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INFLUENCE OF HOST PHENOLOGY ON VOLTINISM PATTERNS
OF ALDER, BIRCH AND HAWTHORN LEAFMINER
(HYMENOPTERA: TENTHREDINIDAE), WITH EVIDENCE FOR
BET-HEDGING STRATEGIES IN BIRCH AND ALDER LEAFMINER.

presented by

Joseph Lee Chichester

has been accepted towards fulfillment
of the requirements for

Master's degree in Entomology

David A. Herms
Major professor

Date 4 October 1994

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INFLUENCE OF HOST PHENOLOGY ON VOLTINISM PATTERNS OF ALDER, BIRCH AND HAWTHORN LEAFMINER (HYMENOPTERA: TENTHREDINIDAE), WITH EVIDENCE FOR BET-HEDGING STRATEGIES IN BIRCH AND ALDER LEAFMINER.

By

Joseph Lee Chichester

A THESIS

Submitted to
Michigan State Uninverstiy
in partial fulfillment of the requirements
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MASTER OF SCIENCE

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1994

ABSTRACT

INFLUENCE OF HOST PHENOLOGY ON VOLTINISM PATTERNS OF ALDER, BIRCH AND HAWTHORN LEAFMINER (HYMENOPTERA: TENTHREDINIDAE), WITH EVIDENCE FOR BET-HEDGING STRATEGIES IN BIRCH AND ALDER LEAFMINER

By

Joseph Lee Chichester

Alder, birch, and hawthorn leafminer oviposited exclusively in immature foliage. Larvae were aggregated even though no consistent physical and chemical differences were detected between colonized and non-colonized leaves, and despite intra-specific competition. Larval mortality increased as leaves matured, becoming tougher and less nutritious. Pre-mature extra-long diapause occurred in first and second generation birch and alder leafminers, with the number of emerging adults proportional to the quantity of immature foliage available to their progeny.

These data support the hypothesis that larvae can develop only in immature foliage, and that the indeterminate growth of birch and alder permitted the evolution of multivoltinism in birch and alder leafminers, while determinate growth of hawthorn has constrained hawthorn leafminer to one generation. Premature extra-long diapause of birch

and alder leafminers may be a bet-hedging strategy; diapausing individuals forego a breeding opportunity, but avoid competition for the decreased supply of their obliate food resource: immature leaves.

I dedicate this work to Marcy Ochs and my immediate family.
Their patience, support, and understanding
could never be surpassed.

ACKNOWLEDGMENTS

I thank Daniel A. Herms, my major advisor and friend, for giving me the opportunity to pursue a career in entomology. Dan spent countless hours reviewing my work and making valuable suggestions which have helped me grow as a scientist. This work would not of been possible without the financial support of The Dow Gardens and the Herbert and Grace A. Dow Foundation of Midland, Michigan. I thank Douglas J. Chapman, Director of The Dow Gardens, for his willingness to support me in this project.

I thank my committee, William Mattson, Guy Bush, James Miller, and Cathy Bristow for their valuable suggestions throughout the planning of and interpretation of my research. I would like to extend my gratitude to William Mattson for his valuable data analysis assistance and suggestions.

This research would not of been possible without the extended help of Ted Bomba, Tisha Walls, and Bruce Birr. Ted and Tisha spent many tedious hours counting leaves and collecting other data. Their good sense of humor made long days something to look forward to. Bruce spent numerous hours conducting chemical analysis on hundreds of leaf samples. I also thank Bruce for his patience and friendly assistance with my countless questions and interruptions. I would also like to recognize D.R. Smith, Research Entomologist, Systematic Entomology Laboratory, USDA, for the proper identification of the insects used in this study.

I would like to acknowledge two colleagues: Joe Spencer and Cathy Papp. I am grateful for their support, suggestions, and friendship, which I can't thank them enough for.

My immediate family was very supportive in my quest to earn my degree. They constantly reminded me of my success and how I made them very proud. Last, but not least, I gratefully thank Marcy Ochs for her love, friendship, support, understanding, and most of all her extreme patience. I am forever grateful!

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Three related hymenopteran leafminers (Tenthredinidae: Fenusini) occur sympatrically in Midland, Michigan: alder leafminer (*Fenusa dohrnii* Tischbein), birch leafminer (*Fenusa pusilla* Lepeletier) and a leafminer of hawthorn (*Profenusa canadensis* Marlett) (hereafter referred to as the hawthorn leafminer). They specialize on tree species within the genera *Alnus*, *Betula*, and *Crataegus*, respectively. The biology of all three species is similar, with the birch leafminer being most thoroughly studied. Birch leafminer females deposit eggs singly between the epidermal layers of an immature leaf (Friend 1933, DeClerck & Shorthouse 1985). Upon hatching, larvae begin mining the palisade tissue. Mining ceases when the fifth instar (pre-pupal stage) is reached (Friend 1933). Mature larvae exit the leaf, fall to the ground, and pupate a few centimeters below the soil surface (Jones and Raske 1976, DeClerck & Shorthouse 1985). At this point, interspecific and intraspecific differences in life history patterns occur. The hawthorn leafminer is univoltine, remaining in the soil until the following spring (Wilson et al. 1982). However, alder and birch leafminers are multivoltine, with first and second generation individuals emerging as adults in early July and mid-August to initiate a second and third generation, respectively (DeClerck & Shorthouse 1985; Hart et al. 1991).

Since females oviposit almost exclusively in immature foliage (Friend 1933, Wilson et al. 1982, Hart et al. 1991), the evolution of voltinism patterns of the alder, birch, and hawthorn leafminers may have been influenced by the phenology of their respective host plants. Alder and birch display indeterminate growth, producing new foliage throughout the summer (personal observation, Friend 1931). Hawthorn trees do not produce new foliage after the initial flush at bud-break, thus displaying determinate growth (personal observation). The continuous production of leaves by alder and birch may have allowed alder and birch leafminer to evolve a multivoltine life cycle, whereas hawthorn leafminer may be restricted to a univoltine life cycle due to the determinate growth pattern of its host. Such phenological constraints on life history have been reported by Auerbach and Simberloff (1984) for two leafmining species, *Eriocraniella* sp and *Dyseriocranina* sp (Lepidoptera: Eriocraniidae), which also feed only on first-flush leaves.

Qualitative differences between immature and mature leaves may explain why females oviposit in immature foliage. Food quality has been shown to play a role in insect seasonality in many cases (Wolda 1988). Ayres and MacLean (1987) identified several leaf characteristics that change with age, affecting the growth of a geometrid larvae (*Epirrita autumata*). As leaves age, water, nitrogen, and phosphorous concentrations decline and larval performance decreases while toughness and specific mass increase. They proposed that phenological constraints imposed on the larvae by leaf maturation should influence the evolution of larval phenology, selecting for early season development. A similar relationship between leaf and insect phenologies was documented by Merle (1988) for the green oak leafroller, *Tortrix viridana* (Lepidoptera: Tortricidae) and various species of oak.

In central Michigan, the number of first generation adult birch leafminers (overwintering individuals) always greatly

xceed numbers of second and third generation adults (Herms unpublished data). In the case of the alder leafminer, large first and second generations are followed by a small third generation (Hart et al. 1991). This intrapopulation variability in adult emergence may be related to host plant phenology. If the number of immature leaves present at any one time decreases after the first flush, then the quantity of immature leaves available to second and third generation alder and birch leafminer larvae would be only a fraction of that available to the first.

Intrapopulation variability in the timing of adult emergence which coincides with temporal variability in resource availability has been reported in two Lepidopteran (Gracillariidae) leafmining species, *Acrocercops* sp. and *Neurobathra strigifinitella* (Auerbach & Simberloff 1984). The voltinism patterns of these two species are similar to that of the birch leafminer, with a large first generation followed by a small second and third generation.

The decreased availability of immature leaves over the course of the season could create the potential for competition among larvae. The observed intraspecific variation in voltinism patterns of the birch leafminer may be a density-dependent evolutionary response to intraspecific competition among larvae for the limiting food resource (immature leaves). Intraspecific competition has been observed in several leafminer species. Tuomi et al. (1981) documented intraspecific competition in *Scolioneura betuleti*, a hymenopteran leafminer colonizing birch leaves. Intraspecific competition occurred as population density increased, resulting in decreased larval survival. They also found a negative correlation between mean larval weight and larval density. Furthermore, mean larval weight was positively correlated with leaf area available per larva. These findings coincide with those of Quiring and McNeil (1984), who documented competition in alfalfa blotch leafminer (*Agromyza frontella* Rondani) populations. Stiling (1984) reported that larval mortality of *Hydrellia valida* Loew on salt marsh cordgrass increased when two or more mines coalesced, and that intraspecific competition accounted for 11.4% of total mortality. Intraspecific competition has also been documented in populations of leafminers on oak, aspen, and lima beans (Bultman & Faeth 1986a, Auerbach 1991, and Petitt & Wietlisbach 1992, consecutively). It appears that intraspecific competition occurs at high larval densities due to food limitation.

Intraspecific competition may select for density-dependent premature extra-long diapause (*sensu* Hanski 1988). Diapause is a state of low metabolic activity which is hormonally controlled. During diapause, morphogenesis is reduced, resistance to environmental extremes is increased, and behavioral activity is altered or reduce (Tauber et al. 1984). Hanski (1988) reviewed the different kinds of extra-long diapause. Extra-long diapause occurs when individuals miss one or more breeding opportunities experienced by other members of the population by entering diapause early (premature extra-long diapause) or by exiting diapause late (prolonged extra-long diapause).

Premature and prolonged extra-long diapause may be subdivided into density-independent and density-dependent diapause. Density-independent diapause induction is not affected by density and all individuals face the same environmental factors (Hanski 1988). Density-dependent diapause, on the other hand, is affected by the number of individuals in which a target individual interacts and therefore, a function of population size or density. As larval density increases, the frequency of negative interactions (intraspecific competition) between individuals increases. Thus, intraspecific competition among larvae may select for density-dependent premature extra-long diapause. Premature extra-long diapause may be an evolutionary response to larval competition by a portion of the second and third generation of alder and birch leafminer population. If there is no intrapopulation variability in emergence (i.e. all individuals emerge as adults) and if fewer leaves are available for oviposition as the season progresses, then larval competition could potentially be very high during the second and third generations, resulting in high larval mortality. The pitcher-plant mosquito, *Wyeomyia smithii*, is one of the few documented examples of density-dependent premature extra-long diapause. According to Istock et al. (1975), when populations are high, some individuals may shift to a univoltine life cycle by entering diapause until resources are more plentiful.

The small size of the second generation birch leafminer, relative to the first generation, may result because most individuals do not emerge from pupation for a second generation. Instead, these individuals may enter density-dependent premature extra-long diapause (*sensu* Hanski 1988) to avoid intraspecific competition among offspring in a resource-limited habitat (limited availability of immature leaves). These individuals would loose a breeding opportunity and emerge the following spring when a full flush of leaves is once again available. Thus, a "bet-hedging" (Philippi and Seger 1989) diapause strategy may have evolved in birch leafminer. Philippi and Seger (1989) defined bet-hedging as an evolutionary trade off between mean and variance of fitness. Individuals with a lower mean fitness may have a selective advantage over highly fit individuals under certain conditions. In years where conditions are favorable for larval development (warm summers, high quality food, and low levels of competition), the small portion of the population that emerges for a second generation would have a selective advantage over the portion of the population that diapaused. In years when conditions are not favorable, the portion of the population entering diapause would have a selective advantage.

