < 0.0001$) (Figure 7).

In Antrim County on 14 May 2001, aphid density was also relatively high (32 ± 4.5 aphids per tree). No significant difference in aphid density between treatment groups was observed on 14 May (four days before the first lacewing release) or on 23 May, five days after the first lacewing release (Figure 6). On 6 June, however, aphid numbers on the control trees were 66% higher than either lacewing treatment ($F = 12.05$; $df = 2,37$; $p < 0.0001$). This relationship remained consistent on 14 June ($F = 9.44$; $df = 2,37$; $p = 0.0005$). On both 6 June and 14 June, no difference in aphid sample numbers was observed between the trees that received one or two applications of lacewing larvae (Figure 6). The density of *M. abietinus* eggs was significantly lower on the trees treated with *C. rufilabris* ($F = 21.9$; $df = 2,160$; $p < 0.0001$) than on untreated trees, although there was no significant difference in egg density between the trees treated with one or two applications of *C. rufilabris* ($t = -0.39$, $df = 82$, $p = 0.70$) (Figure 7).

In Grand Traverse County on 14 May 2001, average aphid density was lower and relatively more variable than in the other two counties (mean of 15 ± 4.1 aphids per tree), although differences in aphid density among treatment groups were not significant (Figure 6). Differences among treatments were not observed until 14 June, two weeks after the second lacewing release. On 14 June, aphid numbers on the control trees were significantly lower than either lacewing treatment ($F = 4.02$; $df = 2,37$; $p = 0.03$), but the difference was small, approximately 7 aphids per tree. Egg density was not significantly different among the three treatment groups ($F = 1.83$; $df = 2,74$; $p = 0.17$).

Discussion

Objective 1. Natural enemy complex. *Mindarus abietinus* is associated with a diverse complex of natural enemies in Michigan Christmas tree fields. Our observations in the three Christmas tree fields confirm other reports in the literature of known predators of *M. abietinus* (Berthiaume et al. 2000, Kleintjes 1997b, Nettleton and Hain 1982, Ehler and Kinsey 1985). However, while several predators attack *M. abietinus*, they do not always keep the aphid below damaging levels (Räther and Mills 1989). One reason for this may be the early start of the aphid life cycle; the stem mothers (fundatrices) are present at a time when few predators are active. When fundatrices begin to reproduce, the rate of increase is so high (average fecundity 22-41 offspring (Varty 1968)) that predators may not be able to reduce the population before damage occurs.

Another reason for the lack of early season natural enemy activity may be the ability of *M. abietinus* to survive cold temperatures and late frosts in early spring. *Mindarus abietinus* exhibits a remarkable ability to survive in adverse conditions; for example, *M. abietinus* likely evolved in the boreal forests, and the brief life cycle of most *Mindarus* probably reflects an adaptation to short subarctic summers (Hille Ris Lambers 1966). In Michigan, the growing season is relatively longer than in the far north, and the complex of natural enemies that prey on *M. abietinus* in Michigan tend to have longer life cycles and generation times. Also, most are generalists not tightly tied in to the *M. abietinus* cycle. One exception may be the coccinellid *Anatis mali*, an indigenous coccinellid that is associated with coniferous forests and tends to become active relatively

early in the spring (Berthiaume 1998). Another may be the syrphid *Allograpta obliqua*, which we observed ovipositing directly in active colonies of *M. abietinus*.

The brief life cycle of *Mindarus* presents a challenge for conservation biological control strategies. With an aphid that completes the cycle from egg hatching to oviposition only once per year in a relatively brief time span, predators may not have the opportunity to mount a density-dependent response to a rapid increase in prey density. However, we did observe that the average number of aphid predators tended to increase as the aphid population grew, appearing too late to reduce damage but potentially affecting the aphid population for the next year. When we monitored uncaged branch tips in 2001, the density of aphid eggs on these branches was significantly lower (by 63%) than the egg density in the cages where natural enemies were excluded.

The apparent synchrony between aphid numbers and syrphid larvae on infested branch tips presents interesting possibilities. Adult syrphid flies use aphid colonies as cues for oviposition (Sadeghi and Gilbert 2000), and we observed this occurring on several occasions in the field. Syrphid flies may have a significant effect on aphid populations—larvae of *Syrphus ribesii* can eat up to 52 pea aphids per day (Chambers et al. 1983). Possibly, the impact of syrphid larvae has been underestimated, as they are not readily visible when feeding on aphids within curled needles, and tend to be nocturnal. Other studies on aphid predators have also recognized the potential to underestimate the impact of syrphid larvae preying on aphids (Chambers et al. 1983).

Objective 2. Effectiveness of *C. rufilabris* larvae in the lab and field cages.

Our results indicated that under some conditions, *C. rufilabris* is capable of reducing *M. abietinus* population levels. In the laboratory, *C. rufilabris* proved a voracious predator of *M. abietinus*. This agrees with Ehler and Kinsey's findings (1985).

Results in the field cages were varied. *Chrysoperla rufilabris* larvae did significantly reduce egg density in the moderately infested cages in 2000, but not significantly in the heavily infested cages, or in any cage in 2001. However, the mean egg density in the cages with no lacewing was often highly variable, which may have prevented us from seeing significant differences.

Although field cages have the benefit of confining a known number of predators to a given space, they also have disadvantages. In 2000, the heavily infested cages were literally sticky with honeydew and crowded with aphids; such extreme densities were probably much higher than would normally be observed. This may have prevented us from seeing an effect from the lacewing larvae in the cage. The very high density of aphids may have affected the reproductive capacity of the sexuparae (Chambers et al. 1983), which would also have affected the results. In 2001, we caged the branch tips early in the season, but were unable to add the lacewings until later, so the aphid density inside the cages grew to unnaturally high levels. We might have avoided this situation if we had added the lacewings earlier.

The uncaged, tagged branches in 2001 had significantly lower (by 33%) egg densities than caged branches with a lacewing. Of course, uncaged branches were exposed to natural enemies in the field, and the alatae produced on the uncaged branches had the opportunity to disperse while those on the caged branches did not. Nevertheless,

the mean egg density in cages with one lacewing was consistently lower than in cages with no lacewing, if not statistically significant. Further work is needed to describe the relationship of *M. abietinus* egg density to aphid populations the next year.

Objective 3. Open field release of *C. rufilabris*. In our open field releases, *C. rufilabris* effectively reduced the *M. abietinus* population in two out of three fields. The two fields with significant effects (Ingham and Antrim Counties) had relatively high populations of *M. abietinus*, indicating perhaps that the observable effect of *C. rufilabris* may be limited to higher densities. However, the effect may also have been apparent because variability among aphid densities in Ingham and Antrim Counties was lower than in the Grand Traverse County field. In addition, the small lacewing larvae may not have been able to search and disperse efficiently enough to have an effect on the relatively sparse population of *M. abietinus* in Grand Traverse County.

Another possible reason for the lack of significant effects in the Grand Traverse County field is the timing of our release, when aphids were already well into the second generation. We would have liked to release the lacewing larvae earlier, to test our hypothesis that early season release would be effective. However, practical considerations prevented this. Further research is needed to determine if *C. rufilabris* larvae can effectively reduce the aphid population if applied early in the year when fundatrices are present and fewer natural enemies are active. In the early spring in Christmas tree plantations, *M. abietinus* may be the only prey readily available to *C. rufilabris* larvae. *Mindarus abietinus* has a relatively short life cycle (about eight weeks from egg hatch to oviposition) making a relatively short-lived augmentative control method more feasible for *M. abietinus* than for an aphid that has several generations.

The lack of significant differences between one or two lacewing applications may have been due to several factors. The second release of lacewings took place a week later, possibly too late to cause a significant reduction in the aphid population. More likely, *Chrysoperla* larvae are well known to be cannibalistic, and the large number of larvae probably resulted in intraspecific predation. The higher competition pressure may also have resulted in lowered survival of the lacewings in the second release. On the other hand, this indicates that there may be a ‘threshold’ beyond which additional lacewing larvae will not increase the effectiveness. Further studies may be warranted to determine where this threshold occurs.

Practical applications. Although many studies have documented the potential of chrysopids to consume insect pests in the laboratory, the release of chrysopid larvae at commercially feasible rates has not been tested extensively (Tauber et al. 2000). Despite its potential for effective control, we recognize that augmentative releases of *C. rufilabris* larvae may not yet be economical for large plantations. However, they may be useful for smaller farms or even ornamental trees with patchy infestations of *M. abietinus*. Possibly, the emerging interest in organically grown Christmas trees may eventually provide a lucrative market for Christmas trees grown without pesticides.

The bottles of 1,000 *C. rufilabris* larvae that we used retail for an average of US \$30, including shipping. This is relatively expensive, since we used one bottle to treat 20 infested trees, and Christmas trees average 1200 trees per acre. However, *C. rufilabris* can be purchased as eggs (a card of 1,000 eggs sells for approximately \$5), and wholesale prices on bulk orders would likely be lower. We did not test the effectiveness of using eggs of *C. rufilabris* for control in the field, partly because other workers have reported

less success with eggs than with larvae (Daane et al. 1996, Daane and Yokota 1997, Ridgway and Murphy 1984), and eggs are vulnerable to predation by ants (Dreistadt et al. 1986). However, the lower cost of using eggs may warrant further investigation.

Mechanical methods such as backpack sprayers and tractor-pulled sprayers can be used for the release of *C. rufilabris* (Gardner and Giles 1996, 1997). Methods for the release of *C. rufilabris* larvae mixed with sawdust have been developed for use in cotton (Ridgeway et al. 1977, Kinzer 1976). Currently, a wide variety of release methods are being tested (Tauber et al. 2000), some of which may be potential candidates for use in Christmas trees. However, the effectiveness of mass-released lacewings can vary significantly with the release rate, release method, and even intraguild predation in the field (Tauber et al. 2000). Further refinement of the release methods and field tests are needed to determine an appropriate and cost-effective method of using lacewings in Christmas tree fields.

An interesting possibility with the augmentative use of *Chrysoperla* includes the judicious use of chemical insecticides, since many *Chrysoperla* species have shown resistance to common insecticides (Tauber et al. 2000, Rumpf et al. 1997, Ridgway and Jones 1968). Conservation of existing natural enemies can also be achieved by reducing the number of insecticide sprays. Many natural enemies such as adult syrphid flies consume flower nectar, and providing flowering plants nearby may help to encourage populations of natural enemies. An integrated management program combining conservation of existing natural enemies, effective timing of insecticide applications, and occasional use of *C. rufilabris* larvae can work to provide effective and environmentally sound control of *M. abietinus* in fir Christmas trees.

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Table 1. Potential predators of *M. abietinus* collected in the field in 1999 by hand or beat samples, from 14 April to 30 June

Insect	Field location	Date collected	
		First	Last
Adult Coccinellids			
<i>Harmonia axyridis</i> (Pallas)	Grand Traverse, Antrim, Ingham, Kalamazoo, Manistee	14 April	29 June
<i>Coccinella transversoguttata richardsonii</i> Brown	Grand Traverse, Ingham	27 April	26 May
<i>Anatis mali</i> (Say)	Grand Traverse	12 May	26 May
<i>Anatis labiculata</i> (Say)	Grand Traverse	26 May	26 May
<i>Mulsantina picta</i> (Randall)	Antrim	27 April	27 April
<i>Mulsantina hudsonica</i> (Casey)	Grand Traverse	15 June	15 June
<i>Coccinella trifasciata perplexa</i> Mulsant	Grand Traverse	26 May	26 May
Adult Syrphids			
<i>Eupeodes</i> spp.	Ingham	16 May	16 May
<i>Sphaerophoria</i> spp.	Kalamazoo	25 June	25 June
<i>Toxomerus marginata</i>	Antrim	30 June	30 June
Larval Syrphids^a			
<i>Allograpta obliqua</i> (Say)	Ingham	30 May	30 May
<i>Syrphus</i> spp	Manistee	1 June	1 June
<i>Eupeodes</i> spp	Ingham, Manistee	21 May	1 June
<i>Sphaerophoria</i> spp.	Ingham	30 May	30 May
Adult Lacewings			
Chrysopidae	Grand Traverse	1 June	22 June
	Manistee	1 June	1 June
	Kalamazoo	21 May	30 May
	Antrim	30 June	30 June
Hemerobiidae	Ingham	18 June	25 June

^a reared to adult in lab

Table 2. Adult predators found on the sticky traps in each field, 2000.

Field	Dates	# traps	Syrphids	Hemerobiids	Chrysopids	Coccinellids
Ingham County	13-22 April	4	12	8	0	0
	22-29 April	10	8	3	0	1
	29 April-7 May	8	0	19	1	7
	7-17 May	6	12	4	0	3
	17-28 May	9	10	2	0	1
	28 May-9 June	9	5	1	1	14
	9 June-22 June	10	34	0	0	24
	22 June-21 July	10	27	0	0	7
	4-18 April	4	9	0	0	0
	18-25 April	4	56	0	0	0
Grand Traverse Co.	25 April-2 May	4	4	2	0	1
	2-10 May	4	8	3	0	7
	10-17 May	8	9	6	0	3
	17-26 May	8	4	0	2	1
	26 May-5 June	6	3	3	2	2
	5-14 June	6	5	0	0	5
	14-21 June	7	2	3	0	1
	21-29 June	8	2	0	0	1
	4-18 April	4	0	0	0	0
	18-25 April	4	1	3	0	1
Antrim County	25 April-2 May	4	1	2	0	2

Table 2 cont'd.

Field	Dates	# traps	Syrphids	Hemeroibiids	Chrysopids	Coccinellids
Antrim County	2-10 May	4	4	3	0	4
	10-17 May	4	0	5	0	1
	17-26 May	4	6	0	0	1
	26 May-5 June	4	3	1	4	1
	6-14 June	4	1	0	0	2
	14-21 June	4	4	0	1	4
	21-29 June	4	4	0	0	4

Table 3. Species of Coccinellidae found on sticky traps in each field, 2000.

Species	County	Number collected	Date trap set out
<i>Anatis mali</i> (Say)	Grand Traverse	1	10 May
	Antrim	1	5 June
		2	5 June
<i>Coccinella novemnotata</i> Herbst	Grand Traverse	1	2 May
		1	10 May
<i>Coccinella transversoguttata richardsonii</i> Brown	Grand Traverse	1	26 May
		1	14 June
	Antrim	2	25 April
		2	2 May
	Kalamazoo	1	19 May
<i>Coccinella trifasciata perplexa</i> Mulsant	Grand Traverse	1	9 April
<i>Coleomegilla maculata</i> (Degeer)	Ingham	1	29 April
<i>Cycloneda munda</i> (Say)	Antrim	1	17 May
		1	26 May
		1	6 June
	Kalamazoo	1	25 May
		1	9 June
<i>Harmonia axyridis</i> Pallas	Ingham	2	29 April
		1	7 May
		1	17 May
	Grand Traverse	3	2 May
		3	10 May
		1	26 May
		1	5 June
	Antrim	1	25 April
		1	21 June
	Kalamazoo	1	9 April
		2	29 April

Table 3 cont'd.