A evolutionary bet-hedging strategy linking winter diapause termination with resource availability has been identified in two Lepidoptera (Eriocraniidae) leafmining species. *Acrocercops* sp. and *Neurobathra* sp. have staggered termination of winter diapause which allows them to respond to atypically large leaf flushes at certain times of the growing season (Auerbach & Simberloff 1984).

Climatic conditions such as photoperiod, temperature, and resource availability and quality may play a significant role in insect phenology (Tauber and Tauber 1973). Climatic conditions influence the termination date of leaf production and larval development rates (Scriber & Lederhouse 1992). Thus, climatic variation has the potential to influence the evolution of a density-independent premature extra-long diapause in second generation alder and birch leafminers. Decreased numbers of adults giving rise to third generation larvae suggest that some second generation alder and birch leafminer individuals may hedge their bets by entering density-independent premature extra-long diapause (*sensu* Hanski 1988). This portion of the population would loose a breeding opportunity but emerge the following spring when the development of their offspring would not be constrained by climatic conditions. The offspring of the smaller portion of the population giving rise to the third generation, may have to "race" to finish development before unfavorable fall climate conditions prevail (cessation of immature leaf production, low temperatures, and leaf abscission). These individuals would have a selective advantage over diapausing individuals in years when development can be completed.

Bet-hedging may also take the form of risk-spreading through phenotypic variability in offspring. Offspring from a single alder or birch leafminer female may be phenotypically different from one another to ensure that at least some individuals will be adapted to a uncertain future. Thus, it is an adaptive strategy that may increase fitness in a temporally variable environment (Bradford & Roff 1993). Bradford and Roff (1993) suggested that a bet-hedging diapause strategy occurs in a partially bivoltine population of cricket (*Allanemobius fasciatus*). As first generation females aged, the proportion of diapausing eggs increased, reflecting a decrease in the probability of second generation offspring being able to complete development before the end of the growing season.

OBJECTIVES

Theoretical Hypothesis

The primary objective of this research was to test the following theoretical hypothesis:

> The phenological pattern of immature leaf production by their respective host plants has directed the evolution of the voltinism patterns of alder, birch, and hawthorn leafminer. Specifically, the determinate growth pattern of hawthorn has constrained the hawthorn leafminer to a univoltine life history, while the indeterminate growth pattern of alder and birch has allowed the evolution of multivoltinism in alder and birch leafminer. Furthermore, intraspecific temporal variation in the pattern of new leaf production by alder and birch has resulted in a bet-hedging diapause strategy in alder and birch leafminer. This strategy takes the form of density-dependent premature extra-long diapause (*sensu* Hanski 1988) selected for by intraspecific competition for the immature leaf food resource.

Specific Objectives

The following experimental objectives were used to test the theoretical hypothesis:

(1) to characterize the seasonal presence and abundance of alder, birch, and hawthorn leafminer;

(2) to characterize the spatial distribution of larvae of all three species;

(3) in order to explain observed distribution patterns, to compare physical and chemical traits of colonized and noncolonized leaves;

(4) to test the hypothesis that immature leaves are the only suitable resource for the survival and development of all three species by:

    (a) in choice and no-choice experiments, comparing oviposition preference for immature relative to mature foliage,

    (b) comparing chemical and physical differences of immature and mature leaves,

    (c) quantifying larval survival of all three species in relation to leaf maturity;

(5) to quantify seasonal variation in the availability of the immature leaf resource of all three host species;

(6) to test for intraspecific competition among larvae of all three species for their respective immature leaf resource; and

(7) to test for correlation between variation in the relative seasonal abundance of the three leafminer species and temporal variation in the production of immature leaves by their respective host plants.

CHAPTER ONE

ABUNDANCE, LARVAL DISTRIBUTION, AND VOLTINISM PATTERNS OF ALDER, BIRCH, AND HAWTHORN LEAFMINER IN CENTRAL MICHIGAN

Alder, birch, and hawthorn leafminer are closely related hymenopteran sawflies (Tenthredinidae: Fenusini) occurring sympatrically in Midland, Michigan. They specialize on tree species within the genera *Alnus*, *Betula*, and *Crataegus*, respectively. *Alnus glutinosa* L., *Betula papyrifera* Marsh., and *Crataegus crusgalli* L. were the respective hosts of the leafminer populations studied in this project. The hawthorn leafminer is native to North America, while the alder and birch leafminers were introduced from Eurasia (Smith 1979).

The biology of the three leafminer species are similar. Each overwinters as pupae in the soil directly below its host. Adult emergence begins in late April to mid-May as bud break occurs (Slingerland 1905, Parrott & Fulton 1915, Friend 1933, Hart et al. 1991). Females of all three species oviposit almost exclusively in immature leaves (Slingerland 1905, Parrott & Fulton 1915, Friend 1933, Wilson et al. 1982, Hart et al. 1991). Eggs of the birch leafminer do not hatch if deposited in mature or nearly mature leaves (Friend 1933).

Females deposit eggs singly between the epidermal layers of a leaf (Slingerland 1905, Parrott & Fulton 1915, Friend 1933, DeClerck & Shorthouse 1985). Upon hatching, larvae begin mining the palisade tissue. Mining ceases when the fifth instar (pre-pupal stage) is reached (Friend 1933). Mature larvae exit the leaf, fall to the ground, and pupate a few centimeters below the soil surface

(Slingerland 1905, Parrott & Fulton 1915, DeClerck & Shorthouse 1985). At this point, interspecific and intraspecific differences in life history patterns occur. The hawthorn leafminer is univoltine, remaining in the soil until the following spring (Parrott & Fulton 1915, Wilson et al. 1982). However, alder and birch leafminers are multivoltine with first and second generation individuals emerging as adults in early July and mid-August to initiate respective second and third generation (DeClerck & Shorthouse 1985; Hart et al. 1991).

The distribution of organisms within their environment may be random or non-random. A random distribution implies that the environment is homogenous and/or individuals do not selectively choose their habitat. Organisms may also be non-randomly distributed due to some constraints. Non-randomly distributed organisms can be either clumped (aggregated) or uniform (evenly distributed) (Ludwig & Reynolds, 1988). Such factors as wind and water currents, light intensity, reproductive strategy (asexual, sexual), territoriality, and intraspecific competition may influence the distribution of organisms (Ludwig & Reynolds, 1988).

A result of oviposition site selection by females, the larval distribution of several leafminer species has been found to be clumped. Stiling et al. (1987) reported that the larvae of *Stilbosis quadricustatella*, a leafmining moth, are distributed non-randomly within leaves of sand live oak (*Quercus geminata*) and water oak (*Quercus nigra*). When leaves contained two or more larvae, fewer larvae were found together on the same side of the mid-vein than expected by chance.

Bultman and Faeth (1986b) reported that four species of leafminers on *Quercus emoryi* selected leaves based on leaf size. *Stigmella* sp. and *Tischeria* sp., which produce small mines, selected small leaves. *Stilbosis juvantis* produces intermediate sized mines and selected average sized leaves while *Cameraria* sp. selected large leaves and produced large mines. In addition, *Cameraria* sp. densities were lower and dispersion was more aggregated on small-leaved branches and, in contrast to the birch leafminer, densities were greater in the shaded regions of trees (Faeth 1991).

Oviposition and larval distribution may be influenced by such factors as leaf location (sun leaves vs shade leaves) (Hamilton 1943, Faeth 1991), leaf size (Bultman & Faeth 1986b, Faeth 1991), leaf abscission (Stiling et al. 1991), and resource or interference competition (Stiling et al. 1987, Faeth 1991).

Birch leafminer distribution is concentrated on the tip and periphery of the crown (Jones and Raske 1976). Friend (1931) reported that birch leafminer females only oviposited in immature leaves, resulting in only the tips of the branches being infested. Hawthorn leafminer females prefer to oviposit in leaves that first appear and which are still in the unfolded state (Parrott & Fulton 1915) and which are in a warm sunny location (Hamilton 1943).

OBJECTIVES

The objectives of this research were to characterize larval and adult phenology and abundance of alder, birch, and hawthorn leafminers, as well as the spatial distribution of larvae within the host plant. Physical and chemical differences between infested and uninfested leaves were also characterized in an attempt to understand the mechanisms underlying the observed distributions.

METHODS AND MATERIALS

Research Sites

Populations of all three species were identified in Midland, Michigan. The alder leafminer population had colonized an experimental planting of European alder (*Alnus glutinosa*), and the birch leafminer population was located in an experimental paper birch planting (*Betula papyrifera*) at the Dow Gardens. Hawthorn leafminer had established a population in an ornamental hawthorn (*Crateagus crusgalli*) planting on the estate of H. H. Dow. These populations and host plants were used for all studies.

Adult Abundance and Phenology

To monitor the presence and abundance of each species, one yellow sticky trap was deployed in each of eight trees for all three species. Traps were hung near the terminal portion of branches. Therefore, during the second and third generation of alder and birch leafminer, traps were near immature leaves (oviposition sites). In 1993, four additional alder trees were used to monitor the population. Each of the four alder trees received two yellow sticky traps, while each of the original eight trees received one yellow sticky trap, for a total of 16 traps. In 1992 and 1993, from May to August, the number of adults per trap were counted weekly and traps were changed as needed. The mean number of adults per trap was plotted against time.

Larval Distribution and Abundance

In 1993, larval abundance and distribution were quantified by examining each individual leaf on each of four branches on six alder, eight birch, and eight hawthorn trees. Branches of approximately equal size that exhibited long extension growth the previous year were sampled. The number of larvae in each leaf of each branch was recorded. In 1992, the larval density and distribution of birch, alder, and hawthorn leafminers was attempted, but complete data sets were not acquired. The density and distribution of all generations of each species were quantified in 1993.

Physical and Chemical Comparisons of Infested and Uninfested Leaves

In 1992 and 1993, during the first generation, ten infested and ten uninfested leaves of the same age class were randomly sampled on a weekly basis from each of eight trees of all three species. While in the field, leaves were placed in labelled plastic bags and stored on ice. Leaves were promptly transported to the laboratory.

For all leaves the following variables were measured: (1) toughness (g) with a penetrometer, (2) leaf area (cm^2) with a digital image analyzer, (3) specific leaf mass (g/m^2), and (4) nitrogen and phosphorous concentration (% dry wt.) using standard micro-kjeldahl techniques. Chemical analyses were conducted in the laboratory of W.J. Mattson by Bruce Birr, USDA Forest Service, at Michigan State University, East Lansing, MI.