Species	County	Number collected	Date trap set out
<i>Harmonia axyridis</i> Pallas	Kalamazoo	1	19 May
		1	9 June
<i>Hippodamia parenthesis</i> (Say)	Ingham	1	29 April
	Grand Traverse	1	25 April
	Antrim	2	18 April
		1	2 May
		1	26 May
<i>Mulsantina hudsonica</i> (Casey)	Grand Traverse	1	25 April
<i>Mulsantina picta</i> (Randall)	Grand Traverse	2	2 May
		2	10 May
	Antrim	1	2 May

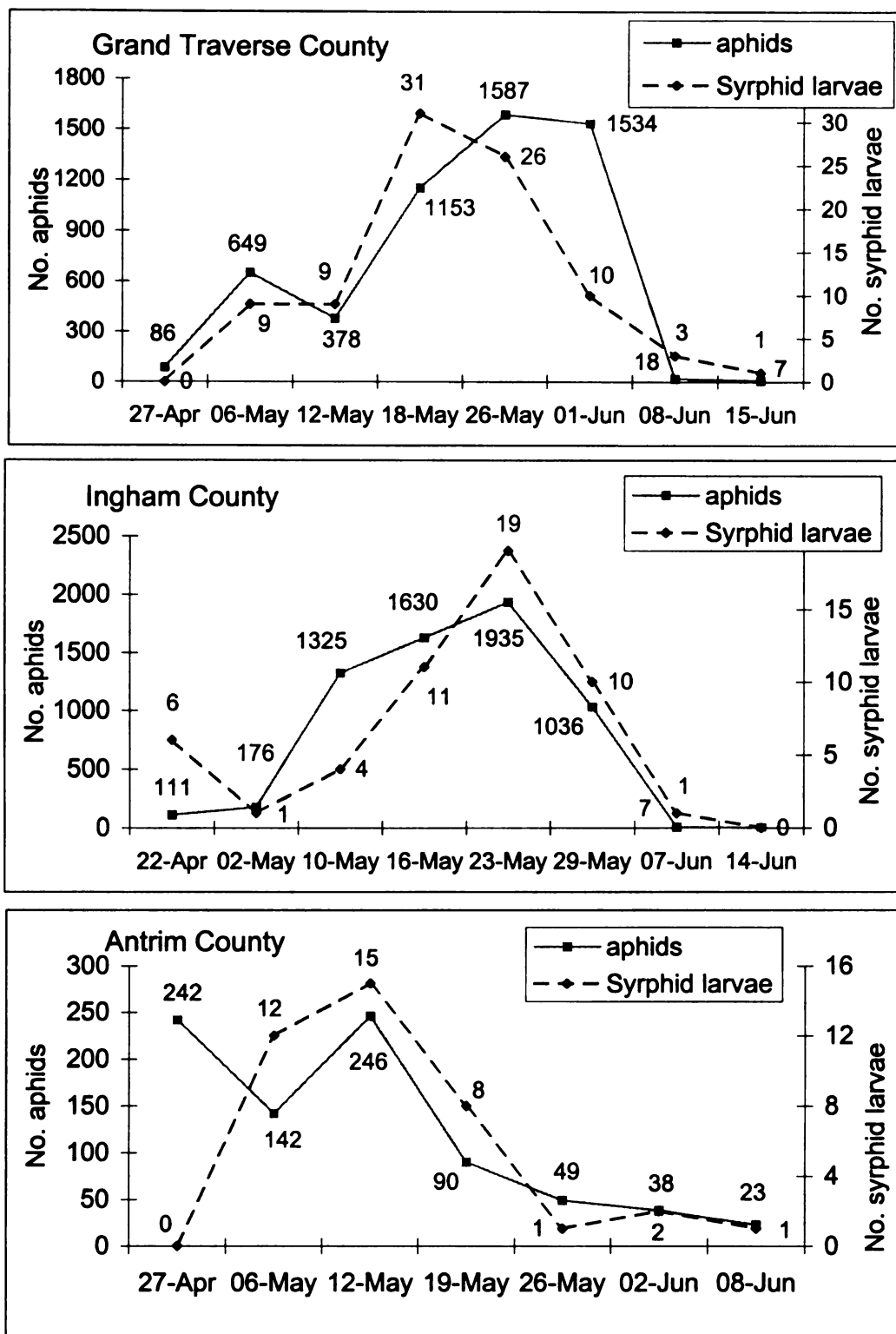


Figure 1. *Mindarus abietinus* and syrphid larvae counts in clipped branch tip samples, 1999.

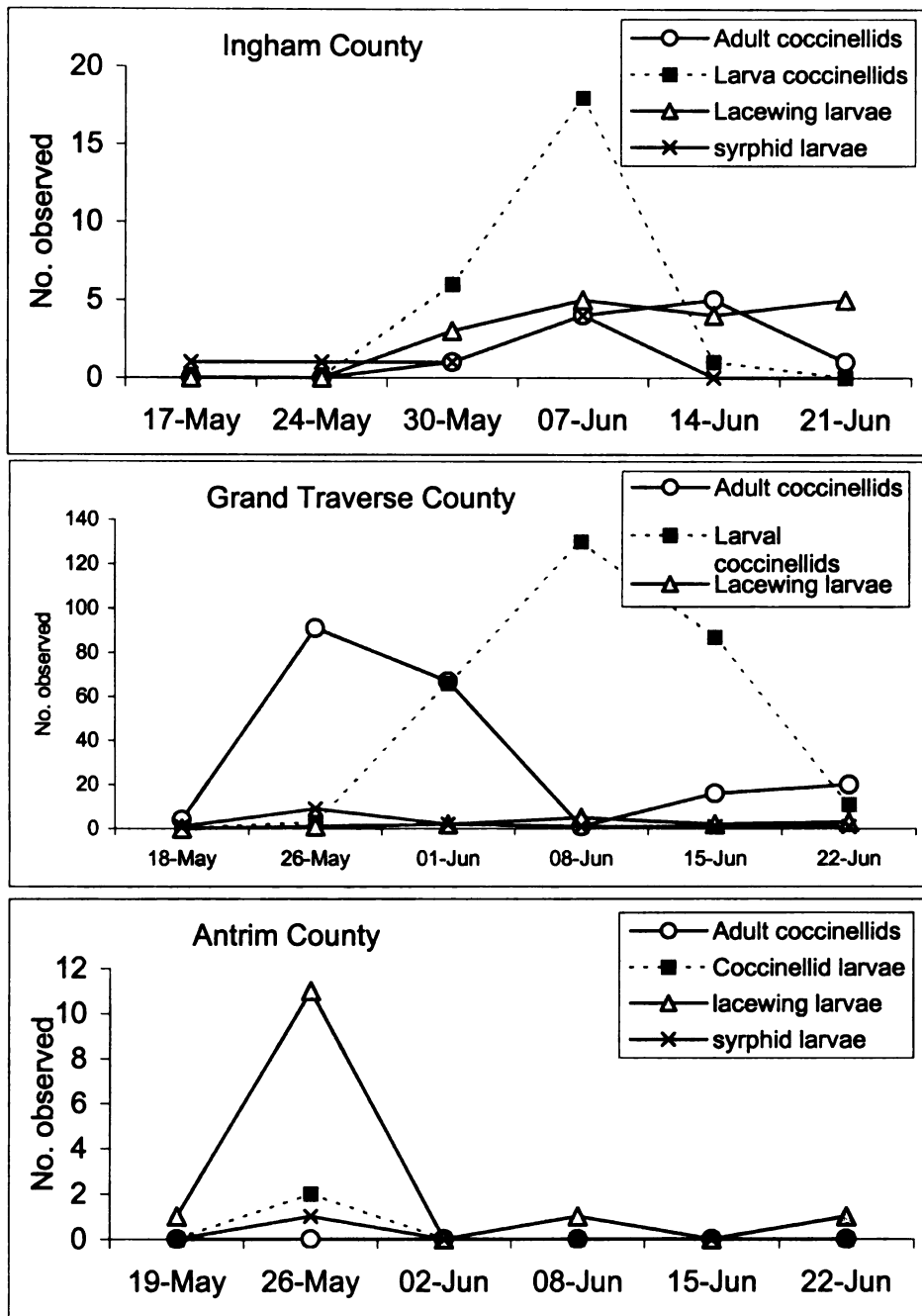


Figure 2. Numbers of coccinellids, lacewings and syrphids found in beat and visual examinations of sample trees, 1999. In Antrim Co. in 1999 no survey was done on 2 June because of rain. On 15 June a survey was done but no predators were observed.

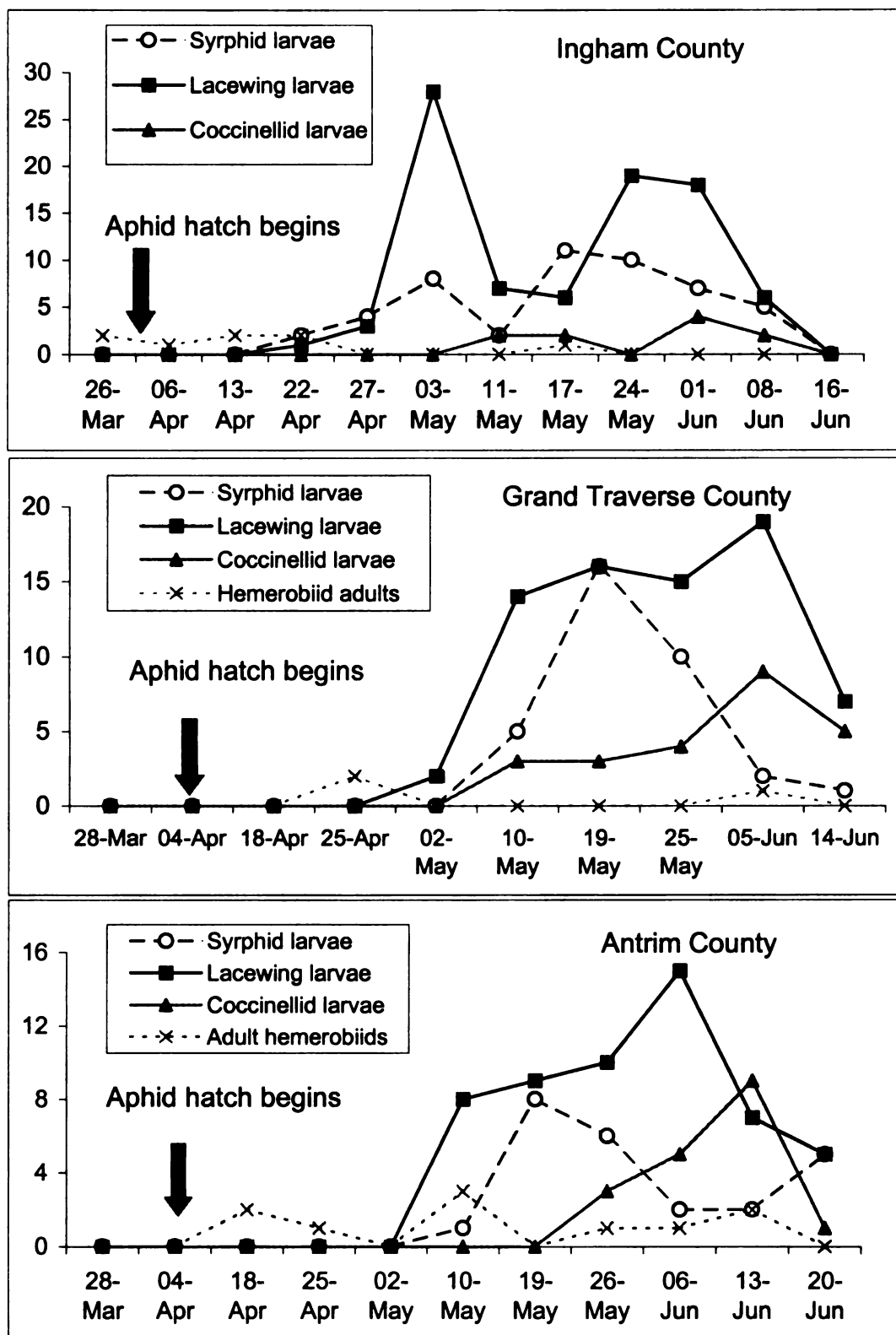


Figure 3. Predators found in beat samples, 2000. Data are presented as the number of each taxon collected per sample date.

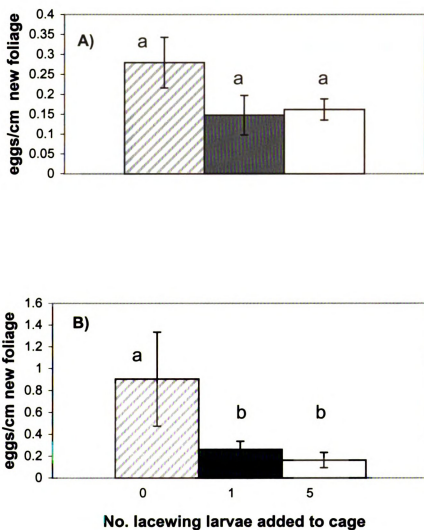


Figure 4. Number of *M. abietinus* eggs per cm of current-year shoot growth (mean $\pm$ 1 SE) on branch tips from field cages in 2000. A) Heavily infested branches (n = 26). B) Moderately infested branches (n = 23).

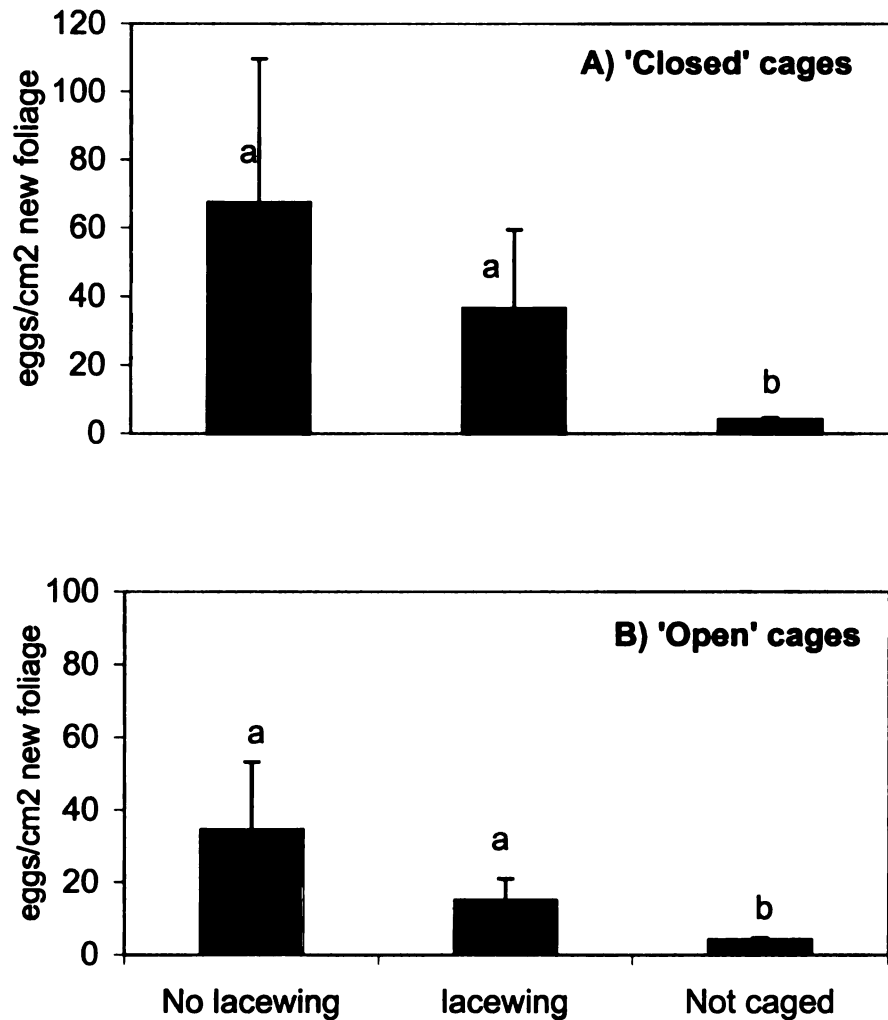


Figure 5. Mean ($\pm$ SE) number of aphid eggs/cm² of current-year foliage in 2001 field cages. Letters above bars indicate significant differences. A) 'closed' cages were kept closed before lacewings were added; B) 'open' cages had one end left open until lacewings were added.