DATA ANALYSIS

Adult Abundance and Phenology and Larval Distribution and Abundance

To test the hypothesis that larvae were distributed randomly among leaves, the observed larval distribution was compared with the theoretical Poisson distribution using Chi-Square Analysis. If the hypothesis of a Poisson distribution was rejected (indicating a non-random distribution), and the variance-to-mean ratio was greater than one, indicating clumping, then the hypothesis that larvae were distributed according to the negative binomial model was tested using Chi-Square analysis and by calculating the *k* parameter. As *k* approaches zero, the degree of clumping increases. Evidence of clumping was further evaluated by calculating the index of dispersion (ID) (variance:mean ratio) and *d*-statistic. An ID value of less than one indicates a random distribution, a value equal than one indicates a uniform distribution, and a value of greater than one indicates a clumped distribution (Ludwig & Reynolds, 1988). An absolute *d*-statistic value of less than 1.96 indicates a random distribution, a *d*-statistic value less than -1.96 indicates a uniform distribution, and a *d*-statistic value greater than 1.96 indicates a clumped distribution (Ludwig & Reynolds, 1988). The degree of clumping was also estimated using Green's index. Green's index ranges from zero to one, with zero indicating a random distribution and one indicating a clumped distribution (Ludwig & Reynolds, 1988). In addition, larval abundance and distribution were also visually examined by plotting a frequency histogram of the number of larvae per leaf.

Physical and Chemical Comparisons of Infested and Uninfested Leaves

Means of the following variables of infested and uninfested leaves were compared using a t-test: (1) leaf toughness, (2) leaf area, (3) specific leaf mass, and (4) chemical composition (percent nitrogen and phosphorous).

RESULTS

Adult Abundance And Phenology

Alder leafminer

In 1992, adults emerged before sampling began on 11 May (Figure 1A). Distinct generations were difficult to distinguish, but at least two population peaks were observed. The first peak occurred on 11 May (16 ± 3 adults/trap), and the second on 27 July (28 ± 6 adults/trap). No adults were observed after 13 September.

In 1993 traps were deployed on 28 April. Adults were first collected on 11 May (Figure 1B). As in 1992, distinct generations were difficult to distinguish, but at least two population peaks were observed with the second being larger (205 ± 63 adults/trap on 26 July) than the first (80 ± 25 adults/trap on 10 May). No adults were observed after 26 August.

Birch leafminer

In 1992, adults emerged before sampling began on 11 May; the first population peak was large (122 ± 24 adults on 11 May) (Figure 2A) while the second

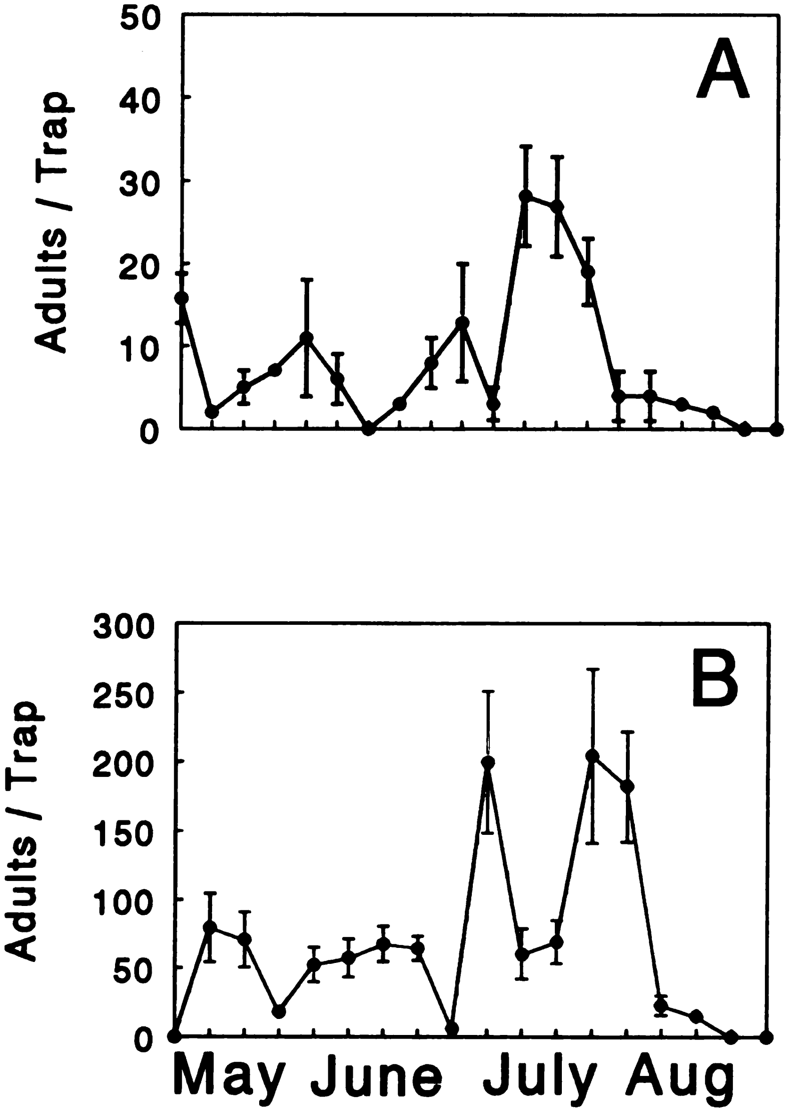

Figure 1: Seasonal phenology of adult alder leafminer in 1992 (A) and 1993 (B).

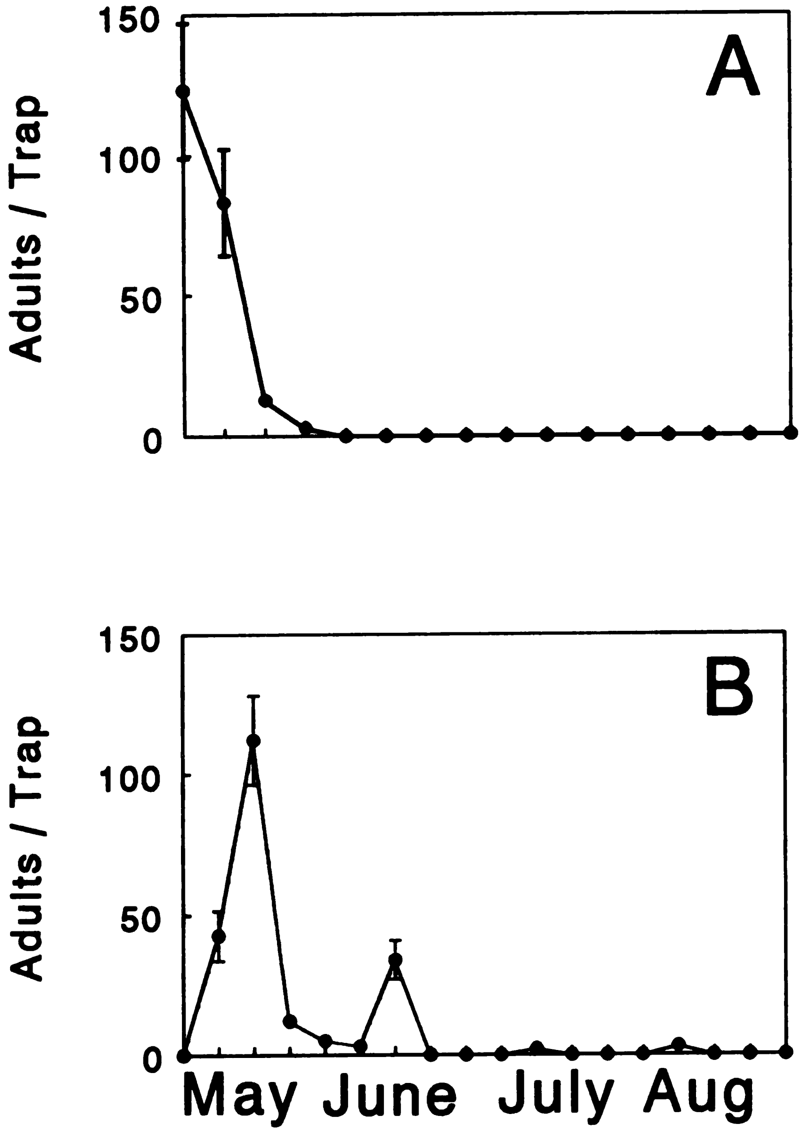

Figure 2: Seasonal phenology of adult birch leafminer in 1992 (A) and 1993 (B).

and third population peaks were small relative to that of the first (< 1 adult/trap). Adults were last observed on 14 July (Figure 2A).

In 1993, traps were deployed on 28 April and adult emergence was first observed on 11 May. Again a large first generation (112 ± 16 adults/trap on 18 May) was followed by a relatively small second and third generation (0-5 adults/trap) (Figure 2B). Peak emergence of the second and third generations were observed on 13 July and 10 August, respectively and no adults were observed after 17 August (Figure 2B).

Hawthorn leafminer

In 1992, adults emerged before sampling began on 11 May, which was also when the largest adult population count of 1071 ± 84 adults per trap was observed. No adults were trapped after 22 June (Figure 3A).

In 1993, traps were deployed on 28 April and adults were first captured on 11 May. A single generation was observed with peak emergence occurring on 18 May (1100 ± 50 adults/trap) and no adults were trapped after 29 June (Figure 3B), resulting in one generation.

Larval Distribution and Abundance

Alder leafminer

During the first generation of 1993, larval distribution was found to be non-random as the hypothesis of a Poisson distribution was rejected ($P < 0.001$; $X^2 = 1857$; $d.f. = 3$). The hypothesis of a negative binomial distribution was also rejected ($P = 0.01$; $X^2 = 15$; $d.f. = 10$; $k = 0.082$). The overall variance of the number of larvae per leaf increased at a faster rate than the mean number of larvae per leaf

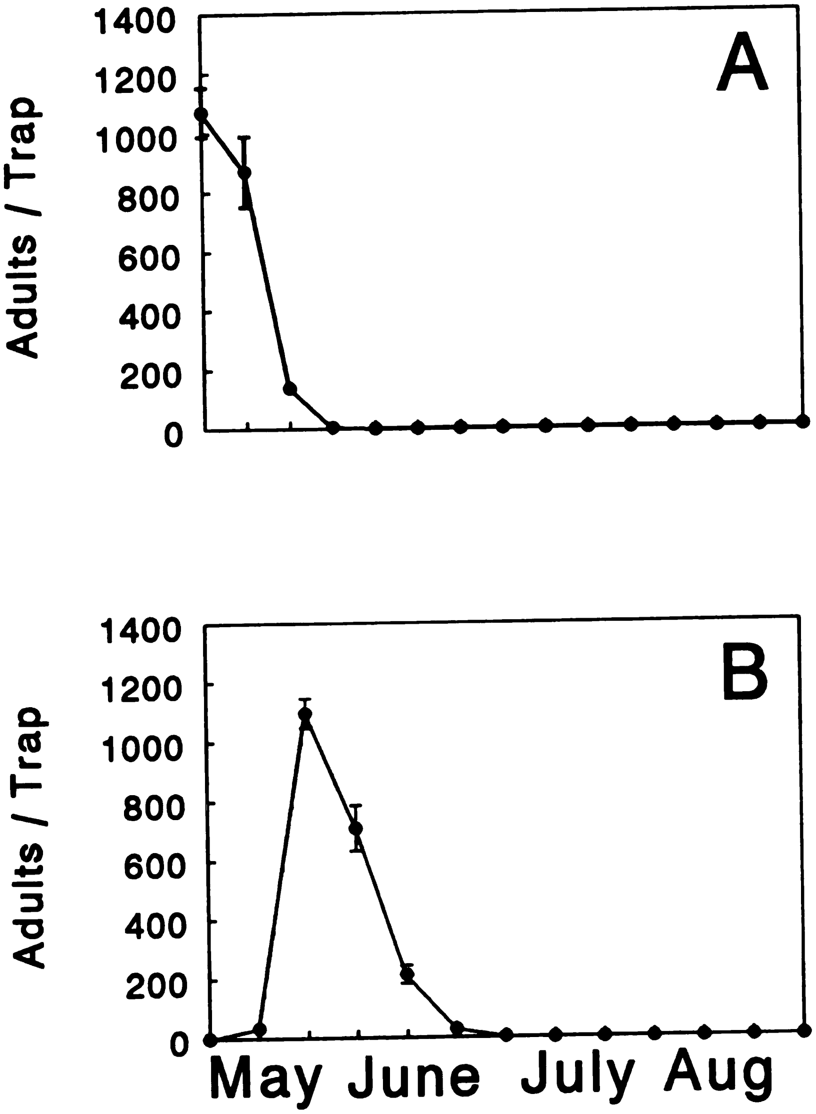

Figure 3: Seasonal phenology of adult hawthorn leafminer in 1992 (A) and 1993 (B).