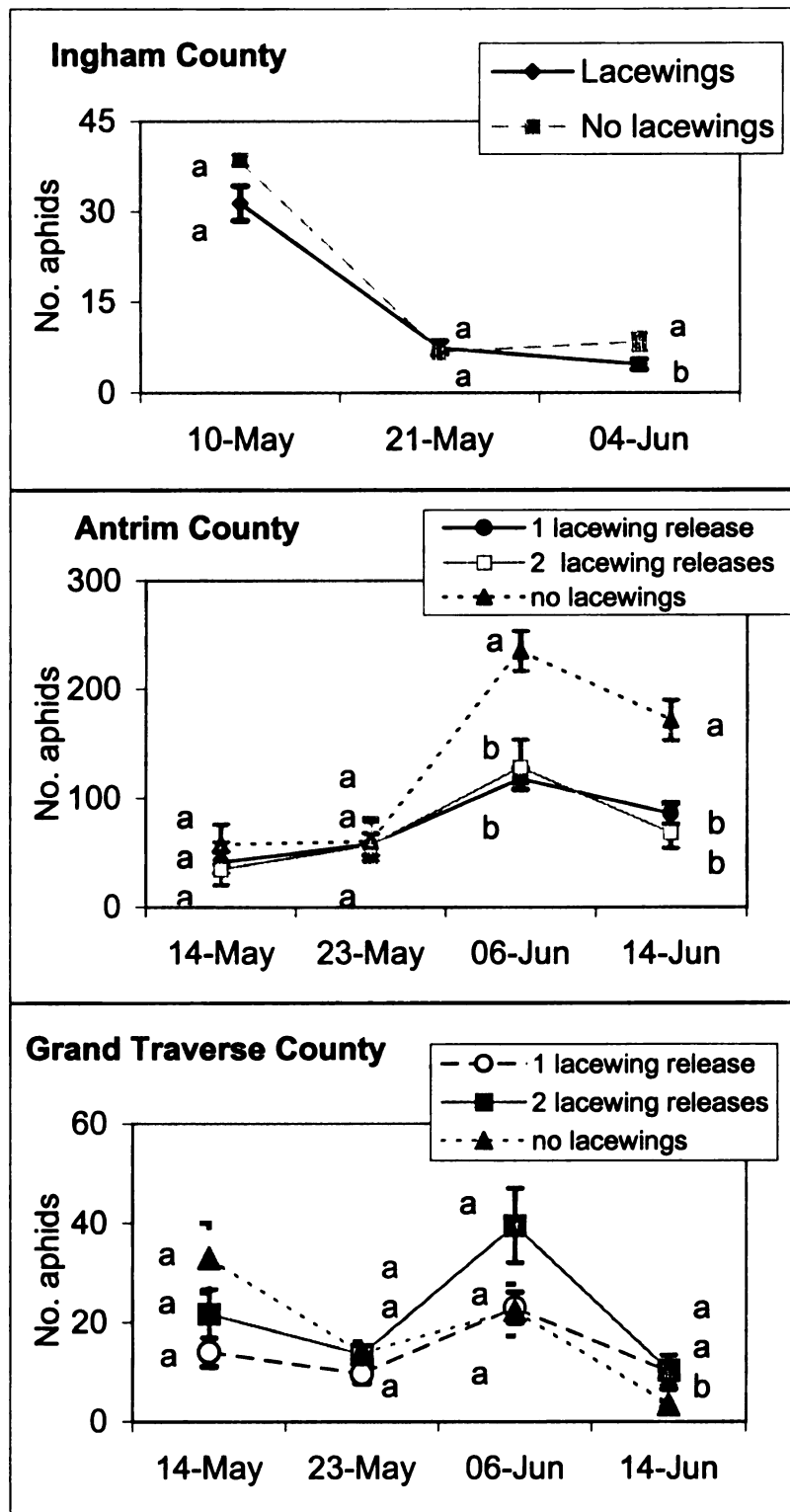


Figure 6. Mean ($\pm$ SE) aphid counts before and after the lacewing applications in the open field releases in 2001. Data within a column marked with different letters are significantly different ($p < 0.05$).

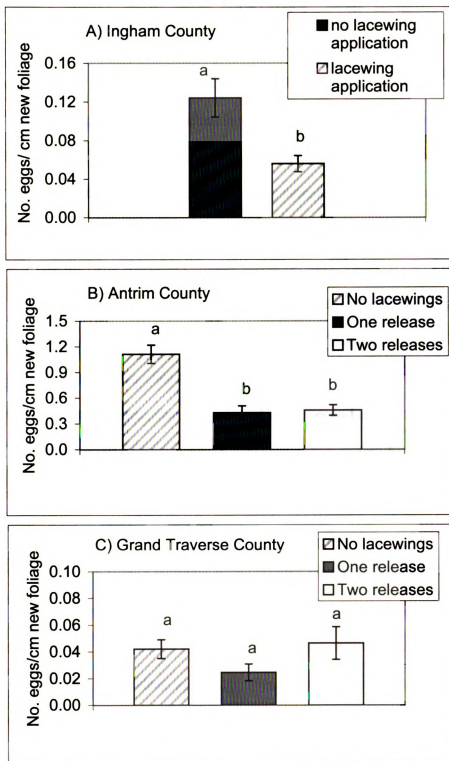


Figure 7. Mean ($\pm$ SE) density of *M. abietinus* eggs on new foliage in open releases of *C. rufilabris* larvae in 2001.

Chapter 3

Phenology and natural enemies of pine needle scale (*Chionaspis heterophyllae* (Fitch)) (Homoptera: Diaspididae) in Christmas tree fields

Abstract. Our objectives in this study were to: 1) determine the phenology of the second generation of pine needle scale in calendar days and degree-day accumulations; 2) characterize the natural enemy complex acting on the 2nd generation of pine needle scale in Christmas tree fields in Michigan; and 3) determine the rates of predation and parasitism on pine needle scale infestations on commercial Scotch pine (*Pinus sylvestris* L.) Christmas tree fields. We monitored scale populations in three counties in Lower Michigan for three years. The development of the second generation of pine needle scale was more consistently associated with cumulative degree-days base 10°C (50°F) (DD₅₀) than calendar date. Egg hatching extended for a period of approximately three weeks. The second instar (the hyaline stage) was the predominant stage present at roughly 1500 DD₅₀. The natural enemy complex acting on pine needle scale was similar in all fields and years. The coccinellids *Chilocorus stigma* (Say) and *Microweisla misella* (LeConte) were most frequently found in all fields. An endoparasitic wasp, *Encarsia bella* Gahan (Hymenoptera: Aphelinidae), and a hyperparasitoid, *Marietta mexicana* Howard (Hymenoptera: Aphelinidae), were also present. Predaceous mites were observed but not monitored closely. The percentage of predation was high in unsprayed fields in 1999, averaging 70% predation and leaving few adult females to overwinter. Parasitism rates varied among fields and in different months.

Introduction

The pine needle scale (*Chionaspis pinifoliae* (Fitch)) is native to North America and has a relatively wide host range, including 28 species of conifers in the genera *Pinus*, *Picea*, *Tsuga*, *Abies*, *Cedrus*, *Taxus*, and *Toreya* (Zahradník 1990, Luck and Dahlsten 1974). A nearly identical insect, *C. heterophyllae* (Cooley) ('pine scale') shares at least 14 host plant species, primarily *Pinus* species (Shour 1986). *Chionaspis heterophyllae* is native to the eastern United States, including Michigan (Shour and Schuder 1987). The two species are nearly morphologically identical, and have extremely similar life histories; several papers that have been published using the species name *C. pinifoliae* were later determined to actually be *C. heterophyllae* (i.e. Nielsen and Johnson 1972 and 1973, Walstad et al. 1973; per Burden and Hart 1989). Our study populations were originally designated *C. pinifoliae*, but have since been identified as *C. heterophyllae* (courtesy of Douglass Miller, Systematic Entomology Laboratory, Agricultural Research Service, USDA). We will refer to our study organism as 'pine needle scale', to be consistent with the literature and in light of the extreme similarity of the two insects.

In managed settings such as nurseries, tree plantations, and ornamentals, pine needle scale can become a major problem (Tooker and Hanks 2000, Johnson and Lyon 1988, Sheffer and Williams 1987). Pine needle scale can be found in the forest, but it rarely if ever becomes a noticeable pest there (Burden and Hart 1993, Cooper and Cranshaw 1999, Ruggles 1931). It is one of the most common insect pests of Scotch pine (*Pinus sylvestris* L.) grown for Christmas trees in Michigan (McCullough and Fondren

1998). In a 1998 survey of Michigan Christmas tree growers, the number of acres treated and number of sprays for this insect alone exceeded those for any other insect pest in Scotch pine Christmas trees (McCullough and Fondren 1998). On Christmas trees, light to moderate populations of pine needle scale can reduce the value of trees due to the white scale armor that remains on the needles even after the insects die. High populations can cause needles or even branches to die (Kosztarab 1990, Walstad et al. 1973, Cumming 1953).

Life cycle. The number of generations of pine needle scale can vary with geographic location and other factors such as temperature, host plant, density, and local climactic conditions (Shour 1986, Nielsen and Johnson 1973, Luck and Dahlsten 1974). Univoltine populations are reported from relatively cold climates such as upstate New York and Saskatchewan (Cumming 1953, Gambrell 1938). Bivoltine populations are most common throughout most of the United States including Lower Michigan (Shour 1986). Most commonly, the scale insects overwinter as eggs that hatch in early spring, around the time that lilacs bloom in May (Mussey and Potter 1997, Herms 1990). The pinkish newly hatched crawlers move about for a few days before “settling” or inserting their stylets into a needle on the host plant. After the crawlers settle, they undergo a first molt after approximately ten days. This stadium, the second instar, is also called the ‘hyaline’ (transparent) stage. After about five to seven days, the female second instar nymphs begin to secrete a thin transparent covering at their posterior end while the males begin to secrete a white waxy covering (Cumming 1953, Nielsen and Johnson 1973). Like most diaspidids, the females undergo a total of three instars while the males have five instars, including a pupal stage (Beardsley and Gonzalez 1975). The males reach

adulthood at about the same time as females reach the third instar, and mating occurs shortly after the males emerge. Following mating, the females usually begin to secrete the familiar white waxy covering. In Indiana, both *C. pinifoliae* and *C. heterophyllae* have been occasionally observed to have a partial third generation (Shour 1986). This may occur in southern Michigan in especially warm years, but was not observed during our study or in previous studies in Michigan (Eliason and McCullough 1997).

The crawler stage of pine needle scale has often been recommended as the ideal stage to target with insecticides (Beard and McLeod 1992, Burden and Hart 1989, McCullough et al. 1998, Gambrell 1938). This strategy is suited to the first generation of pine needle scale, which tend to hatch within a few days' time. However, when control is needed on the second generation, targeting the population when most are in the hyaline stage will be most effective, as by then most of the eggs would have hatched from underneath the protective covering (Nielsen and Johnson 1972, Martel 1972)

We focused our efforts on monitoring the phenology of the second generation because it poses the most challenges for pest control. Phenology of the second generation of pine needle scale is more difficult to predict and monitor than the first generation. Second generation crawlers begin to hatch in midsummer, usually in July in Lower Michigan, and continue to hatch for several weeks. After the insects secrete their protective waxy covering, control with insecticides is less effective (McCullough et al. 1998, Beard and McLeod 1992, Martel 1972).

Natural enemies. Many natural enemies have been reported to prey on *C. pinifoliae* and *C. heterophyllae*. Most coccinellids associated with pine needle scale and other armored scales (Diaspididae) are specialists on this family (DeBoo and Weidhass

1976, Drea and Gordon 1990). The coccinellid *Chilocorus stigma* (Say) (= *C. bivulnerus*), the twice-stabbed ladybeetle, was noted in the original description of *C. pinifoliae* (Fitch 1856, from Shour 1986). Several other workers have also reported this coccinellid to be an important or common predator on both *C. pinifoliae* and *C. heterophyllae* (Cumming 1953, Shour 1986, Nielsen 1970, DeBoo and Weidhaas 1976). Most known *Chilocorus* species are specialists on diaspidid scales (Greathead and Pope 1977, Drea and Gordon 1990). Other coccinellids found in association with *C. pinifoliae* include: *Coccidophilus* (as *Microweisia* or *Cryptoweisia*) *marginata* (LeConte), *Microweisia* (as *Cryptoweisia*) *atronitens* (Casey), and *Microweisia misella* (LeConte) (Nielsen 1970, Luck and Dahlsten 1974, Gorham 1921).

The parasitoid complex associated with pine needle scale appears to vary in different geographic regions (Martel and Sharma 1975, Burden and Hart 1990, Cooper and Cranshaw 1999). Ten species of primary parasitoids and two species of hyperparasitoids are reported to attack either *C. pinifoliae* or *C. heterophyllae* in North America, and are usually found at densities of about 15-30% of the host population (Burden and Hart 1993). Most of the parasitoids found to attack pine needle scale have multiple hosts (Martel and Sharma 1975, Burden and Hart 1993).

One predatory mite, *Hemisarcoptes malus* (Shimer) (Astigmata: Hemisarcoptidae), was reported to feed on *C. heterophyllae* in Indiana (Nielsen 1970, from Shour 1986). Gerson et al. (1990) note that *H. malus* is known to be phoretic on *C. stigma*.

Control methods. Currently, pine needle scale is usually controlled in Christmas tree plantations with broad-spectrum insecticides (McCullough and Fondren 1998,

Eliason and McCullough 1997, Beard and McLeod 1992). Many of these products are undergoing intensive review under the federal Food Quality Protection Act of 1996, and may eventually lose registration in minor-use crops such as Christmas trees (DiFonzo and McCullough 1998). Developing other options for control of pine needle scale will give growers an alternative to conventional insecticides, encourage conservation of natural enemies, and reduce worker exposure to pesticides.

Christmas tree plantations are a relatively stable ecosystem compared with annual crops (McCullough 1999) which may increase the potential for using biologically –based control methods (Raupp et al. 1992). Plantations can provide overwintering sites for insect natural enemies such as coccinellids, which usually overwinter as adults in leaf litter. The structural heterogeneity of tree plantations encourages a diversity of insect species, providing refuge for predators and alternate prey sources if necessary.

Our objectives in this study were to: 1) determine the phenology of the second generation of pine needle scale in terms of calendar days and degree-day accumulations; 2) characterize the natural enemy complex acting on the 2nd generation of pine needle scale in Christmas tree fields in Michigan; and 3) determine the rates of predation and parasitism on pine needle scale infestations in commercial Christmas tree fields.

Materials and Methods

Field sites. Four field sites were used throughout the course of this study from 1999 to 2001. All trees were Scotch pine (*Pinus sylvestris* L.) planted in a standard 1.3 x

1.3-m (6 by 6 ft) spacing and sheared annually. Three sites were on commercial Christmas tree plantations: Montcalm County in 1999 (43°14'N, 85°03'W), Van Buren County in all three years (42°22'N, 85°52'W), and another field in Montcalm County in 2001 (43°21'N, 85°14'W). The fourth site was on MSU's Tree Research Center in Ingham County, monitored only in 1999 and 2000 (42°40'N, 84°27'W).

Age and variety of trees varied among the fields (Table 1). All trees selected for phenology and natural enemy sampling were selected in cooperation with the growers. Trees were selected nonrandomly based on the presence of a noticeable scale insect infestation. In 1999, no insecticides were applied in any field during our study. In 2000 and 2001, some trees in the fields were used for a related study on insecticides, but we monitored scale phenology only on unsprayed trees.

Objective 1. Phenology of the second generation. Sampling began before initial hatch of second generation scale insects and continued weekly until second generation scales began ovipositing in late summer. For the first few weeks, at least 25 adult female scales (when possible) from each field were examined to estimate the progression of crawler hatch and number of eggs present. As the crawlers hatch, they leave white chorions behind under the female scale armor. These chorions, however, are very flimsy and could not usually be counted accurately enough to determine an exact percentage of egg hatch, so the mean number of eggs was used as an indicator of the progression of egg hatch.

Once eggs began to hatch, samples of scale insects were taken in each field by removing approximately 20 current-year and previous year fascicles (2 needles per fascicle) from infested areas in each cardinal direction on each tree. Samples were placed

individually in coin envelopes, kept in coolers for transport to the laboratory and held at 4°C to limit insect development. On each sample date, five to ten needles per tree were examined under a microscope to determine the density (scales/cm of needle), sex, life stage and cause of mortality of each scale insect present. In 2000 and 2001, three to five trees were sampled each week (rather than 6 to 10) and 3-5 needles per tree (rather than 10) were examined under a microscope. At least 100 scales were examined from each sample on each sample date, when possible, to monitor the phenology of the population.

Objective 2. Natural enemies. Visual counts of coccinellid adults and larvae on each sample tree (Table 1) were conducted weekly in 1999. Number and stage (adult, larvae, or pupae) of each insect was recorded. In 2000 and 2001, we monitored sample trees at biweekly intervals to record predator activity, scale development, mortality, and to collect scale predators. Natural enemy activity was recorded on each field visit and while processing samples in the lab. Although the precise impact of each natural enemy could not be determined, we recorded the presence and type of natural enemies collected from each sample. Representative specimens of each taxon were identified using the keys in Gordon (1985). Voucher specimens were deposited in the A.J. Cook Arthropod Research Collection at Michigan State University, voucher number 2002-01.

To look for overwintering parasitoids, ten randomly selected branch tips were clipped from infested trees in the Van Buren County field on 11 March 2000. Fifty randomly selected needles with a total of 641 scales were examined under a microscope to search for overwintering parasitoids. When a parasitized female scale was found, it was isolated in a microfuge tube and the parasitoid was allowed to emerge. In the summer of 2000, we also reared out parasitoids found while inspecting our weekly needle

samples. Parasitoids were allowed to air dry and were mounted on slides. Parasitoids were identified to genus by Dr. John Luhman, Minnesota Department of Agriculture, St. Paul, MN, and to species by Dr. Michael Gates, Systematic Entomology Laboratory, Agriculture Research Service, U.S. Department of Agriculture.

Objective 3. Rates of predation and parasitism. Predation or parasitism on each scale insect was recorded when needle samples were examined weekly. Predation and parasitism rates were recorded as the percentage of the total number of scales examined. On most sample dates, and on every sample date in 1999, we noted the instar of each predated scale body when possible, to obtain an estimate of the rate of predation or parasitism on different instars. Obvious signs of predation on 3rd instar and adult scales included jagged holes or tears in the scale armor. Stage-specific predation on the earlier instars was difficult to assess accurately after the scale body had dessicated. Because our sampling was destructive, the rates of predation recorded each week represent different sets of needles.

Rates of parasitism were determined by flipping over the white scale armor of adult females and looking for scale mummies. Scales parasitized by endoparasitoids appeared rigid and orange-brown in color, and filled the whole armor (Nielsen and Johnson 1973). Dead female scales were typically dark and shriveled up in the anterior end of the armor. Only the adult females appeared to be attacked by parasitoids. Rates of parasitism could only be determined for Van Buren County in 2000.

Statistical Analyses. Data for this chapter are presented as either tables or figures that list percentages of scale insects in various life stages, numbers of scale eggs, percentages of predated scales, and numbers of natural enemy species collected.

Results

Objective 1. Phenology of the second generation. Second generation eggs hatched over a period of approximately three weeks, usually beginning the second or third week of July and continuing until early August. As eggs began to hatch, the average number of eggs we found beneath female armor declined, giving us an indicator of the progression of hatch (Table 2). In 1999, eggs hatched first in Van Buren County, the southernmost county, beginning on or just prior to 5 July (1228 degree-days (DD_{50})). By 13 July in Van Buren County, the number of eggs per female had declined by 59% (Table 2). On that date, both first and second instar scale nymphs were present in the field (Table 3). In Montcalm County in 1999, eggs hatched between 7 July and 12 July (1227-1315 DD_{50}), as shown by the appearance of first instar scale nymphs on the foliage (Table 4). In 2000, egg hatch was first observed in Van Buren County on 12 July (1280 DD_{50}), and in Ingham County, the beginning of egg hatch took place between 11 July and 24 July (1232-1436 DD_{50}). Egg hatch in Montcalm County was not observed in 2000, but in 2001, we did monitor egg hatch in Montcalm County in conjunction with another study (see Chapter 4). The second-generation egg hatch in Montcalm County began between 12 July and 27 July (1124-1465 DD_{50}), but since we could not sample between those dates we were unable to determine the duration of egg hatch more precisely. In Van Buren County on 19 July 2001 (1337 DD_{50}), crawlers had already begun to hatch.

Based on these data, the initiation of second-generation egg hatch consistently occurred at approximately 1230-1300 DD₅₀, and generally progressed from Van Buren County (the southernmost county) northward to Montcalm and Ingham Counties.

Development of the scale population. Our first observations of crawlers on the foliage were the most reliable evidence that egg hatch had started, and throughout the sampling period we determined the length of egg hatch in part based on observations of live crawlers. On 5 July 1999 in Van Buren County, we observed crawlers (first instars) in the field (Table 2), although we did not take rigorous counts of the number of scales per needle. By 13 July (1392 DD₅₀), the second-generation scale population consisted of 31% first instar and 69% second instar (hyaline stage) nymphs (Table 3). This indicated that egg hatch had been continuing for at least 12 days, but our observations of adult females with eggs showed that on average 11 eggs still remained beneath the armor, so egg hatch was likely not yet complete (Table 2). On 19 July (1509 DD₅₀), 76% of the second generation scales had reached the second instar, while 13% had molted to the third instar and 11% were first instars (Table 3). Roughly six eggs remained per female (Table 2), but they were probably not viable.

In 1999 in Montcalm County, 76% of the second generation had reached the second instar on 22 July (1520 DD₅₀), or three days later than in Van Buren County. Abundance of crawlers decreased as the population molted to the hyaline stage, and by 30 July (1695 DD₅₀), roughly 38% of the population had reached the third instar.

In 2000 and 2001, we did not monitor the development of the scale population as intensively, and instead focused on the duration of egg hatch and beginning of the second instar (hyaline stage) (Table 5). In Van Buren County in 2000, the crawlers began to

hatch on 12 July, and by 20 July, approximately 25% were second instars. In Ingham County in 2000, we found crawlers just beginning to emerge on 11 July (1232 DD₅₀), and by our next sample date on 24 July (1436 DD₅₀), 23% were crawlers and 42% were already second instars (Table 2). On 31 July (1566 DD₅₀) in Ingham County, 59% of the second generation was in the hyaline stage. In 2001 in Van Buren County, we found 66% of the population had reached the second instar on 25 July (1588 DD₅₀). Approximately 20% of the population had molted to at least the third instar at that time (Table 5), although some eggs still remained beneath the armor. On 2 August, 49% of the population was in the second instar (hyaline stage) while the rest were adults. Scales overwintered as eggs until the following spring. In early 2000, a random sample of 641 scales taken from the Van Buren County field on 11 March showed less than 1% of the females overwintered as mated adults.

Objective 2. Natural enemies. In 1999, two species of coccinellids were abundant throughout the season in both fields (Figure 2). The twice-stabbed lady beetle (*Chilocorus stigma* (Say)) and a small black coccinellid (*Microweisla misella* LeConte) were the two most common insect predators observed on infested needles. In Van Buren County, *C. stigma* appeared to complete at least two generations. The number of adults observed declined from 27 July to 11 August, when pupae were first observed. The abundance of larval *C. stigma* peaked on 3 August. We saw an increase in the number of *C. stigma* adults in late August as the second generation matured. Adult *M. misella* were observed throughout the sampling period, appearing to peak on 3 August (Figure 2).

The parasitic wasps we collected were identified as *Encarsia bella* Gahan (Hymenoptera: Aphelinidae) and *Marietta mexicana* Howard (Hymenoptera:

Aphelinidae). Rates of parasitism observed in Van Buren County in 2000 fluctuated throughout the season (Table 6).

Objective 3. Rates of predation and parasitism. In the 1999 field studies on unsprayed trees, rates of predation reached better than 70% in each field (Tables 7 and 8). In Van Buren County, the predation rate averaged roughly 70% from 3 August to 27 August. Predation rates in the Van Buren County field jumped from 23% to 73% between 27 July and 3 August. This corresponded with a significant increase in the numbers of *C. stigma* and *M. misella* larvae and *M. misella* adults observed in the field (Figure 2).

In both fields, the scale covers of the adult males appeared nearly 100% predated by the end of the season. This is not a result of the males emerging because they back out from underneath their scale armor when mature and do not tear the armor (Cumming 1953). Their cover is also thinner than females, which may provide easier access for predators, especially while the scales are in the pupal stage.

By definition, the number of adult female scales remaining at the end of the season indicates the potential for increase of the population the following year, since they are sessile and the eggs remain under the scale armor throughout the winter. In Van Buren County in 1999, 51% of the adult females had already been killed by predators on 20 September (Table 7). On 9 September in Montcalm County, roughly 17% of 30 remaining females had been predated (Table 8). On 23 September in Montcalm County, only five adult females could be found in a sample of 278 scales (Table 8).

Discussion

The extended hatching period of the second generation of pine needle scale is interesting in light of its contrast to the first generation. Although we did not intensively sample the first generation hatch, other workers have found the duration of the first generation hatch to be shorter than that of the second generation (i.e. Beard and McLeod 1992, Nielsen and Johnson 1973). An extended hatching period allows some eggs to remain relatively protected beneath the female's armor while other hatched crawlers emerge to seek places to settle on the new needles. The second generation of pine needle scale generally moves out to the new current-year needles to settle. The extended hatch period may be an adaptive strategy to allow the maximum number of scale crawlers to reach new growth; as the new needles extend, more crawlers emerge to take advantage of the resource. Since Scotch pine typically carries only two year's growth of needles, the scale insects on the newest growth have the best chance of remaining on the tree through the winter.

In terms of the survival of the population, the second generation is especially important since they must successfully settle in a place where they will overwinter. Protection from natural enemies may not be the reason for an extended hatching period, since scales are vulnerable to natural enemies such as *Chilocorus stigma* in nearly every life stage (eggs, crawlers, adults). In case of adverse weather conditions, which can have a devastating effect on the new crawlers (Nielsen and Johnson 1973), spreading out the hatching period in late summer may avoid strong summer storms or other acute disturbances. Another possible advantage of spreading out the hatch period may be

increasing the genetic variability of the population. Males typically mature and mate at about the same time as the females reach the third instar (Brown 1959, Nielsen and Johnson 1973). With different segments of the population maturing at different rates, mixing of the gene pool may be encouraged.

Overall, however, we did find consistent associations between degree-days and major events in the life cycle (at least ± 100 degree-days). Degree-days have been shown to be a good predictor for the phenology of armored scales in general (Beardsley and Gonzalez 1975, McClure 1990), although individuals may be affected by microclimatic factors (Burden and Hart 1989).

In general, we found that natural enemies, especially coccinellids and, to a lesser extent, endoparasitoids, could be found in unsprayed fields throughout the development of the second generation. The ubiquitous presence of *Chilocorus stigma* and *Microweisla misella* indicate a high potential for mortality caused by natural enemies in unsprayed Michigan plantations. These coccinellids were found in every field and year, but were especially prevalent in 1999, when our field plots were left unsprayed by chemical insecticides. *Chilocorus stigma* seems well adapted to the pine needle scale life cycle, with at least two generations per year coinciding with the pine needle scale hatching periods. Larval *M. misella* are very small and were difficult to observe, although a pattern of increasing larval numbers around 3 August was observed in 1999 in Van Buren County, and 6 August 1999 in Montcalm County. In contrast to many coccinellids that overwinter as adults, including *C. stigma*, larvae of coccinellids in the tribe Microweisini are known to overwinter beneath the scale armor of *C. pinifoliae* (Eliason and McCullough 1997). These coccinellids may be amenable to conservation strategies. If

C. stigma and *M. misella* are observed in a Christmas tree plantation, avoiding the use of a broad-spectrum insecticide is likely to improve control of the pine needle scale.

The rates of predation and parasitism observed in unsprayed fields in 1999 were high, effectively leaving a relatively small number of live female scales available to produce a new generation in the next season. Adult male scales consistently displayed a very high rate of predation, which may be due to their relatively thin armor. However, in most armored scales, one male may inseminate several females (Beardsley and Gonzalez 1975), so a high predation rate on males may not significantly affect the population. Although we did not make a direct comparison, predation rates in fields sprayed for control of pine needle scale would probably have been lower, given other factors equal. Predation data in 2000 and 2001 were confounded by a simultaneous insecticide trial occurring in the same fields; the trial was unlikely to affect the phenology of the scale but it may have affected the natural enemy population.

Armored scale insects are often affected by a complex of natural enemies that becomes ineffective if insecticides are repeatedly applied (Luck and Dahlsten 1975, Ripper 1956). The resurgence of phytophagous insects such as armored scales following applications of broad-spectrum insecticides has been frequently documented (Roberts et al 1973, Luck and Dahlsten 1975, Ripper 1956, McClure 1977, Sheffer and Williams 1987). Reducing the rate of disturbance in a tree plantation and increasing the diversity of the natural enemies present will discourage widespread outbreaks.

Implications for management strategies. Documenting the relationship between second generation phenology and degree-days is an important step toward managing the pine needle scale population in Christmas tree plantations. Approximately 1500 DD₅₀,

when hatch is complete and the majority of the population is still in the second instar, would be an appropriate time for applying controls such as an insecticide or horticultural oil (Nielsen and Johnson 1972, Martel 1972). This information may help to show growers that delaying a spray application until most eggs have hatched may be more efficient and effective than spraying as soon as crawlers are visible.

Our results will help managers to develop control strategies for pine needle scale that will not exacerbate the problem. An integrated approach to management that relies on scouting appropriately and preventing unnecessary insecticide applications is likely to be an effective long-term strategy. Further research on the natural enemies of this pest is needed to elucidate the impact of each species on the pine needle scale population and the potential for using natural enemies to prevent scale outbreaks.

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Table 1. Study sites used in 1999, 2000 and 2001 for pine needle scale

Ingham County			Van Buren County			Montcalm County		
Age and variety	Infestation level	No. trees used	Age and variety	Infestation level	No. trees used	Age and variety	Infestation level	No. trees used
1999 Lake Superior Blue, 11 years	Light	20	Pike Lake, 3 years	Heavy	10	Approx. 4 years old; variety unknown	Heavy	6
2000 Lake Superior Blue, 12 years	Light	10	Lake Superior Blue, 7 years	Moderate to heavy	40	a	a	a
2001 a	a	a	Pike Lake, 5 years	Moderate to heavy	50	Group A: 6 years in field, French Green	Moderate to heavy	15
						Groups B and C: 13 years in field, Scotch Highland		

^aThese sites were not used in this year

Table 2. Number of eggs present under female scale armor during oviposition and hatching period

Field	Year	Date	Cumulative DD₅₀	No. females examined	No. eggs per female	Observations
Van Buren Co.	1999	5 July	1228	38	18.0 ± 1.6	Crawlers already present in field
		13 July	1392	30	10.7 ± 1.8	69% hyaline stage
		19 July	1509	70	5.9 ± 0.29	76% hyaline; males nearly adult
		3 August	1990	17	3.4 ± 0.45	54% males adults
Montcalm Co.	1999	7 July	1227	39	12.5 ± 1.33	None had hatched yet
		15 July	1354	21	11.6 ± 1.44	Crawlers out; 25% hyaline
		22 July	1520	62	8.4 ± 0.72	75% hyaline; crawlers still out
		26 June	975	100	0.82 ± 0.18	---
Van Buren Co.	2000	6 July	1168	100	25.6 ± 1.05	---
		12 July	1280	83	25.4 ± 1.51	Initiation of crawler hatch
		17 July	1360	100	19.4 ± 0.96	Crawlers settling
		20 July	1422	85	19.2 ± 1.10	Hyaline stage beginning
		24 July	1481	75	12.7 ± 0.61	Peak hyaline stage
		1 August	1630	84	10.1 ± 0.46	1 st observation of white armor; some eggs still hatching
		8 August	1800	40	6.44 ±	Still some hatch; most at 3 rd instar

Table 2 (cont'd).

Field	Year	Date	Cumulative DD ₅₀	No. females examined	No. eggs per female	Observations
Ingham County	2000	27 June	975		0	---
		5 July	1154	20	12.9 ± 2.49	---
		11 July	1232	25	28.7 ± 3.38	---
		24 July	1436	49	19.6 ± 1.31	Hyalines and settled pink crawlers
		27 July	1530	14	10.6 ± 1.98	Peak hyaline stage
		31 July	1566	48	10.26 ± 0.58	1 st observation. of white armor
		8 August	1710	14	4.75 ± 0.63	
Montcalm Co.	2001	17 May	350	15	1.6 ±	<i>First generation</i> Crawlers settled; 85% new pink crawlers
		22 May	400	13	1.07 ±	82% of settled crawlers turned yellow
						<i>Second generation</i>
		12 July	1124	19	13.7 ± 1.83	---
		27 July	1465	38	17.6 ± 1.21	Crawlers out
		3 August	1630	47	11.7 ± 0.78	Peak hyaline stage
		10 August	1835	9	9.7 ± 2.16	Most females adult

Table 2 (cont'd).