($Y = 0.4e^{2.6X}$; $r^2 = 0.76$) (Figure 4A) and the variance-to-mean ratio was 15. Both results suggest a clumped distribution. The *d*-statistic ($d = 171$) also indicated a highly clumped larval distribution. However, Green's index (GI = 0.008) suggested that larval distribution approached random.

During the first generation, 81% of all leaves were uninfested ($n = 1768$). One larvae per leaf was the most frequently observed class among infested leaves ($n = 109$) and up to 80 larvae per leaf were observed (Figure 5A). There was 1 ± 14 larvae per leaf (Figure 5A).

On the second sample date (29 June) larval distribution, over all leaves (mature and immature combined) and over immature leaves only, was non-random and the hypothesis of a Poisson distribution was rejected ($P < 0.001$; $X^2 = 3954$; $d.f. = 3$ and $P < 0.001$; $X^2 = 2264$; $d.f. = 4$, respectively). The hypothesis of a negative binomial distribution was also rejected ($P < 0.001$; $X^2 = 36$; $d.f. = 9$; $k = 0.0364$ and $P < 0.001$; $X^2 = 29$; $d.f. = 9$; $k = 0.199$, respectively). Both the variance-to-mean ratio and the *d*-statistic, however, indicated a highly clumped larval distribution for all leaves (ratio = 10; $d = 156$), as well as for immature leaves only (ratio = 9; $d = 89$). As for the first sample date, Green's index indicated that the larval distribution was near random for mature and immature leaves combined (GI = 0.006), as well as for immature leaves only (GI = 0.005).

Considering immature and mature leaves combined, only 10% of 2374 were infested; 25% of 1000 immature leaves contained larvae. Among the infested leaves, one larvae per leaf was the most frequently observed class ($n = 44$), and as many as 29 larvae per leaf was observed (Figure 5B). There were 1 ± 6 larvae per leaf for all leaves considered (mature and immature combined), but 2 ± 13 larvae per leaf for immature leaves only.

Similar trends in larval distribution occurred on the third sample date (13 July). The hypothesis of a Poisson distribution was again rejected using Chi-Square

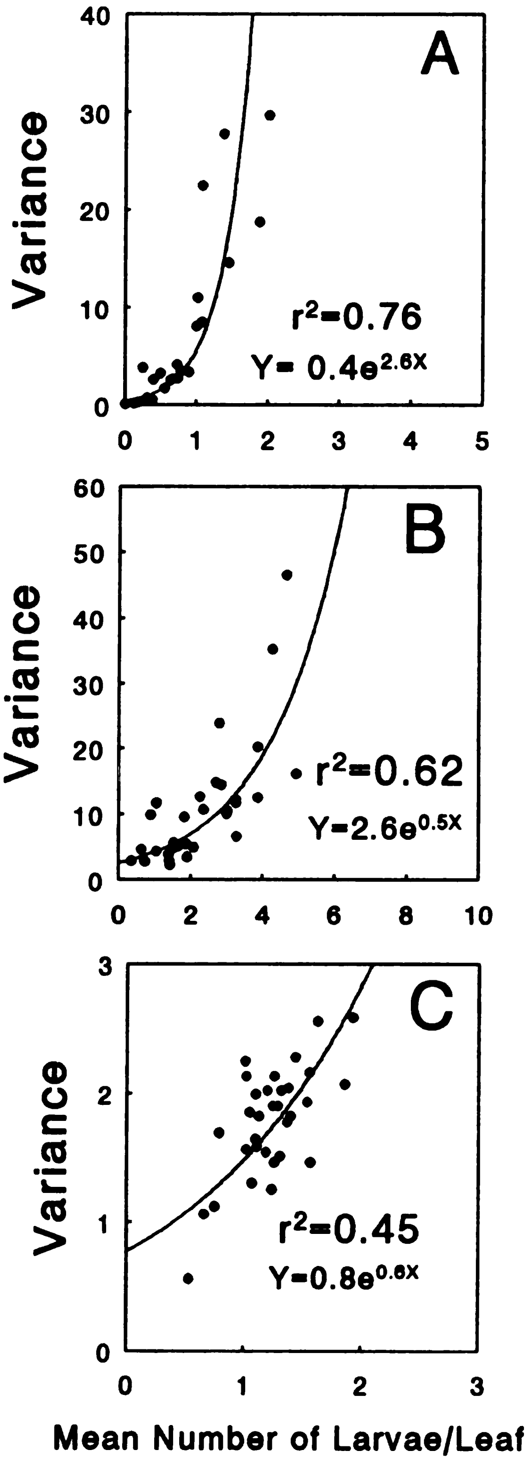

Figure 4: Mean versus variance for the number of first generation larvae per leaf in 1993: (A) alder leafminer, (B) birch leafminer, and (C) hawthorn leafminer.

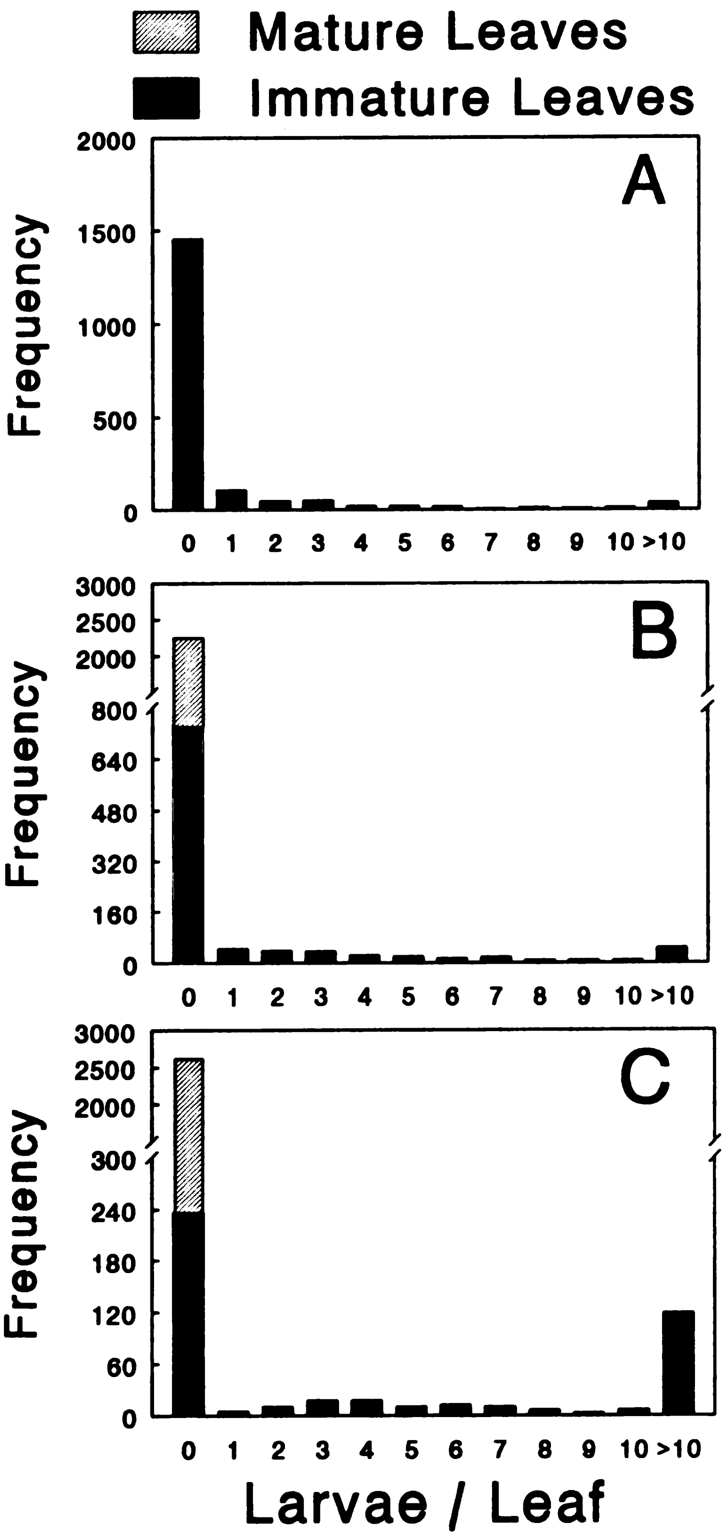

Figure 5: Distribution and abundance of alder leafminer larvae per leaf: (A) first generation (2 June, 1993), (B) second generation (29 June, 1993) and (C) second generation (13 July, 1993).

analysis when tested against the observed larval distribution over all leaves ($P<0.001$; $X^2=9709$; *d.f.* $=5$), and over immature leaves only ($P<0.001$; $X^2=7186$; *d.f.* $=12$). The negative binomial distribution was also rejected for all leaves and for immature leaves only ($P<0.001$; $X^2=67$; *d.f.* $=7$; $k=0.0178$ and $P<0.001$; $X^2=49$; *d.f.* $=8$; $k=0.1604$, respectively). However, the variance-to-mean ratio indicated that larvae were highly clumped over all leaves (ratio = 35) and over immature leaves only (ratio = 28). In addition, the *d*-statistic indicated that larvae were highly clumped over all leaves ($d=374$) and over immature leaves only ($d=128$). Again, Green's index indicated that larvae were distributed near randomly over all leaves (GI = 0.009), as well as over immature leaves only (GI = 0.007).

Uninfested leaves were the most frequently observed class when considering immature and mature leaves together ($n=2657$, 93%). When considering immature leaves only ($n=451$), forty seven percent were infested. Three and four larvae per leaf were most frequently observed class ($n=17$ each), and 98 larvae per leaf was the maximum (Figure 5C). The mean number of larvae per leaf for all leaves combined was 1 ± 49 and for immature leaves only, the mean was 9 ± 245.

Birch leafminer

During the first generation of 1993, larval distribution was not random ($P<0.001$; $X^2=4862$, *d.f.* $=6$). The negative binomial distribution was also rejected ($P<0.001$; $X^2=126$, *d.f.* $=15$; $k=0.3178$). However, the variance-to-mean ratio was 5.3, with the variance increasing at a faster rate than the mean number of larvae per leaf ($Y=2.6e^{0.5X}$; $r^2=0.62$;) (Figure 4B), and the *d*-statistic was 72. Each of these results indicates a highly clumped larval distribution. However, Green's index suggested that larvae were distributed randomly (GI = 0.001).