Field	Year	Date	Cumulative DD ₅₀	No. females examined	No. eggs per female	Observations
Van Buren County	2001	19 July	1337	68	17.9 ± 0.98	Crawlers out
		25 July	1588	97	14.37 ± 0.76	Peak hyaline stage
		6 August	1817	33	8.8 ± 0.97	Hyalines, 3 rd instar females and male pupae
		13 August	2051	5	7-13 eggs	Adult males, females 3 rd instar or adult

Table 3. Pine needle scale phenology, Van Buren County, 1999 (means +/- 1 SE where applicable)

Date	Approx. degree days base 50°F	N (scales)	Avg density/cm per needle (n=100)	%crawlers	%hyaline	%3 rd instar	%adult females	%adult males
13 July	1392	1247	4.67 +/- 0.62	30.79	69.05	0.16	0	0
19 July	1509	705	5.53 +/- 0.62 ^a	10.5	75.60	12.06	0	1.84
27 July	1744	3050	9.99 +/- 0.48	8.00	37.67	15.02	0	39.25
3 August	1990	2435	7.52 +/- 0.5	4.60	23.90	16.96	0	54.41
11 August	2066	2503	8.71 +/- 0.45	3.76	24.61	12.90	0.08	57.29
18 August	2200	2705	8.91 +/- 0.5	4.84	22.59	10.57	0.15	60.52
27 August	2377	2182	7.06 +/- 0.47	2.75	25.16	11.09	0.27	58.94
7 Sept.	2640	123	3.59 +/- 0.73 ^b	2.44	21.95	15.45	24.39	3.25
20 Sept.	N/A	1460	4.9 +/- 0.39	1.15	18.79	10.04	4.86	50.34

Percentages of life stages are percent of total N and include predated scales

^a n=45

^b n=10

Table 4. Pine needle scale phenology, Montcalm County, 1999 (means +/- 1 SE where applicable)

Date	Degree days (approx.)	N (scales)	Avg density/cm (n=60)	%crawlers	%hyaline	%3 rd instar	%adults	
							Males	females
7 July	1227	0	0	0	0	0	0	0
12 July	1315	249*	1.3 +/- 0.2	87.6	11.2	0.8	0	0
15 July	1354	746	4.9 +/- 0.96 (n=50)	73.2	25.9	0.4	0.13	0
22 July	1520	877	6.6 +/- 0.96 (n=40)	22.0	75.5	2.2	0	0.23
30 July	1695	1060	4.28 +/- 0.83	14.3	47.3	22.5	15.9	0
6 August	1876	1392	6.1 +/- 0.76	12.6	41.5	13.0	32.0	0.57
16 August	2025	1280	6.3 +/- 0.8	13.5	31.5	10.5	42.8	0
25 August	2197	1002	4.9 +/- 0.7	11.08	37.72	11.48	35.93	3.79
3 Sept.	2399	935	4.3 +/- 0.73	11.12	27.49	14.01	44.81	1.39
9 Sept.	2424	1063	5.0 +/- 0.7	6.2	34.3	14.5	42.1	2.8
23 Sept.	N/A	278	3.0 +/- 0.58 (n=30)	12.6	36.7	16.2	32.7	1.8

Percentages of life stages are out of the total number examined and include predated scales

Table 5. Pine needle scale phenology 2000 and 2001

Field location	Date	Approx. degree days base 50°F (10°C)	N (scales)	% crawlers	% hyaline	% 3 rd instar	% adult females	% adult males
Van Buren	12 July 2000	1280	180	6.7	21.1 ^a	3.9 ^a	48.3 ^a	20.0 ^a
Van Buren	20 July 2000	1422	407	20.2	25.8	3.7 ^a	22.9 ^a	27.5 ^a
Ingham	11 July 2000	1232	127	2.4	33.1	9.5	31.5	23.6
Ingham	24 July 2000	1436	97	22.7	42.3	2.1	30.9	2.1
Ingham	31 July 2000	1566	283	8.1	59.4	1.8	20.9	9.9
Van Buren	25 July 2001	1588	619	12.4	65.8	1.0	19.9	1.0
Van Buren	2 August 2001	1700	717	0	48.8	3.8	7.5	39.3
Van Buren	6 August 2001	1817	709	0	35.1	19.2	2.1	43.6
Van Buren	19 August 2001	2100	491	3.7	18.7	18.1	19.6	39.9

Percentages of life stages are percent of total and include scales killed by predators

^aThese are most likely first generation scales; see Table 2

Table 6. Parasitoids recovered from scale mummies in 2000.

Date collected	Percent parasitized	No. parasitized scales	No. <i>Encarsia</i>	No. <i>Marietta</i>	Percent <i>Encarsia</i>
11-Mar-00	25.3	162	30	2	93.8
26-Jun-00	7	7	None emerged		
06-Jul-00	46	46	32	0	100
12-Jul-00	25.3	21	11	1	91.7
17-Jul-00	40	40	22	4	84.6
20-Jul-00	52.9	45	None emerged		
24-Jul-00	33.3	25	1	3	25
01-Aug-00	Unknown ^a	Unknown ^a	11	2	84.6

^aThe number of host insects was not recorded

Table 7. Percent predation of pine needle scale in each life stage, Van Buren County 1999. See Table 3 for the percentage of the population that was in each life stage on each sample date.

Date	Degree days (approx.)	N (scales)	Percent predated ^a	% hyaline predated ^b	% 3 rd instar predated	% adult females predated	% adult males predated
13 July	1392	1247	N/A	100 (n=861)	N/A	0 (n=2)	N/A
19 July	1509	705	48.9	58.3 (n=533)	37.6 (n=85)	N/A	15.4 (n=13)
27 July	1744	3050	22.85	24.2 (n=1149)	22 (n=458)	N/A	26.7 (n=1197)
3 August	1990	2435	73.80	49.1 (n=582)	68.3 (n=413)	N/A	92.7 (n=1325)
11 August	2066	2503	71.03	37.7 (n=616)	56.4 (n=323)	0 (n=2)	94.8 (n=1434)
18 August	2200	2705	72.35	42.6 (n=611)	66.1 (n=286)	25 (n=4)	91.4 (n=1637)
27 August	2377	2182	70.6	38.1 (n=549)	58.3 (n=242)	0 (n=6)	91.6 (n=1286)
7 September	2640	123	65.85	58.3 (n=24)	36.8 (n=19)	63.3 (n=30)	90.9 (n=44)
20 September	Not avail.	1460	62.14	100 (n=410)	82.6 (n=219)	50.7 (n=71)	99.2 (n=735)

^aPercentage of the total population that appeared predated

^bPercentage of the hyaline stage (second instar) only that appeared predated or dead

Table 8. Percent predation of pine needle scale at each life stage, Montcalm County, 1999. See Table 4 for the percentage of the population that is in each stage on each sample date.

Date	Degree days (approx.)	N (scales)	Percent predated ^a	% hyaline predated ^b	%3 rd instar predated	% adult predated	
						Male	female
7 July	1227	0	0				
12 July	1315	249	10.8	92.9 (n=28)	33.3 (n=3)		
15 July	1354	746	26.1	99.5 (n=193)	66.7 (n=3)	0 (n=1)	
22 July	1520	877	33.9	43.5 (n=662)	47.4 (n=19)	N/A	0 (n=2)
30 July	1695	1060	35.2	44.7 (n=501)	39.9 (n=238)	23.1 (n=169)	0
6 August	1876	1392	42.9	45.1 (n=577)	42.0 (n=181)	58.9 (n=445)	0.6 (n=8)
16 August	2025	1280	71.1	91.1 (n=403)	65.2 (n=135)	81.8 (n=548)	0 (0)
25 August	2197	1002	55.9	61.4 (n=378)	51.3 (n=115)	72.5 (n=360)	21.1 (n=38)
3 September	2399	935	63.7	54.5 (n=257)	56.5 (n=131)	90.5 (n=419)	0 (13)
9 September	2424	1063	60	54.8 (n=365)	59.7 (n=154)	76.1 (n=448)	16.7 (n=30)
23 September	Not avail.	278	60.8	65.7 (n=102)	75.6 (n=45)	73.6 (n=91)	20.0 (n=5)

^aPercentage of the total population that appeared predated

^bPercentage of the second instar (hyaline stage) only that appeared predated or dead

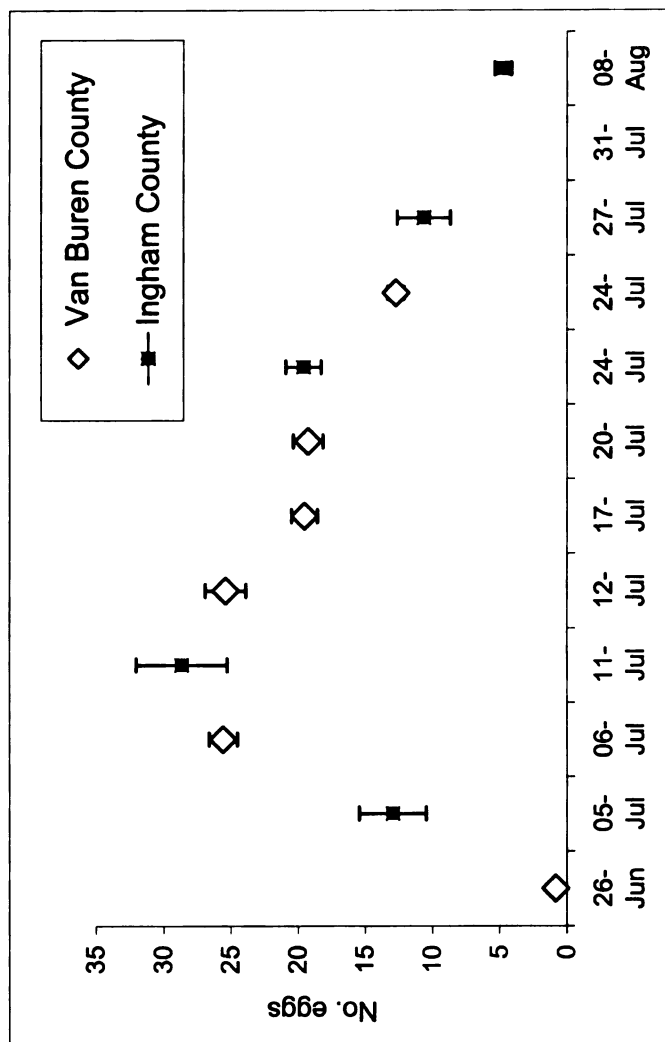


Figure 1. Mean ($\pm$ SE) number of eggs present beneath the female scale armor in 2000 in the Van Buren and Ingham County fields on each sample date.

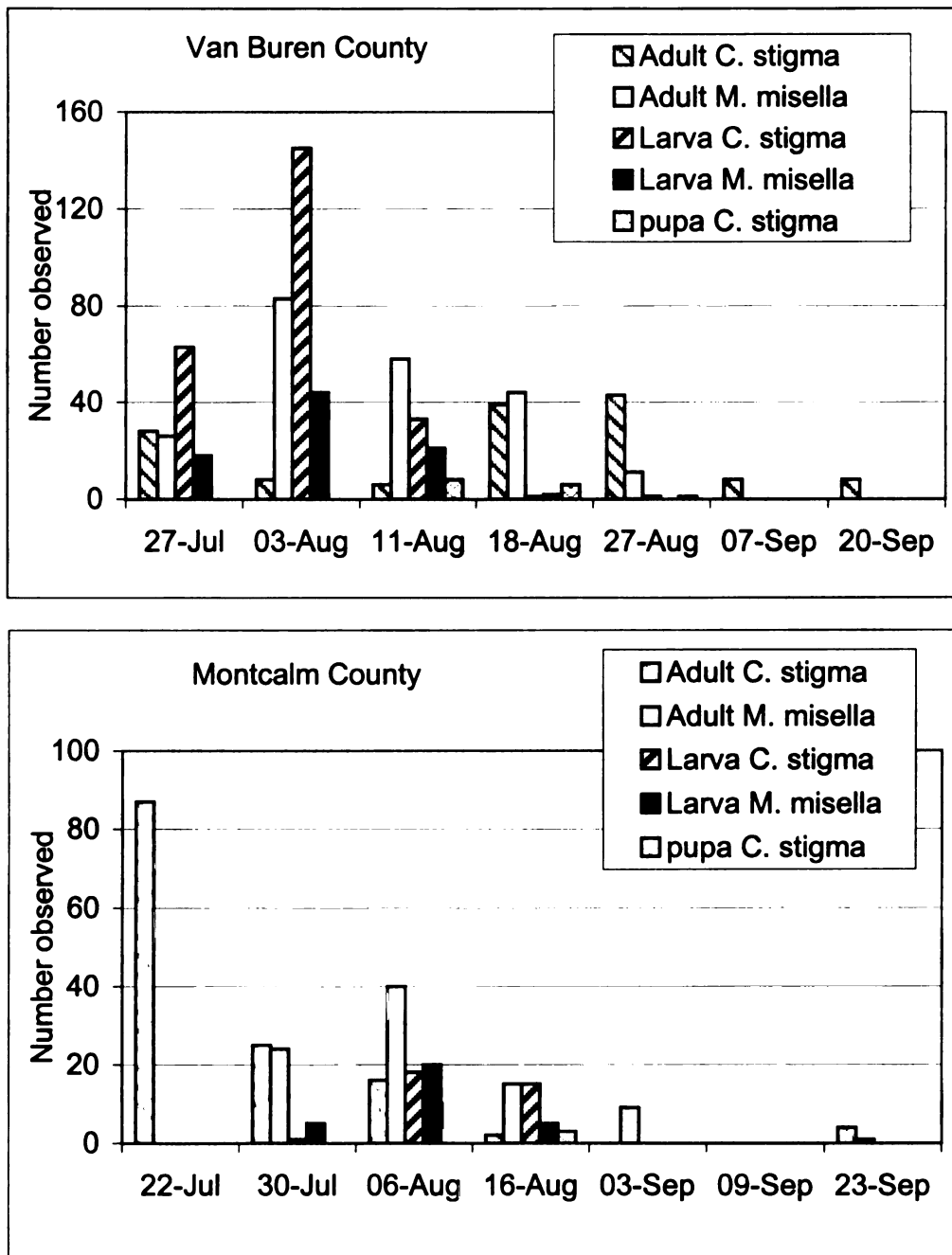


Figure 2. Number and stage of *Chilocorus stigma* and *Microweisia misella* observed in our 1999 field study. The total number of each observed on ten trees in Van Buren County and six trees in Montcalm County is recorded.

Chapter 4

Potential efficacy of horticultural oil for control of pine needle scale (*Chionaspis heterophyllae* (Fitch)) (Homoptera: Diaspididae) in Christmas tree fields

Abstract. Pine needle scale ((*Chionaspis pinifoliae* (Fitch) and *C. heterophyllae* (Cooley)) is an important pest of Scotch pine (*Pinus sylvestris* L.) grown for Christmas trees in Michigan. The most common method of control, application of broad-spectrum insecticides, is most effective before the scale secretes its protective covering. Timing of spray applications is critical especially on the second generation because of its asynchronous hatching period. We also investigated the potential for using horticultural oil as an alternative control method for pine needle scale in Christmas tree fields. Our objectives were to 1) monitor the phenology of second generation pine needle scale to identify optimal timing for insecticide applications in Christmas tree fields; 2) determine the mortality rate of pine needle scale when horticultural oil is applied with a backpack mist blower; and 3) assess the effectiveness of applying horticultural oil with a tractor-mounted airblast sprayer on a large commercial Christmas tree plantation. We monitored the phenology of the second generation of pine needle scale weekly on a minimum of six trees on each of three plantations in Lower Michigan. We found that egg hatch began at approximately 1230 degree-days base 10°C (50°F) (DD₅₀) and the peak of the second instar coincided with 1500-1600 DD₅₀. In 2000 and 2001, we compared the efficacy of a highly refined horticultural spray oil and conventional insecticides applied with a

backpack mist blower at 1500-1600 DD₅₀. In both years, the spray oil did as well or better than the chemical insecticide, and scale mortality on trees treated with oil ranged from 66% to 80%. In 2001, horticultural oil was applied with a tractor-mounted airblast sprayer to a portion of a field. Scale mortality ranged from 36% to 56% and was not significantly different from conventional insecticides.

Introduction

Pine needle scale (*Chionaspis pinifoliae* (Fitch) and pine scale (*C. heterophyllae* Cooley) are important insect pests of Scotch pine (*Pinus sylvestris* L.) and several other conifer species grown in nurseries, landscapes, and tree plantations (Beard and McLeod 1992, Burden and Hart 1990, Johnson and Lyon 1988). *Chionaspis pinifoliae* has a wide geographic distribution, including most of the United States, Canada, Mexico, the Caribbean, and England (Nakahara 1982), while *C. heterophyllae* is native to the eastern and midwestern United States (Shour and Schuder 1987). The two species are nearly morphologically identical, and have extremely similar life histories; several papers that have been published using the species name *C. pinifoliae* were later determined to actually be *C. heterophyllae* (i.e. Nielsen and Johnson 1972 and 1973, Walstad et al. 1973). The literature using the species name *pinifoliae* is more extensive than that of *heterophyllae*, but because the ecology of the two species is so similar, we will refer to the species collectively as pine needle scale.

While pine needle scale can be found in forested areas, it rarely if ever becomes a noticeable pest there (Cooper and Cranshaw 1999, Burden and Hart 1993, Luck and Dahlsten 1975, Ruggles 1931). In contrast, in highly managed settings such as Christmas tree plantations or landscapes, populations can reach high densities (Tooker and Hanks 2000, Johnson and Lyon 1988, Sheffer and Williams 1987). Even moderate populations of pine needle scale can cause Christmas trees to be unsaleable and high populations can cause needles or branches to die or reduce growth and tree vigor (Nielsen and Johnson 1973, Dahlsten et al. 1969, Walstad et al. 1973, Cumming 1953).