About half (49%) of all leaves were infested (n = 1548), with one larvae per leaf being the most frequently observed class. The mean number of larvae per leaf was 2 ± 12, but up to 23 larvae per leaf were observed in a leaf.

Larvae were also not distributed randomly during the second generation ($P < 0.001$; $X^2 = 3243$, $d.f. = 3$) when considering mature and immature leaves combined, and ($P < 0.001$; $X^2 = 1787$, $d.f. = 11$) when considering immature leaves only. The negative binomial distribution was also rejected for all leaves ($P < 0.001$; $X^2 = 60$, $d.f. = 4$), and for immature leaves only ($P < 0.001$; $X^2 = 39$, $d.f. = 4$). The variance-to-mean ratio indicated a clumped distribution over all leaves (25) and over immature leaves only (16). In addition, the *d*-statistic indicated a clumped distribution for all leaves ($d = 285$) and for immature leaves only ($d = 65$). In contrast, Green's index indicated a more random larval distribution over all leaves (0.011), and over immature leaves only (0.007).

During the second generation, 95% of the leaves, immature and mature combined, were not colonized ($n = 2458$). However, when considering immature leaves only, 46% were infested, and as many as 56 larvae were observed in one leaf (Figure 6B). When considering all leaves, there were 1 ± 20 larvae per leaf, but 9 ± 151 when considering immature leaves only. Even though third generation adults were present in August, no colonized leaves were observed.

Hawthorn leafminer

In 1993, larval distribution was not random as the hypothesis of a Poisson distribution was rejected ($P < 0.001$; $X^2 = 384$; $d.f. = 5$). The hypothesis that larvae were distributed according to the negative binomial model was also rejected ($P < 0.001$; $X^2 = 51$; $d.f. = 6$; $k = 1.68$). The variance-to-mean ratio was 1.49, with the overall variance increasing faster than the mean ($Y = 0.8e^{0.6X}$; $r^2 = 0.45$;), suggesting a degree of clumping (Figure 4C). The *d*-distribution statistic equalled 18, also

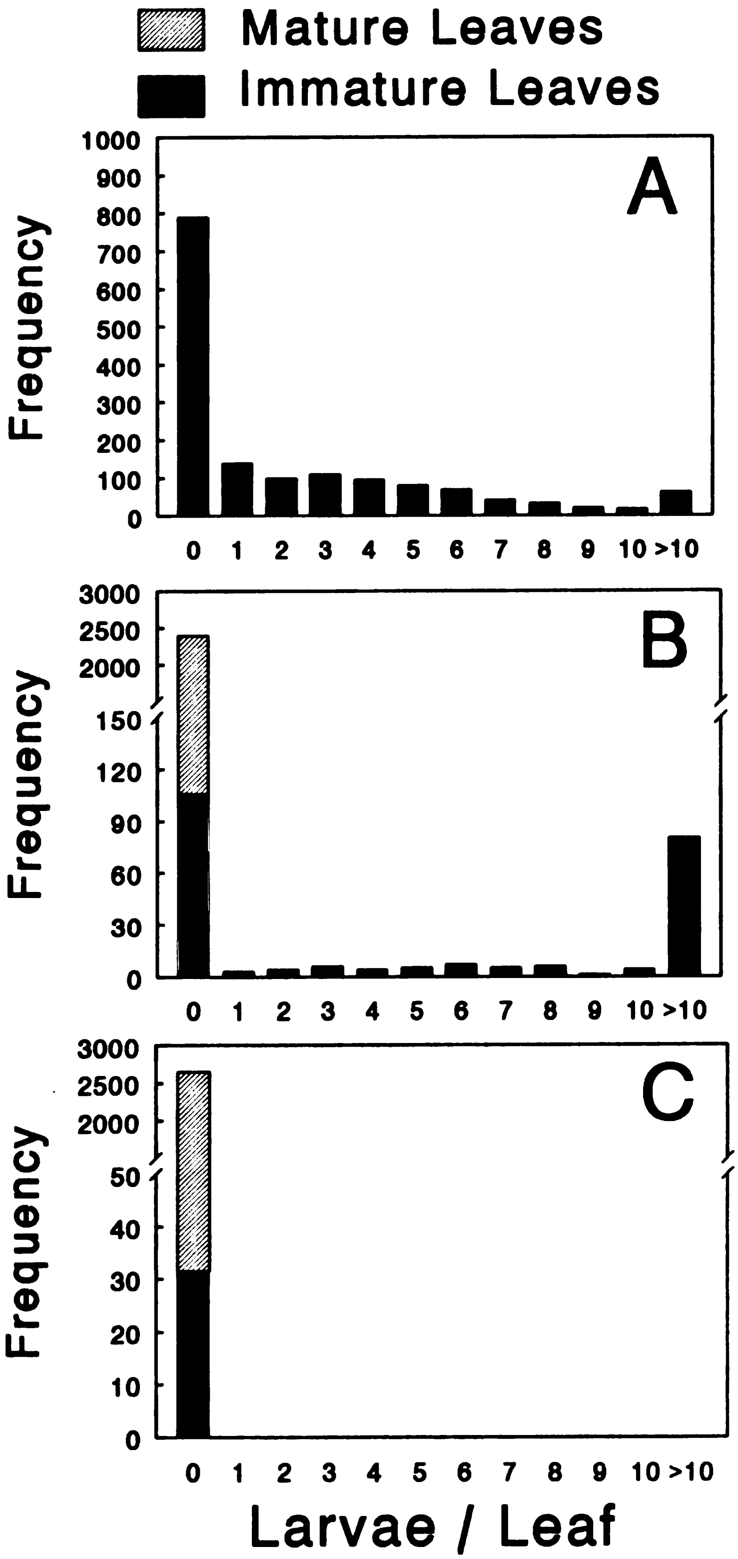

Figure 6: Distribution and abundance of birch leafminer larvae per leaf: (A) first generation, (B) second generation, and (C) third generation (1993).

suggesting clumping. However, Green's index was near zero (0.0001), suggesting that larval distribution was near random.

More infested ($n = 1872$) than uninfested ($n = 1241$) leaves were observed, with the number of larvae per leaf ranging from 1 to 8. One larvae per leaf was the most frequently observed class among infested leaves ($n = 793$) (Figure 7). The mean number of larvae per leaf was 1 ± 2.

Physical and Chemical Comparisons of Infested and Uninfested Leaves

Leaf Toughness

Alder: On the first sample date in 1992 (2 June), during peak oviposition, by first generation alder leafminer, there was no difference in the toughness of infested and uninfested leaves ($t = 0.36$; *d.f.* = 6,6; $P = 0.72$) (Figure 8A). Weekly samples revealed that leaf toughness increased over time, with infested and uninfested leaves remaining similar. Between 2 June and 17 June, the toughness of infested alder leaves increased from 217 ± 9 to 247 ± 13 g, while toughness of uninfested leaves increased from 213 ± 8 to 253 ± 16 g (Figure 8A).

In 1993, there were no differences in the toughness of infested and uninfested leaves ($t = -0.31$; *d.f.* = 5,5; $P = 0.76$) (Figure 9A). Toughness of infested and uninfested leaves again increased over time with no significant differences recorded between them. Between 12 May and 2 July, the toughness of infested leaves increased from 172 ± 10 to 239 ± 32 g, while the toughness of uninfested leaves increased from 176 ± 7 to 229 ± 27 g (Figure 9A).

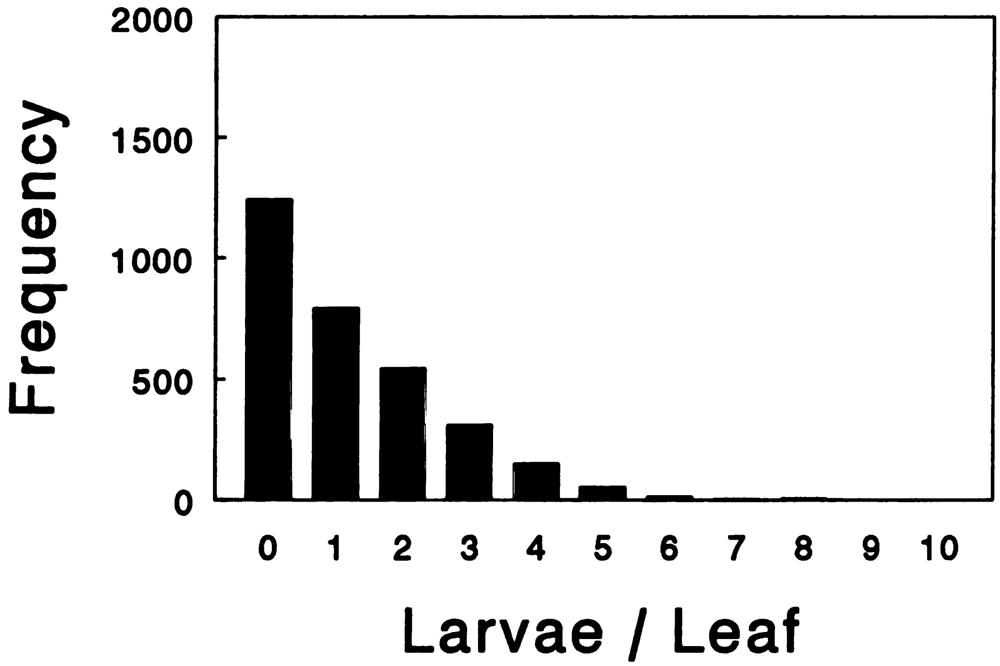

Figure 7: Distribution and abundance of hawthorn leafminer larvae per leaf (1993).

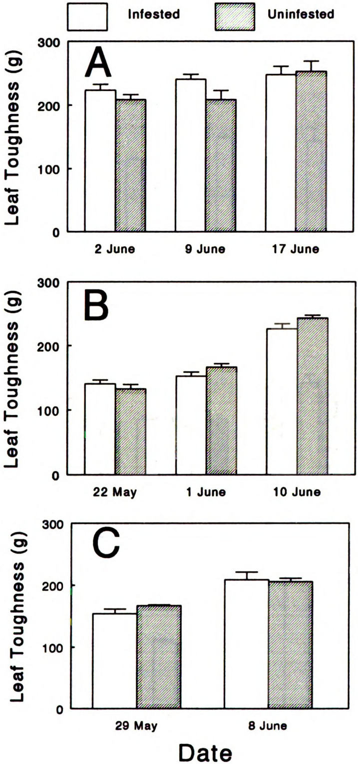

Figure 8: Toughness of infested and uninfested leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

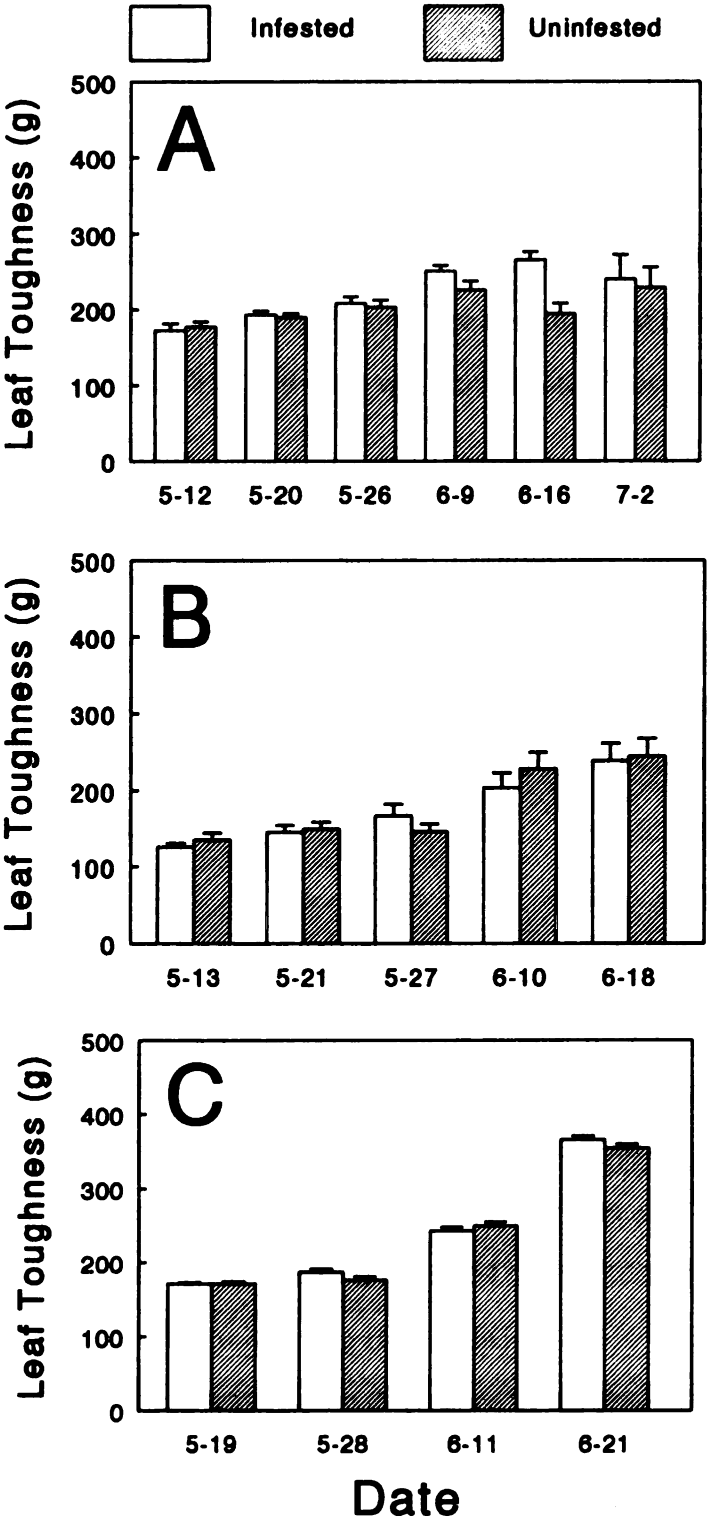

Figure 9: Toughness of infested and uninfested leaves during 1993: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

Birch: Based on the first sample date of 1992 (22 May), during the period of peak oviposition by first generation birch leafminer, infested and uninfested leaves were not different in toughness (*t*=0.77; *d.f.* =7,7; *P*=0.45) (Figure 8B). Leaf toughness increased over time with infested and uninfested leaves remaining similar. Between 22 May and 10 June, the toughness of infested birch leaves increased from 141±6 to 229±8 g, while uninfested leaves increased from 134±7 to 243±5 g (Figure 8B).

In 1993, there was also no difference in the toughness of infested and uninfested leaves (*t*=-0.83; *d.f.* =3,3; *P*=0.44) (Figure 9B). Toughness of infested and uninfested leaves again increased over time with no significant differences being recorded. Between 13 May and 18 June, the toughness of infested and uninfested leaves increased from 126±5 to 238±22 g, and from 135±9 to 243±23 g, respectively (Figure 9B).

Hawthorn: Based on the first sample date of 1992 (29 May), during the period of peak oviposition by hawthorn leafminer, there was no difference in toughness of infested and uninfested leaves (*t*=-1.70; *d.f.* =7,7; *P*=0.11) (Figure 8C). Weekly samples revealed that leaf toughness increased over time with infested and uninfested leaves remaining similar. Between 29 May and 8 June, the toughness of infested hawthorn leaves increased from 154±8 to 217±12 g while uninfested leaves increased from 167±2 to 206±6 g (Figure 8C).

In 1993, there was also no difference in toughness of infested and uninfested leaves (*t*=-0.04; *d.f.* =7,7; *P*=0.97) (Figure 9C). Toughness of infested and uninfested leaves again increased over the sampling period with no significant differences recorded. Toughness of infested and uninfested leaves was equal (171 g) on 19 May (first sample date). By 21 June, the toughness of infested leaves had increased to 364±5 g, while uninfested leaves increased to 354±5 g (Figure 9C).

Leaf Area

Alder: Leaves chosen as oviposition sites by first generation alder leafminer were smaller than non-selected leaves (*t* = -5.53; *d.f.* = 6,6; *P* = < 0.001) (Figure 10A). On the first sampling date of 1992 (2 June), corresponding with peak oviposition, the area of infested leaves was 14.06 ± 0.78 cm^2, while the area of uninfested leaves was 19.78 ± 0.68 cm^2. By 17 June, infested and uninfested leaves were of equal size (21.57 ± 1.60 and 21.33 ± 0.84 cm^2, respectively) (Figure 10A).

In contrast to 1992, leaves selected for oviposition in 1993 by first generation females were larger than those not selected (*t* = 3.37; *d.f.* = 5,5; *P* = 0.0071) (Figure 11A). On 12 May (first sample date), the area of infested leaves was 17.25 ± 0.94 cm^2, nearly 4 cm^2 larger than that of uninfested leaves (13.01 ± 0.83 cm^2) (Figure 11A). Also in contrast to 1992, the area of fully-expanded infested leaves was greater than that of uninfested leaves. By 2 July, infested leaves had reached an area of 40.83 ± 3.35 cm^2, approximately 12 cm^2 larger than that of uninfested leaves (28.55 ± 2.68 cm^2) (*t* = 2.89, *d.f.* = 5,5; *P* = 0.0162) (Figure 11A).

Birch: In 1992 and 1993, the size of leaves chosen as oviposition sites by first generation birch leafminer were not different than those not selected (Figure 10&11).

Hawthorn: On the first sample date (29 May), leaves infested and uninfested by hawthorn leafminer were equal in size (4.14 ± 0.16, 4.14 ± 0.08 cm^2, respectively) (Figure 10C). However, on the last sample date (8 June), infested leaves were slightly larger than uninfested leaves (6.14 ± 0.23 and 5.53 ± 0.21 cm^2, respectively). However, differences were marginally significant (*t* = 1.9601; *d.f.* = 7,7; *P* = 0.0702) (Figure 10C).

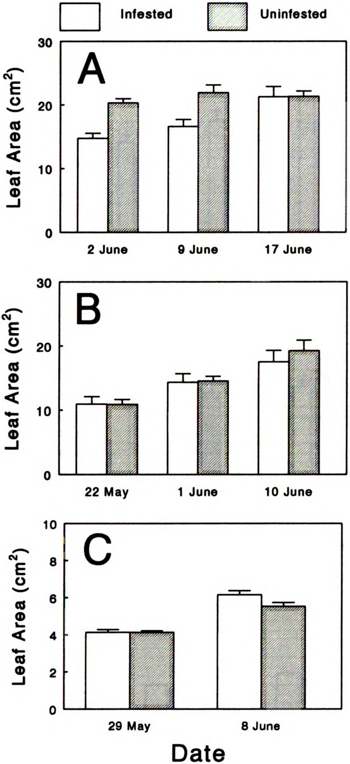

Figure 10: Area of infested and uninfested leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

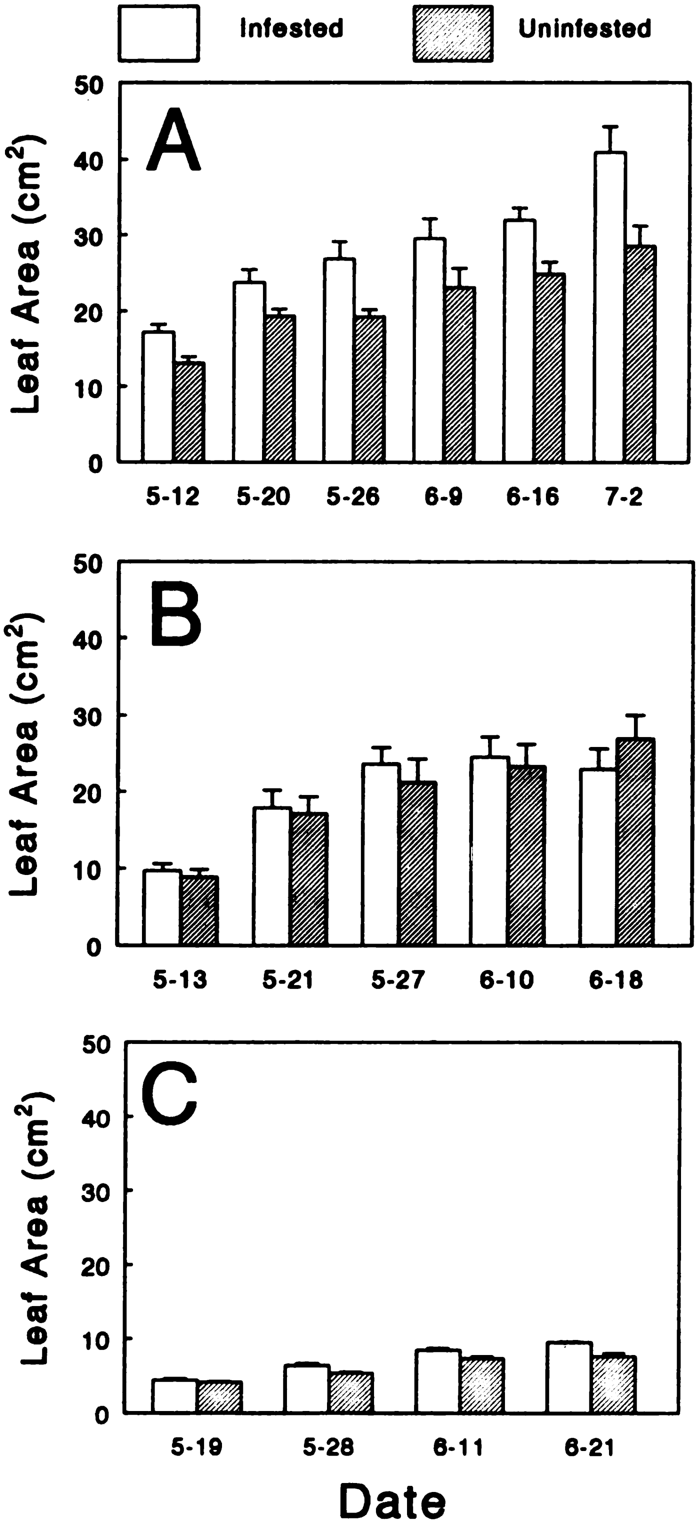

Figure 11: Area of infested and uninfested leaves during 1993: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

As in 1992, infested and uninfested leaves were similar in size (4.44 ± 0.10 and 4.11 ± 0.11 cm^2, respectively) (Figure 11C). By 21 June (the last sample date), infested leaves were larger than uninfested leaves (9.46 ± 0.15 and 7.62 ± 0.37 cm^2, respectively) ($t = 4.59$; *d.f.* =7,7; $P < 0.001$) (Figure 11C).