Life cycle. The number of generations of pine needle scale can vary with geographic location and other factors such as temperature, host plant, density, and local climactic conditions (Shour 1986, Nielsen and Johnson 1973, Luck and Dahlsten 1974). In Michigan, *C. pinifoliae* has two generations per year (Eliason and McCullough 1997). In Indiana, both *C. pinifoliae* and *C. heterophyllae* have been occasionally observed to have a partial third generation (Shour 1986), but this was not observed during our study or in past studies in Michigan (Eliason 1996).

Pine needle scale overwinters as eggs that hatch in early spring, typically just after lilac (*Syringa vulgaris* L.) is in full bloom in May (Mussey and Potter 1997, Herms 1990). The first generation hatching period extends for about seven days (Beard and McLeod 1992, Nielsen and Johnson 1973). Newly hatched crawlers move about for a few days before settling and inserting their stylets into the host plant. About a week to two weeks after settling, the crawlers molt, pushing the exuviae up on top of their bodies (Nielsen and Johnson 1973, Cumming 1953). This stage (the second instar) is termed the hyaline (transparent) stage.

After about five to seven days, the female second instar nymphs begin to secrete a thin transparent covering at their posterior end while the males begin to secrete a white waxy covering (Cumming 1953, Nielsen and Johnson 1973). Females undergo three instars in total and remain immobile once they begin feeding. Males complete three instars, then pupate and emerge as winged adults (Cumming 1953, Shour 1986). Mating begins almost immediately after the males emerge, usually just after the females reach the third instar (Brown 1959). After mating, the females secrete the familiar white waxy covering and produce eggs that remain under the white scale armor until hatching occurs. The adult females die after oviposition is complete (Brown 1959). The white wax covering can protect the insects from many insecticides (Nielsen and Johnson 1972, Martel 1972), but not always (Beard and McLeod 1992).

The second generation of pine needle scale usually begins to hatch in early July in Michigan. The hatching period extends for up to three weeks, making it difficult to time insecticide applications or other control measures (Eliason and McCullough 1997). By the time the last eggs hatch, the earliest hatching nymphs may have already molted to the third instar (Beard and McLeod 1992, Nielsen and Johnson 1972, 1973).

Effective control of pine needle scale requires application of insecticide when the insect is most susceptible, and for the first generation, this usually means targeting the crawler stage (Beard and McLeod 1992, Burden and Hart 1989, Nielsen and Johnson 1972, McCullough et al. 1998, Gambrell 1938). However, when controlling the second generation, the second instar (hyaline stage) is a good target because it is also susceptible to insecticide sprays, and its peak occurrence on the needles usually indicates that egg hatch is complete (Nielsen and Johnson 1973, Shour 1986). Insecticide sprays applied

after the scale insect produces its protective covering are usually ineffective (Martel 1972, Nielsen and Johnson 1972).

Control methods. Pine needle scale is commonly controlled in large Christmas tree plantations with applications of broad-spectrum insecticides (Beard and McLeod 1992, McCullough and Fondren 1998). Results of a survey of Michigan Christmas tree growers showed that the number of acres treated and the number of insecticide sprays for pine needle scale alone exceed those for any other insect pest of Scotch pine (McCullough and Fondren 1998). Scotch pine may be sprayed as many as four times per year in Michigan plantations for control of pine needle scale (Beard and McLeod 1992, McCullough and Fondren 1998). Broad-spectrum insecticides such as chlorpyrifos and malathion have been recommended for use against pine needle scale for some time (Beard and McLeod 1992, Martel 1972), and are among the most common insecticides used on pine needle scale in Michigan (McCullough and Fondren 1998). Applications of broad-spectrum insecticides may decimate natural enemy populations and often result in outbreaks of armored scales (Roberts et al. 1973, Luck and Dahlsten 1975, Ripper 1956, McClure 1977, Sheffer and Williams 1987). Often, this situation leads to further applications of insecticides, e.g. the 'pesticide treadmill' (Rose 1990).

Horticultural spray oils are often used to control insect pests in landscape or ornamental situations (Raupp et al. 1992, Nielsen 1990), but are rarely used on Christmas tree plantations (McCullough and Fondren 1998, Nielsen 1990). Horticultural spray oils have been recommended for use against armored scale insects since the early 1920's (Riehl 1990, Pearce et al. 1941, Gambrell and Hartzell 1939, Doane 1926). Modern spray oils are not phytotoxic under most conditions (Riehl 1990, Nielsen 1990), and are

relatively harmless to humans and natural enemies such as coccinellids (Smith and Krischik 2000). After more than fifty years of use, there have been no known cases of insecticide resistance to horticultural oils among armored scales (Riehl 1990). The mode of action of spray oils is simple—the oil clogs the spiracles of the insect, effectively suffocating it. Because of this mode of action, adequate coverage is essential. Some of the concerns raised by growers have included the efficacy of oils and the difficulty of obtaining adequate coverage efficiently in a large-scale setting.

Objectives. Our objectives in this study were to 1) monitor the phenology of second generation pine needle scale to identify optimal timing for insecticide applications in Christmas tree fields; 2) determine the mortality rate of pine needle scale when horticultural oil is applied with a backpack mist blower; and 3) assess the effectiveness of applying horticultural oil with a tractor-mounted airblast sprayer on a large commercial Christmas tree plantation.

Materials and Methods

Study sites. This research was conducted in 2000 and 2001 in three Scotch pine fields on commercial Christmas tree plantations in Lower Michigan (Table 1). Two study sites were located on a farm in Van Buren County (42°22'N, 85°52'W). Another site, used in 2000 only, was on MSU's Tree Research Center in Ingham County (42°40'N, 84°27'W). A fourth site was located in Montcalm County (43°21'N, 85°27'W) and was used only in 2001.

All trees used in the study were selected nonrandomly on the basis of a visible pine needle scale infestation on at least part of the tree. The number of trees used each year ranged from 13 to 50 trees per field, depending on pine needle scale abundance and the number of trees that could be provided by growers (Table 1).

The Montcalm County field was used only for a test with a grower's commercial airblast sprayer in 2001. In May 2001, the grower notified us that he intended to use horticultural spray oil on part of his field. He allowed us to sample the trees before and after the spray application to assess its effectiveness when applied with an airblast sprayer. Therefore, the first generation of pine needle scale was monitored in this field, rather than the second generation as in the other field studies. In May 2001, before sprays were applied, 15 trees with obvious pine needle scale infestation were selected with the grower's help. At least four trees were located in each of three sections. The three sections were named Groups A, B, and C (Table 1).

Objective 1. Phenology of second generation scales. In 2000 and 2001, we monitored the duration of second generation egg hatch and crawler emergence in Van Buren County. Ingham County was monitored in 2000 only, because the scale infestation was too low in 2001. In each field, up to 100 adult female scales were collected weekly beginning in late June or early July, before any crawlers had hatched. Samples were taken by removing approximately 20 infested needles from each sample tree on each date. Needles were taken from the midcrown level in all four cardinal directions, when the infestation level permitted. Uninfested needles were not chosen because our objective was to monitor phenology of the scale insect rather than density of the population on the tree. We examined the first 100 female scales from each collection

under a microscope for evidence of oviposition and egg hatch. We determined the duration of egg hatch by counting the number of live eggs per female on each sample date. Each week, the number of scale insects on an additional sample of three to five needles per tree was also examined under the microscope. The stadium and apparent cause of mortality, if applicable, was recorded for each scale insect (stadia were identified after Cumming 1953 and Nielsen and Johnson 1973). We determined when the development of hyaline stage nymphs peaked by monitoring the proportion of hyaline nymphs in the population each week until all had molted to the third instar.

Cumulative degree-days were obtained weekly from the Michigan State University Agricultural Extension website (<http://www.agweather.geo.msu.edu/>) for the closest weather station to each study site. Degree-day data from Lansing were used for the Ingham County field, Paw Paw for the Van Buren County fields and Grand Rapids for the Montcalm County field. Cumulative degree-days expressed as base 50°F (10°C) were used because this is the best threshold for development of pine needle scale (Mussey and Potter 1997, Burden and Hart 1989), and because degrees Fahrenheit are usually more accessible than degrees Celsius to growers and extension personnel in the United States (Mussey and Potter 1997, Herms 1990, Pruess 1983). Cumulative degree-days are indicated by the symbol DD₅₀.

Objective 2. Efficacy of horticultural oil. In 2000, the 40 selected trees in the Van Buren County field were randomly assigned to one of four spray treatments: a 2% solution of highly refined horticultural oil (SunSpray 6E, Sunoco, Philadelphia, PA), chlorpyrifos (a common organophosphate)(Lorsban 4E, Dow AgroSciences LLC, Indianapolis, IN), water, and no spray (control). In Ingham County, we assigned ten trees

each to chlorpyrifos, oil, or water application. Because only 30 trees with adequate scale populations were available in this field, we used water-sprayed trees as a control to ensure that we could differentiate effects of the oil and insecticide from any physical effects of the spray itself. Treatments were applied in both fields with a backpack mist blower (Solo model 423, Sindelfingen, Germany) at recommended field rates (7.57 liters oil/378.5 liters water applied at 935.4 liters/ha; 0.946 liters chlorpyrifos/757.1 liters water at 467.7 liters/ha) (English units: 2 U.S. gal/100 gal water at 100 gal/acre; 1 pt chlorpyrifos/100 gal water at 50 gal/acre). We attempted to simulate the use of an airblast sprayer as closely as possible by using a gasoline powered mist blower (spray output of 24.5 ml/s) rather than a hand pump sprayer. To calibrate the mist blower, we determined the number of ml of solution that should be applied to each tree based on the typical density of 2,963 trees per ha (1,200 trees per acre). We calculated the amount of time needed per tree based on the spray output per s. This was 6.4 s per tree for chlorpyrifos and 12.9 s per tree for oil. We applied the spray to all sides while circling the tree until time ran out and the foliage was wet, but not dripping heavily.

In 2001, we used a similar spray protocol in Van Buren County, although a different brand of highly refined horticultural oil was used (Superior Miscible Spray Oil, Universal Cooperatives, Inc., Minneapolis, MN). The entire field was sprayed aerially with chlorpyrifos two days before treatments were applied. Forty of the trees we had selected were enclosed and protected with white plastic bags during the aerial spray (per Beard and McLeod 1992), and the remaining ten trees were left exposed.

Pre-treatment scale mortality. Approximately 20 infested needle fascicles were pulled from each sample tree immediately before sprays were applied. Needles were

removed from all sides of the tree when infestation levels permitted. Samples were placed in coin envelopes and held in the laboratory at 4°C until they were examined under a microscope the following day. We examined enough needles to count 25 to 50 scale nymphs per tree. We determined if each nymph was live or dead by poking it with a minuten pin to see if liquid oozed out. Dead scale insects typically appeared to be dry, flattened, or otherwise desiccated. Samples were collected weekly for three weeks in the same manner and the mean percentage mortality of scale nymphs was determined for each tree.

Objective 3. Effectiveness of commercial application of horticultural oil. The grower applied different spray treatments in each of three sections, to test the difference between using horticultural oil or a conventional insecticide. In this trial, a true control (unsprayed or water-sprayed) could not be established because of the grower's immediate need to achieve control of the population. Treatments were assigned to each section based on the logistics of using the airblast sprayer and other considerations, including expected harvest date, and designated Groups A, B, and C (Table 1). Trees in Group A received a 4% horticultural oil treatment (Damoil, Drexel Chemical Co., Memphis, TN) (7.57 liters oil/189.3 liters water at the rate of 467 liters/ha) (2 gal oil/50 gal water at 50 gal per acre). Trees in Group B received 2% oil and trees in Group C were sprayed with Metasystox R (S-[2-(Ethylsulfinyl)O,-O-dimethyl phosphorothioate)(Gowan Co., Yuma, AZ), along with the fungicide Bravo (Zeneca Ag Products, Wilmington, DE), at label rates (Metasystox R: 2.34 liters/ha at the rate of 467 liters/ha; Bravo at 3.36 g/ha in 467 liters water/ha) (1 qt/acre in 50 gal H₂O and 3 lbs/acre in 50 gal H₂O). Treatments were applied on 17 May 2001 with an AgTec airblast sprayer (model 2004). On 29 May 2001,

the grower reapplied all sprays. Samples were taken on 17 May before treatment, 22 May, 1 June, 4 June, and 9 June.

Statistical Analyses. Data were tested for normality using the Shapiro-Wilk test. Data were transformed when necessary to meet the assumptions of normality and homogeneity of variance. A one-way analysis of variance (ANOVA) was used to test for differences in scale mortality in the oil and insecticide trials. When the ANOVA was significant, treatment means were separated using Fisher's Least Significant Difference. For the Montcalm County 2001 trial, transformed data did not meet assumptions of normality, so data were analyzed using the nonparametric Kruskal-Wallis test. For these data, exact p-values were calculated using the Monte Carlo estimation. All tests were conducted at a significance level of $\alpha = 0.05$. Data were analyzed using SAS v. 8 (SAS Institute 1999).

Results

Objective 1. Phenology of second generation scales. Phenology of the second generation egg hatch in 2000 and 2001 was generally similar among locations (Table 2). In 2000 we sampled at the start of egg hatch in Van Buren County on 12 July (1280 DD₅₀). In other cases, we did not sample at the very beginning of egg hatch, but crawlers were already present by roughly 1230 to 1300 DD₅₀ in all fields. Once egg hatch began, eggs continued to hatch for approximately 2-3 weeks. By roughly 1800 DD₅₀ in each field and year, the last crawlers had emerged, and egg hatch appeared to be complete

except for an average of five to seven eggs that were probably nonviable. While egg hatch was occurring, the newly settled scale nymphs matured, often reaching the third instar before hatch was completed.

The second instar, or hyaline stage, began to appear at approximately 1400 DD₅₀, and peaked between 1500-1600 DD₅₀ in each field (Figure 1). The calendar date of the peak hyaline stage varied slightly, but occurred in the last week of July in Ingham and Van Buren Counties, and the first week of August in the more northern Montcalm County. Our estimation of the peak hyaline stage depended on our sample date, which also varied from year to year depending on the logistics of sampling several fields simultaneously.

Objective 2. Efficacy of horticultural oil. Horticultural oil applied with a backpack mist blower performed at least as well as the conventional broad-spectrum insecticide chlorpyrifos. In 2000 in Van Buren County, the pre-treatment mortality rate averaged about 40% and there were no significant differences among trees in the four treatment groups ($F = 0.31$; $df = 3, 36$; $P = 0.81$) (Figure 2). After treatments were applied on 25 July, the mortality rates on the unsprayed and water spray control trees did not increase, but mortality in the oil and chlorpyrifos treatment groups was significantly higher than the controls ($F = 8.58$; $df = 3, 35$; $p = 0.0002$) (Figure 2). Mortality of scales on trees treated with oil and chlorpyrifos did not significantly differ.

In 2000 in Ingham County, pretreatment mortality averaged roughly 26% for all trees with no significant differences among treatment groups ($F = 0.07$; $df = 2, 27$; $p = 0.93$) (Figure 3). One week after treatments were applied, the average mortality increased to 53.6% (Figure 3). Initially, oil appeared to perform slightly better than

chlorpyrifos, but differences among treatment groups were not significant ($F = 1.57$; $df = 2, 27$; $p = 0.23$). Four weeks after treatments were applied, the mortality rate averaged 29% for all trees, and did not differ significantly among the treatment groups ($F = 1.22$; $df = 2, 27$; $p = 0.31$).

In 2001 in Van Buren County, pretreatment mortality on 25 July averaged 25%, with no significant differences among treatment groups ($F = 0.21$; $df = 4, 45$; $p = 0.93$) (Figure 4). On 13 August, one week after treatments were applied, mortality rates in the unsprayed, water-sprayed, and aerially sprayed treatment groups increased to an average of 52.7%, but scale mortality on these three groups of trees did not differ significantly (Fisher's LSD). Mortality rates on the trees treated with oil and chlorpyrifos increased to 77.1% on average by 19 August. On 19 August, two weeks after treatments were applied, relative differences in scale mortality among treatments were consistent: mortality rates in the oil and chlorpyrifos treatment groups were significantly greater than the control and aerially-sprayed groups, and mortality on the trees treated with oil and chlorpyrifos were not significantly different from each other (Figure 4). Notably, mortality of scale insects on trees treated aerially with chlorpyrifos did not differ significantly from the two control groups.