Specific Leaf Mass

Alder: In 1992, leaves chosen as oviposition sites by first generation alder leafminer had a slightly higher specific mass than leaves not selected ($t = 1.62$; *d.f.* =6,6; $P = 0.13$) (Figure 12).

In 1993, during peak oviposition on 12 May, no differences in specific leaf mass were recorded between infested and uninfested leaves ($t = 0.24$; *d.f.* =5,5; $P = 0.8137$) (Figure 13A). Over time however, infested leaves acheived a higher specific leaf mass than uninfested leaves.

Birch: In 1992, during peak oviposition by the first generation birch leafminer, infested leaves had a slightly higher specific mass than non-infested leaves ($t = 3.34$; *d.f.* =7,7; $P = 0.0049$) (Figure 12B). The difference was magnified by June 1 ($t = 4.78$; *d.f.* =7,7; $P < 0.001$), but disappeared by 10 June ($t = 0.16$; *d.f.* =7,7; $P = 0.8716$) (Figure 12B).

During peak oviposition by the first generation birch leafminer in 1993 (13 May), there was no difference in the specific leaf mass of infested and uninfested leaves ($t = 1.88$; *d.f.* =3,3; $P = 0.1096$) (Figure 13B). Both infested and uninfested leaves increased in specific leaf mass over time, with no consistent differences observed between them.

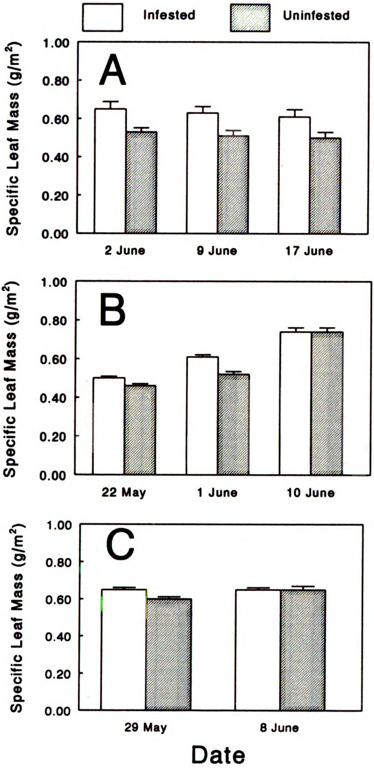

Figure 12: Specific leaf mass of infested and uninfested leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

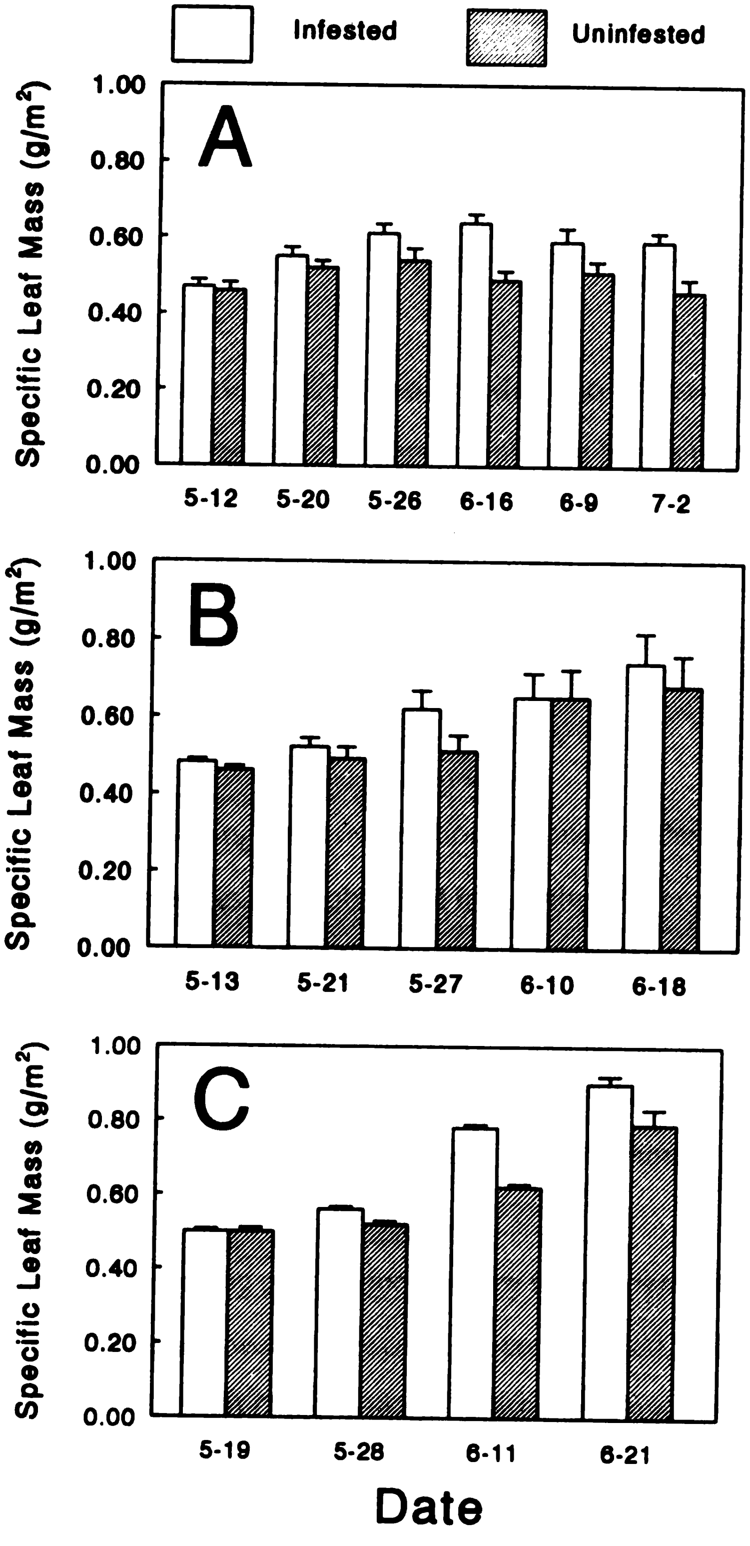

Figure 13: Specific leaf mass of infested and uninfested leaves during 1993: (A) European alder, (B) paper birch (*Betula papyrifera* Marsh), and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

Hawthorn: In 1992 during peak oviposition by hawthorn leafminer, infested leaves were slightly higher in specific mass than uninfested leaves ($t = 2.96$; *d.f.* = 7,7; $P = 0.0103$) (Figure 12C). But by the last sample date (8 July) infested and uninfested leaves were similar in specific leaf mass ($t = -0.3265$; *d.f.* = 7,7; $P = 0.7489$) (Figure 12C).

In 1993, during oviposition (19 May), no differences in specific leaf mass were observed between infested and uninfested leaves ($t = 0.00$; *d.f.* = 7,7; $P = 1.00$) (0.50 ± 0.01 g/m^2) (Figure 13C). However, as leaves matured, infested leaves acheived a higher specific leaf mass.

Nitrogen

Alder: During peak oviposition (2 June) by the alder leafminer, no significant differences were observed in the nitrogen concentration of infested and uninfested leaves ($t = 0.75$; *d.f.* = 15,11; $P = 0.4586$) (Figure 14A). By the second sample date (9 June), infested leaves had a lower nitrogen concentration than uninfested leaves ($t = -4.12$; *d.f.* = 13,15; $P = < 0.001$) (Figure 14A), a difference that was maintained through the final sample date (17 June).

Birch: During peak oviposition by first generation birch leafminer (22 May), no differences were observed between infested and uninfested leaves ($t = 1.26$; *d.f.* = 15,15; $P = 0.2180$) (Figure 14B). Infested and uninfested leaves decreased equally in nitrogen content between 1 June and 10 June ($t = 0.19$; *d.f.* = 15,15; $P = 0.8523$ and $t = 0.1448$; *d.f.* = 15,15; $P = 0.89$, respectively) (Figure 14B).

Hawthorn: No differences in nitrogen concentration were observed between leaves infested and those not infested by hawthorn leafminer (29 May)

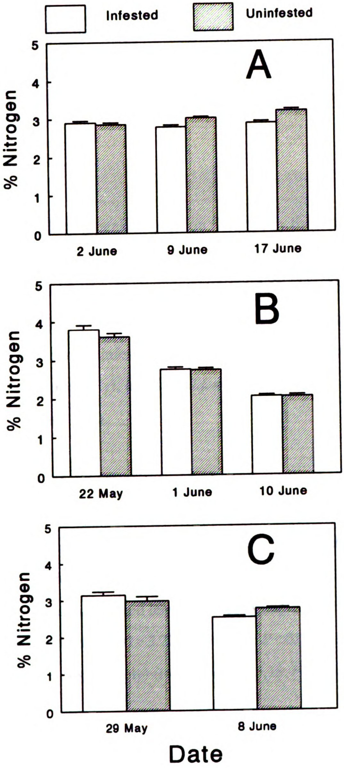

Figure 14: Percent nitrogen concentration of infested and uninfested leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

($t = 1.01$; *d.f.* $= 9,7$; $P = 0.3294$) (Figure 14C). Nitrogen content of hawthorn leaves also decreased over time, with infested leaves having a lower nitrogen concentration than uninfested leaves ($t = -3.84$; *d.f.* $= 15,14$; $P < 0.001$) (Figure 14C).

Phosphorous

Alder: During peak oviposition (2 June) by the first generation alder leafminer, infested leaves were lower in percent phosphorous than uninfested leaves (0.16 ± 0.01 and 0.18 ± 0.01%, respectively) ($t = -3.15$; *d.f.* $= 15,11$; $P < 0.01$) (Figure 15A). On 9 June and 17 June, percent phosphorous concentration of infested leaves remained lower than uninfested leaves (0.13 ± 0.01, 0.17 ± 0.01% and 0.14 ± 0.01, 0.15 ± 0.01%, respectively), ($t = -5.96$, *d.f.* $= 13,15$; $P < 0.001$ and $t = -1.80$, *d.f.* $= 15,15$; $P = 0.0821$) (Figure 15A).

Birch: Infested birch leaves had a higher phosphorous concentration than uninfested leaves during peak oviposition by first generation birch leafminer (22 May) (0.39 ± 0.01 and 0.35 ± 0.01%, respectively) ($t = 2.58$; *d.f.* $= 15,15$; $P < 0.05$) (Figure 15B). Infested leaves maintained a slightly higher phosphorous concentration than uninfested leaves on each sample date: 1 June (0.28 ± 0.00 and 0.25 ± 0.01%, respectively) ($t = 3.72$; *d.f.* $= 15,15$; $P < 0.001$), and 10 June (0.22 ± 0.01 and 0.21 ± 0.01%, respectively) ($t = 1.63$; *d.f.* $= 15,15$; $P = 0.1137$) (Figure 15B).