Objective 3. Effectiveness of commercial application of horticultural oil. In May 2001, we monitored the first generation hatch in Montcalm County to help the grower time spray applications for this generation. We found that by 22 May, most of the eggs had hatched, and crawlers were beginning to turn yellow, an event which immediately precedes the molt to the hyaline stage (per Nielsen and Johnson 1973) (Table 2).

Pretreatment mortality of first generation scale insects averaged 6.4% before the first spray application on 17 May, and did not differ significantly among the three treatment groups (Kruskal-Wallis $H = 0.30$; $df = 2$; $p = 0.88$) (Figure 5). After treatment, on 22 May, the mean mortality rate for the 2% oil treatment was 55.8%, compared with mean mortality rates of 47.4% and 35.9% for the Metasystox R and 4% oil treatments, respectively. Differences in mean mortality rates among the three treatment groups were not significant (Kruskal-Wallis $H = 1.28$; $df = 2$; $p = 0.58$). The repeat spray applications on 29 May, when most scale insects were in the second instar, did not substantially increase scale mortality. Mean mortality rates on 4 June were 40% for 2% oil, 36.5% for Metasystox R, and 23.5% for 4% oil, and differed significantly among treatments (Kruskal-Wallis $H = 6.04$; $df = 2$; $p = 0.0378$).

Discussion

The phenology of the second generation of pine needle scale was complicated by the extended hatching period. Nielsen and Johnson (1973) also found an extended second generation hatching period in *C. heterophyllae* in New York. All populations studied were biparental (Stimmann (1969) had observed a uniparental population). Although several factors can influence the phenology of the scale, our study showed that it was more predictable by use of degree-day accumulation than calendar date. This correlation between degree-days and peak crawler hatch in the second generation is an

important tool for determining the best time to spray and to avoid costly and ineffective sprays. The phenology of the second generation was closely matched to degree-day accumulation in both years. This close relationship is not unusual among diaspidid scales (Beardsley and Gonzalez 1975).

Preserving natural enemies, if they are present in a field, can be an important addition to a program using oils as an alternative to broad-spectrum chemical insecticides. Horticultural oils have been shown to be harmless to coccinellids, an important predator of armored scales (Smith and Krischik 2000). A reduction in the use of broad-spectrum insecticides may increase the potential for control by natural enemies of the scale (i.e. Luck and Dahlsten 1975). Mortality caused by the oil spray combined with conservation of beneficial predators and parasitoids may provide adequate control in most situations.

Our results indicated that using horticultural oil for control of pine needle scale on Scotch pine Christmas trees was at least as effective as using broad-spectrum chemical insecticides. This confirms the observations of other workers (Nielsen 1990, Neilsen 1970, Gambrell 1938) who tested the efficacy of horticultural oil to control armored scales. In each of our trials, we carefully timed our spray applications to coincide with the completion of second-generation egg hatch and highest proportion of hyaline stage nymphs. This would be an ideal time because the hyaline stage is still susceptible to insecticides, and the eggs are no longer protected under the white waxy scale armor (Nielsen and Johnson 1972). In our study, the application of horticultural oil increased pine needle scale mortality by roughly 40%, regardless of the initial level of mortality.

In the field, the timing and manner of spray oil application are critical for success; because of its mode of action, adequate coverage must be achieved for the oil to be effective. Our results from Montcalm County showed that a common tractor-mounted airblast sprayer was capable of achieving adequate spray coverage to cause significant mortality on trees with moderate pine needle scale infestation. However, no significant differences were observed among treatment groups either before or after treatment, illustrating that the oil treatments were as effective as the chemical insecticide. The small sample size is an artifact of using an active commercial operation for the test rather than an experimental plot, but the use of a working farm demonstrates that horticultural oil can be effective if applied with an airblast sprayer. At least, horticultural oil is no worse than chemical insecticides—neither product achieved much more than 55% mortality. The difficulty of achieving adequate coverage regardless of the product used may be one reason for this low level of mortality.

Concerns of Christmas tree growers about phytotoxicity of horticultural oils may stem largely from anecdotal reports or past experience. Early horticultural spray oils did result in phytotoxicity, depending on the properties of the oil and the tree species used (Riehl 1990). The unsulfonated residue (UR) content of spray oils is associated with damage to foliage on citrus trees (Riehl 1990). Currently, there is no evidence of injury to plant foliage from oils with a 92% or higher UR (Riehl 1990). The UR content of spray oils used today is mandated at a minimum of 92% (Riehl 1990). The highly refined oils we used in our study had a UR of 98% or better, reducing the risk of phytotoxicity. We did not observe phytotoxic effects in our fields, nor did any grower bring this to our

attention. However, several varieties of Scotch pine tend to have yellowish foliage naturally (Eliason 1996) which may have masked any phytotoxic effects.

The cost of using horticultural oil has also been perceived as an obstacle to increasing its use as an insecticide in Christmas tree plantations. In Montcalm County, the second oil spray by the grower on 29 May did not significantly increase scale mortality, indicating that a single spray application would have been equally effective and less expensive. As an example, the grower reported that Damoil cost US \$5.19 per U.S. gallon (gal), chlorpyrifos was US \$44.50/gal, and MSR was US \$78.00/gal. This corresponds to \$10.38 to \$15.57 per acre for Damoil, \$11.12 for chlorpyrifos and \$19.50 for MSR, given the standard delivery rates used in Christmas tree production (per the product labels). On a per-acre basis, the cost of using horticultural oil was similar to the costs of the broad-spectrum insecticide products for this grower.

Control recommendations. Several factors can make pine needle scale difficult to control in commercial Christmas tree fields. Its capacity to rapidly increase its population size, its protective covering, and small inconspicuous size all contribute to the difficulty of detecting and controlling pine needle scale before it becomes a notable problem (Eliason and McCullough 1997, Nielsen and Johnson 1972).

Careful timing of insecticide application is important to adequately control pine needle scale, regardless of the insecticidal product used (Nielsen 1970, Martel 1972). Our data showed that an ideal window for spray application occurs between 1500-1600 degree-days, coinciding with the maximum number of second instar or hyaline stage nymphs (Nielsen and Johnson 1972, Martel 1972). Using the published degree-day

accumulations available in newsletters or on the web would be helpful to growers planning to control pine needle scale.

The mortality rates following treatment applications at our study sites did not result in complete scale mortality in any field. The perception among growers that total mortality is necessary to achieve adequate control has often led to overuse of pesticides, not only in Christmas trees but in many agricultural crops (Rose 1990). The establishment of an economic threshold level for pine needle scale would be an important addition to an integrated control program. This would demonstrate that 100% mortality is not necessary to achieve an acceptable level of control (i.e. Sadof et al. 1987). The use of horticultural oils has benefits that render it a viable option for control of pine needle scale, especially when populations are at low to moderate densities.

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Table 1. Varieties, ages and numbers of trees used in each trial

	Tree variety	Tree age	Infestation level ¹	No. trees used
Ingham County				
2000	Lake Superior Blue	11 years	Light	30
Van Buren County				
2000	Lake Superior Blue	7 years	Moderate to heavy	40
2001	Pike Lake	5 years	Moderate to heavy	50
Montcalm County				
2001-Group A	French Green	6 years	Moderate to heavy	4
2001-Groups B and C	Scotch Highland	13 years	Light to moderate	9

¹Lightly infested = fewer than 5 scales per needle, or on only one side of the tree; Moderate = 5-10 scales per needle; on at most two sides of the tree; Heavy = > 10 scales per needle; on 2 or more sides of the tree

Table 2. Number of eggs present under female scale armor during oviposition and hatching period

Field	Year	Date	Cumulative DD₅₀	No. females examined	No. eggs per female	Observations
Van Buren County	2000	26 June	975	100	0.82 ± 0.18	---
		6 July	1168	100	25.6 ± 1.05	---
		12 July	1280	83	25.4 ± 1.51	Initiation of crawler hatch
		17 July	1360	100	19.4 ± 0.96	Crawlers settling
		20 July	1422	85	19.2 ± 1.10	Hyaline stage beginning
		24 July	1481	75	12.7 ± 0.61	Peak hyaline stage
		1 August	1630	84	10.1 ± 0.46	1 st observation of white armor;
		8 August	1800	40	6.44 ±	some eggs still hatching
Ingham County	2000	27 June	975		0	---
		5 July	1154	20	12.9 ± 2.49	Still some hatch; most at 3 rd instar

Table 2 cont'd.

Field	Year	Date	Cumulative DD₅₀	No. females examined	No. eggs per female	Observations
Ingham County		11 July	1232	25	28.7 ± 3.38	---
		24 July	1436	49	19.6 ± 1.31	Hyalines and settled pink crawlers
		27 July	1530	14	10.6 ± 1.98	Peak hyaline stage
		31 July	1566	48	10.26 ± 0.58	1 st observation of white armor
		8 August	1710	14	4.75 ± 0.63	
Montcalm County	2001	17 May	350	15	1.6 ±	<i>First generation</i> Crawlers settled; 85% new pink crawlers
		22 May	400	13	1.07 ±	82% of settled crawlers turned yellow
						<i>Second generation</i>
		12 July	1124	19	13.7 ± 1.83	---
		27 July	1465	38	17.6 ± 1.21	Crawlers present
Van Buren County	2001	3 August	1630	47	11.7 ± 0.78	Peak hyaline stage
		10 August	1835	9	9.7 ± 2.16	Most females in adult stage
		19 July	1337	68	17.9 ± 0.98	Crawlers present

Table 2 cont'd.

Field	Year	Date	Cumulative DD₅₀	No. females examined	No. eggs per female	Observations
Van Buren County		25 July	1588	97	14.37 ± 0.76	Peak hyaline stage
		6 August	1817	33	8.8 ± 0.97	Hyalines, 3 rd instar females and male pupae
		13 August	2051	5	7-13 eggs	Adult males and females

^ano second generation scales were out on this date

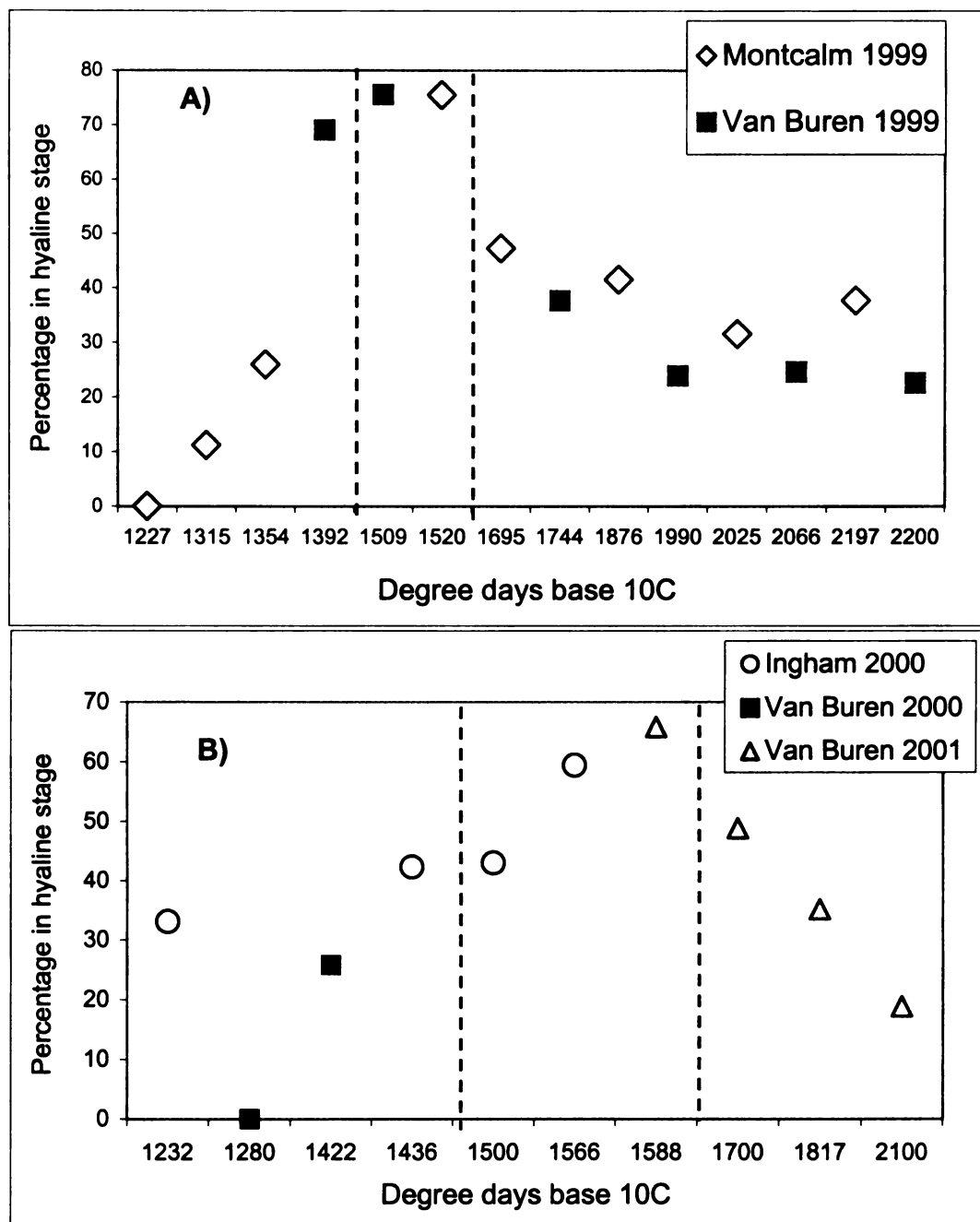


Figure 1. Percentage of scale population in the 2nd instar, or hyaline stage, in each field. A) Montcalm and Van Buren Counties, 1999. B) Ingham and Van Buren Counties, 2000 and 2001. Dotted lines indicate approximately 1500-1600 degree days. Data were not available for every sample date.

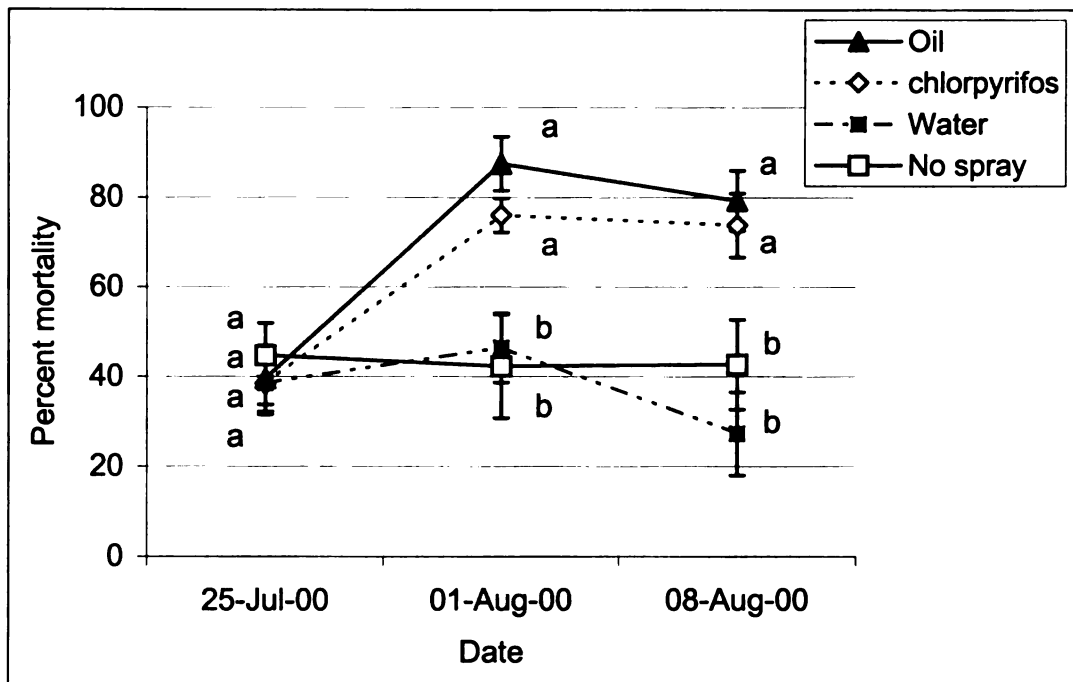


Figure 2. Mean ($\pm$ SE) pine needle scale mortality in the Van Buren County field in 2000. Treatments were applied on 25 July after pretreatment samples were taken. Significant differences among treatment groups on each date are indicated by different letters. N = 10 trees per treatment.

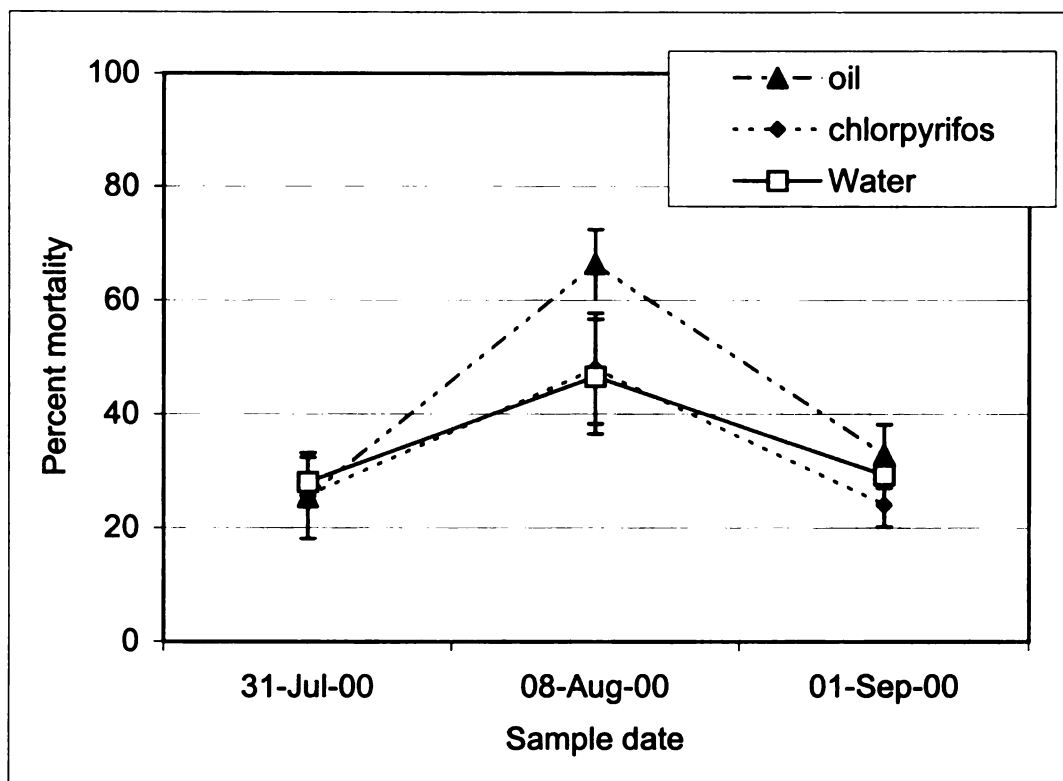


Figure 3. Mean ($\pm$ SE) pine needle scale mortality in the Ingham County field in 2000. Treatments were applied on 31 July 2000 after pretreatment samples were taken. There were no significant differences among treatment groups on any date. N = 10 trees per treatment.

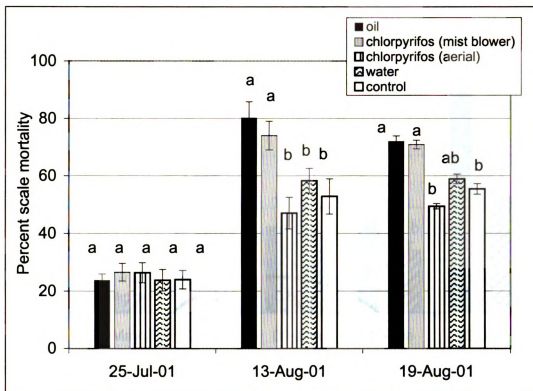


Figure 4. Pine needle scale mortality in Van Buren County, 2001. Treatments were applied on 6 August 2001. Means were separated with Fisher's LSD where the global ANOVA was significant ($p < 0.05$). Means with different letters are significantly different. Data are presented as mean $\pm$ 1 SE.

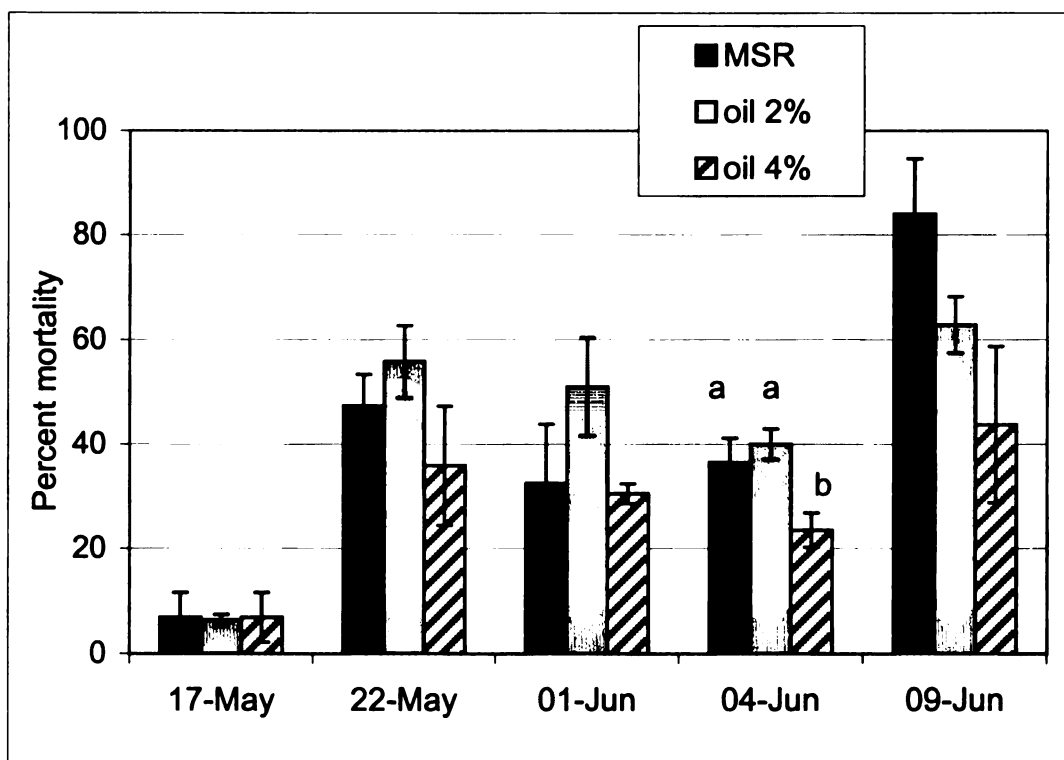


Figure 5. Percentage of mortality (mean $\pm$ 1 SE) in the Montcalm County field in 2001. Treatments were applied on 17 May and repeated on 29 May 2001. There were no significant differences among treatment groups (Kruskal-Wallis; $n=4$) except on 4 June. Significant differences on 4 June are marked with different letters.

APPENDIX

Appendix 1

Record of Deposition of Voucher Specimens*

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

Voucher No.: 2002-01

Title of thesis:

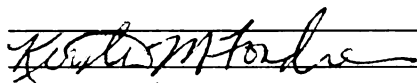
Alternative Control Methods for Two Important Insect Pests of Christmas Trees

Museum where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Investigator's Name:

Kirsten M. Fondren



Date 7/16/2002

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.

Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

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

Copies: Include as Appendix 1 in copies of thesis or dissertation.

Museum(s) files.

Research project files.

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

Appendix 1.1

Voucher Specimen Data

Page 1 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of Vials:						
		Museum where deposited						
		Other						
		Adults ♂						
		Adults ♀						
		Alate sexuparae						
		Sexuparae (wing buds)						
		Fundatrigeniae						
		Fundatrices						
<i>Mindarus abietinus</i> Koch	Ingham County, 2 May 1999	1						1
<i>Mindarus abietinus</i> Koch	Ingham County, 10 May 1999							
<i>Mindarus abietinus</i> Koch	Ingham County, 23 May 1999		1					
<i>Mindarus abietinus</i> Koch	Ingham County, 29 May 1999			1				1
<i>Mindarus abietinus</i> Koch	Ingham County, 26 May 1999							
<i>Mindarus abietinus</i> Koch	Grand Traverse County, 6 May 1999	1						
<i>Mindarus abietinus</i> Koch	Grand Traverse County, 18 May 1999				1			
<i>Mindarus abietinus</i> Koch	Grand Traverse County, 18 May 1999					1		
<i>Mindarus abietinus</i> Koch	Grand Traverse County, 26 May 1999						1	
<i>Mindarus abietinus</i> Koch	Antrim County, 12 May 1999							1
<i>Mindarus abietinus</i> Koch	Antrim County, 19 May 1999	1						
<i>Mindarus abietinus</i> Koch	Antrim County, 14 June 2000 (1 vial only)							

Voucher No. 2002-01

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

[Signature] Date 16 July 2002
Curator

Kirsten M. Fondren

Date 7/16/2002

Voucher Specimen Data

Page 2 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of Vials:							
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
<i>Chionaspis heterophyllae</i> (Cooley)	Montcalm County, 9 September 1999	4							
<i>Chionaspis heterophyllae</i> (Cooley)	Montcalm County, 17 May 2001	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Montcalm County, 12 July 2001	2							
<i>Chionaspis heterophyllae</i> (Cooley)	Montcalm County, 10 August 2001	2							
<i>Chionaspis heterophyllae</i> (Cooley)	Van Buren County, 7 September 1999	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Van Buren County, 13 September 1999	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Van Buren County, 20 July 2000	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Van Buren County, 19 August 2001	2							
<i>Chionaspis heterophyllae</i> (Cooley)	Ingham County, 27 June 2000	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Ingham County, 5 July 2000	1							
<i>Chionaspis heterophyllae</i> (Cooley)	Ingham County, 31 July 2000	2							

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Appendix 1.1

Voucher Specimen Data

Page 3 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:						
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other
<i>Chilocorus stigma</i> (Say)	Ingham County, 29 May 1999						1?	
<i>Chilocorus stigma</i> (Say)	Ingham County, 29 May 1999					1		
<i>Chilocorus stigma</i> (Say)	Ingham County, 7 June 1999					1		
<i>Chilocorus stigma</i> (Say)	Ingham County, 7 June 1999						1?	
<i>Chilocorus stigma</i> (Say)	Montcalm County, 7 July 1999					1		
<i>Chilocorus stigma</i> (Say)	Montcalm County, 12 July 1999					1		
<i>Chilocorus stigma</i> (Say)	Montcalm County, 9 September 1999					1		
<i>Chilocorus stigma</i> (Say)	Montcalm County, 27 July 2001					1		
<i>Chilocorus stigma</i> (Say)	Montcalm County, 27 July 2001					1		
<i>Chilocorus stigma</i> (Say)	Van Buren County, 13 July 1999					1		
<i>Chilocorus stigma</i> (Say)	Van Buren County, 18 August 1999					1		
<i>Chilocorus stigma</i> (Say)	Van Buren County, 20 September 1999					1		
<i>Chilocorus stigma</i> (Say)	Van Buren County, 25 July 2001					1		

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Appendix 1.1

Voucher Specimen Data

Page 4 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:						Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀♂	Other	
<i>Microweisia misella</i> (LeConte)	Ingham County, 21 June 1999					1		
<i>Microweisia misella</i> (LeConte)	Ingham County, 3 July 1999					1		
<i>Microweisia misella</i> (LeConte)	Ingham County, 6 July 1999					1		
<i>Microweisia misella</i> (LeConte)	Montcalm County, 12 July 1999					1		
<i>Microweisia misella</i> (LeConte)	Montcalm County, 12 July 1999					1		
<i>Microweisia misella</i> (LeConte)	Montcalm County, 16 August 1999					1		
<i>Microweisia misella</i> (LeConte)	Montcalm County, 3 September 1999					1		
<i>Microweisia misella</i> (LeConte)	Van Buren County, 13 July 1999					1		
<i>Microweisia misella</i> (LeConte)	Van Buren County, 7 September 1999					1		
<i>Microweisia misella</i> (LeConte)	Van Buren County, 6 August 2001					1		

Voucher No. 2002-01
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Appendix 1.1

Voucher Specimen Data

Page 5 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:						
		Eggs	Larvae	Nymphs	Pupae	Adults ♀♂	Other	Museum where deposited
<i>Hemerobius stigma</i> Stephens <i>Hemerobius stigma</i> Stephens <i>Hemerobius stigma</i> Stephens <i>Hemerobius stigma</i> Stephens	Grand Traverse County, 25 April 2000					1		
	Grand Traverse County, 10 May 2000					1		
	Grand Traverse County, 26 May 2000					2		
	Grand Traverse County, 6 June 2000					1		
<i>Chrysopa oculata</i> Say <i>Chrysopa oculata</i> Say <i>Chrysopa oculata</i> Say	Grand Traverse County, 26 May 2000					2		
	Grand Traverse County, 5 June 2000					1		
	Grand Traverse County, 6 June 2000					3		
<i>Chrysoperla rufilabris</i> Burmeister	Reared from larval culture					5		
<i>Mulsantina picta</i> (Randall) <i>Mulsantina picta</i> (Randall) <i>Mulsantina picta</i> (Randall) <i>Mulsantina hudsonica</i> (Casey) <i>Mulsantina hudsonica</i> (Casey) <i>Mulsantina hudsonica</i> (Casey)	Grand Traverse County, 2 May 2000					3		
	Grand Traverse County, 10 May 2000					1		
	Grand Traverse County, 14 May 2001					1		
	Antrim County, 27 April 1999					1		
	Grand Traverse County, 15 June 1999					1		
	Grand Traverse County, 25 April 2000					1		

Voucher No. 2002-01

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Appendix 1.1

Voucher Specimen Data

Page 6 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀♂	Other		
<i>Hippodamia parenthesis</i> (Say)	Antrim County, 18 April 2000					2			
<i>Hippodamia parenthesis</i> (Say)	Antrim County, 2 May 2000					1			
<i>Hippodamia parenthesis</i> (Say)	Antrim County, 26 May 2000					1			
<i>Hippodamia parenthesis</i> (Say)	Ingham County, 29 April 2000					1			
<i>Hippodamia parenthesis</i> (Say)	Grand Traverse County, 25 April 2000					1			
<i>Hippodamia parenthesis</i> (Say)	Van Buren County, 18 August 1999					1			
<i>Hippodamia parenthesis</i> (Say)	Kalamazoo County, 30 May 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 12 May 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 18 May 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 26 May 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 22 June 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 8 June 1999					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 10 May 2000					1			
<i>Anatis mali</i> (Say)	Grand Traverse County, 14 May 2001					1			
<i>Anatis mali</i> (Say)	Antrim County, 25 April 2000					1			
<i>Anatis mali</i> (Say)	Antrim County, 14 May 2001					1			
<i>Anatis labiculata</i> (Say)	Grand Traverse County, 26 May 1999					1			

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Appendix 1.1

Voucher Specimen Data

Page 7 of 8 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
<i>Coccinella trifasciata perplexa</i> Mulsant	Kalamazoo County, 9 April 2000					1			
<i>Coccinella trifasciata perplexa</i> Mulsant	Grand Traverse County, 26 May 1999					1			
<i>Coccinella transversoguttata</i>	Grand Traverse County, 27 April 1999					1			
<i>richardsonii</i> Brown	Grand Traverse County, 6 May 1999					1			
	Grand Traverse County, 18 May 1999					2			
	Grand Traverse County, 26 May 1999					3			
<i>Coccinella novemnotata</i> Herbst	Grand Traverse County, 2 May 2000					1			
<i>Coccinella novemnotata</i> Herbst	Grand Traverse County, 10 May 2000					1			
<i>Cycloneda munda</i> (Say)	Antrim County, 17 May 2000					1			
<i>Cycloneda munda</i> (Say)	Antrim County, 26 May 2000					1			
<i>Cycloneda munda</i> (Say)	Antrim County, 6 June 2000					1			
<i>Cycloneda munda</i> (Say)	Kalamazoo County, 25 May 2000					1			
<i>Cycloneda munda</i> (Say)	Kalamazoo County, 9 June 2000					1			

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Appendix 1.1

Voucher Specimen Data

Page 8 of 8 Pages

Number of:	Museum where deposited	
	Other	
	Adults ♂	
	Adults ♀	1 1 2
	Pupae	
	Nymphs	
	Larvae	
	Eggs	
Species or other taxon	Label data for specimens collected or used and deposited	
<i>Allograpta obliqua</i> (Say)	Ingham County, 29 May 2000	
<i>Allograpta obliqua</i> (Say)	Grand Traverse County, 14 May 2001	
<i>Allograpta obliqua</i> (Say)	Kalamazoo County, 15 May 2000	

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