Hawthorn: No differences in phosphorous concentration were observed between infested and uninfested hawthorn leaves during peak oviposition by hawthorn leafminer (29 May) (0.28 ± 0.01 and 0.28 ± 0.01%, respectively) ($t = -0.47$; *d.f.* $= 9,7$; $P = 0.6474$) (Figure 15C). Infested and uninfested leaves remained similar

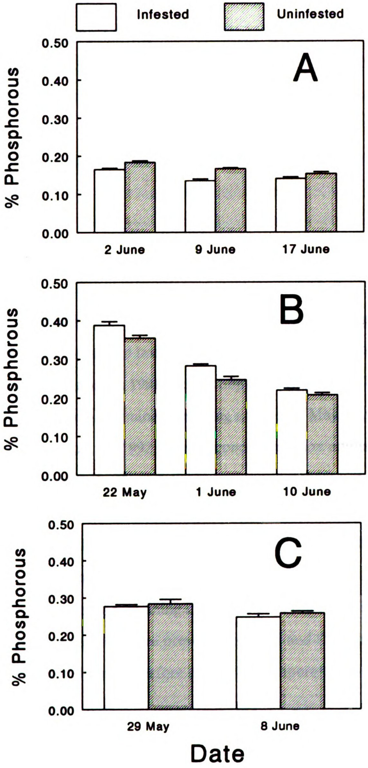

Figure 15: Percent phosphorous concentration of infested and uninfested leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of first generation alder (A), birch (B), and hawthorn (C) leafminer.

on the second sample date (0.25 ± 0.01 and 0.26 ± 0.01, respectively) (*t* = -1.01; *d.f.* = 14,15; *P* = 0.3225) (Figure 15C).

DISCUSSION

Adult Abundance and Phenology

First generation adults of each species typically begin emergence in late April to mid-May as bud break occurs (Slingerland 1905, Parrott & Fulton 1915, Friend 1933, Hart et al. 1991, Herms unpublished). In 1992 and 1993, a large first generation of birch leafminer, begining around 11 May, was followed by a small second generation. In 1992, a few second generation adults were observed, but no adults were trapped. The relatively cool temperatures experienced throughout the summer of 1992 may have been a reason why no birch leafminer adults were trapped after 14 July. Adult emergence typically occurs until mid-August to early September (Herms, unpublished data). Instead of emerging as adults, individuals may have diapaused, remaining in the soil until the following spring. The relatively cool temperatures may have prevented second and third generation individuals from completing development before cooler fall temperatures set in and leaf abscission occurred.

In 1993, a large first generation in May was followed by a smaller second and third generation in July and August respectively. Friend (1931) reported that during late summer and early fall, an increasing proportion of pupae enter diapause. In Newfoundland in 1972, two generations of birch leafminer was reported with part of the population completing a third generation, a pattern similar to that observed in Europe (Jones and Raske 1976). Three generations were also observed in Connecticut (Friend 1931) and Quebec (Jones and Raske 1976).

The first generation of birch leafminer is largest possibly because emergence occurs when all leaves on the host plant are immature, which is the preferred oviposition site (Friend 1933), and possibly the required food source for larvae. Larvae may not be able to complete development in mature leaves.

If, as the season progresses, the availability of immature leaves decreases, some individuals may enter premature extra-long diapause (*sensu* Hanski 1988) to avoid placing their offspring in a resource-limited environment. If all first generation individuals emerged as second generation adults, and had fewer leaves available as oviposition sites, then resource competition among larvae could be intense, resulting in the evolution of a density-dependent "bet hedging" diapause strategy. Under this scenario, the majority of first generation individuals would enter diapause and by-pass a breeding opportunity, only to emerge the following spring when a full flush of immature leaves is once again available, therefore placing their offspring in a potentially less competitive environment. On the other hand, some individuals emerge as second generation adults, take advantage of the breeding opportunity, but risk placing their offspring in a potentially more competitive environment.

In 1992 and 1993, alder leafminer adults also began emerging around 11 May. Relatively cool spring temperatures in 1992 and 1993 may have prolonged adult emergence of some alder leafminer individuals, causing each generation to become less distinct from one another. The cool spring temperatures may have been a reason why the abundance of alder leafminer adults emerging in May and June was less than that which emerged in July and August, causing the generations to overlap and resulting in the second and/or third generation to appear larger than the first. Head capsule measurements of larvae, taken weekly in order to determine instar for other experiments, revealed the presence of a small third generation in August. After second generation larvae began reaching the fourth and fifth instar, first instar individuals were again more commonly observed.

Slingerland (1905) reported that alder leafminer adults were present throughout the summer, but was unable to distinguish the number of generations because the broods overlapped. Slingerland suggested that two to three generations were present. In 1991, Hart et al. reported three distinct generations of alder leafminer (late May, early July, and mid-to-late August) in Iowa and Michigan. The Michigan population studied by Hart et al. (1991) was the same used for this research. This pattern would be consistent with second generation alder leafminer expressing a density-independent "bet-hedging" diapause strategy as described for the second generation of birch leafminer. The large first and second generation followed by a small third generation suggests that only the second generation may "hedge their bet". Some individuals may enter diapause, by-passing a breeding opportunity only to emerge the following spring. Individuals that emerge as third generation adults take advantage of the breeding opportunity but their offspring run the risk of not being able to complete development before the onset of cool fall temperatures and leaf abscission.

In both 1992 and 1993, the hawthorn leafminer was observed to have only one generation in the spring. Each year, adult emergence began on approximately 11 May and adults were not observed after the end of June. The population was large relative to that of the alder and birch leafminer, with over 1000 adults collected per trap per week during peak emergence in 1992 and 1993. These results are consistent with those of Hamilton (1950) who reported *Profenusa canadensis* as being a univoltine insect with emergence beginning in late April to early May, shortly after bud-break, and ending in early to mid June. If immature leaves are required for oviposition and for larval development, as seems to be the case for the alder and birch leafminer, then hawthorn leafminer may be univoltine because its host plant does not produce immature leaves throughout the summer.

Larval Distribution and Abundance

During both 1992 and 1993, for each generation of all three species, larvae were not distributed randomly or according to the negative binomial distribution. However, the variance-to-mean ratio, and the *d*-statistic suggested that larval distribution was clumped in all cases. Meanwhile, Green's index suggested that larval distribution was near random in all cases.

The majority of the alder and birch leaves sampled were uninfested even though numerous larvae were observed per leaf, and intraspecific competition occurs among larvae (see chapter 3). During the first generation, the distributions of both the birch and alder leafminer were highly clumped. Slingerland (1905) reported 15-20 alder leafminer larvae per leaf. In this study, as many as 80 alder leafminer larvae and 23 birch leafminer larvae were observed in a single leaf during the first generation. In later generations, up to 98 alder leafminer larvae and 56 birch leafminer larvae per leaf were observed. The mean and variance for the number of larvae per leaf also increased with each generation. The number of alder leafminer larvae per leaf increased from 1 ± 14 during the first generation, to 2 ± 13 during the second generation, and finally to 9 ± 245 larvae per leaf during the third generation. As for birch leafminer, the number of larvae per leaf during the first generation was 2 ± 12 and increased to 9 ± 151 during the second generation; to few larvae were observed during the third generation for analysis. The number of larvae per leaf may have increased due to a decrease in the number of immature leaves available as the season progressed.

Bultman and Faeth (1986b) reported the distribution of five species of leafminers on oak (*Quercus emoryi*) to be clumped. The five species studied were *Bucculatrix cerina*, *Cameraria* sp. nov., *Stigmella* sp., and *Stilbosis quadricustatella*. The non-random distribution of *Cameraria* sp. larvae resulted because fewer leaves were mined and because more leaves had multiple mines than expected by chance (Faeth 1991). Faeth (1991) concluded that the clumped distribution of larvae was due to leaf size with larvae being more clumped on small leaves. This preference may be the result of females preferring to oviposit in sun leaves which tend to be smaller than shade leaves in Emory oak trees (Faeth 1991). However, higher rates of premature leaf abscission of sun leaves may contribute to lower over-all densities of larvae in sun leaves relative to shade leaves (Faeth 1991).

Hawthorn leafminer larvae were more randomly distributed than were alder and birch leafminer larvae. This may be because the smaller size of hawthorn leaves accommodates the successful development of only one or two larvae, compared to five to ten commonly accommodated by birch and alder (personal observation). Fully expanded hawthorn leaves are approximately 10 cm^2 in size while birch and alder leaves are typically 20 and 40 cm^2 in size, respectively. The average leaf area consumed by a single hawthorn leafminer per leaf was approximately 4 cm^2, which is approximately fifty percent of the average leaf. On the other hand, a single alder leafminer per leaf consumed approximately 3 cm^2 (about 8% of the leaf) and a single birch leafminer consumed approximately 2 cm^2 (10% of the leaf) before exiting the leaf to pupate in the soil. Thus, the number of alder and birch leafminer larvae per leaf often exceeded that which could be supported by the leaf, while hawthorn leafminer larvae tended not to exceed their carrying capacity.

The over abundance of alder and birch leafminer per leaf may result in intraspecific competition among larvae for the available food resource. This creates the potential for selection for density dependent premature extra-long diapause within the population (*sensu* Hanski 1988). The smaller population size of the second and third generation of birch leafminer observed in this study and the smaller size of the third generation of alder leafminer, reported by Hart et al. (1991) are consistent with the occurrence of such a bet-hedging strategy.

Physical and Chemical Comparisons of Infested and Uninfested Leaves

During the first generation there were no consistent differences in the toughness, size, and specific mass of immature alder, birch, and hawthorn leaves selected for oviposition by alder, birch, and hawthorn leafminer and those which did not receive any eggs. Differences in the size and specific mass of selected and non-selected alder leaves varied from year to year. In 1992, infested leaves were smaller in size and were higher in specific leaf mass. However, in 1993 infested leaves were larger in size and there was no difference in specific leaf mass. In 1992, birch leaves of a slightly higher specific mass were preferred, but this was not the case in 1993.

For all three species, the nitrogen concentration of leaves selected by females were similar to leaves not receiving eggs. Since larval distribution tends to be aggregated, this suggest that females are selecting leaves as oviposition sites based on some other variable and not nitrogen concentration, even though nitrogen is generally a limiting nutrient for larval development (Mattson 1980).

Differences in the phosphorous concentration of infested and uninfested leaves were also inconsistent between species during the first generation. Infested alder leaves were lower in phosphorous than leaves not receiving eggs. However, birch leaves selected for oviposition had a higher phosphorous concentration than leaves which did not receive eggs. Hawthorn leaves receiving eggs and those not receiving eggs were similar in phosphorous content.

In summary, the distribution of larvae of all three species were highly aggregated. However, there were no consistent differences detected between infested and uninfested leaves in the physical (toughness, area, and specific leaf mass) and chemical (nitrogen and phosphorous) variables measured in this study. Due to these inconsistencies, it can not be concluded that alder, birch, and hawthorn leafminers selecte leaves for oviposition based on the traits measured in this study.

Hamilton (1943), suggested that these leafminer species seemed to prefer to oviposit in sun leaves rather than shade leaves. Adults of the red oak leafminer (*Profenusa alumna* MacGillivray) also deposit their eggs in the upper sunlit portion of the trees (Labonte and Lipovsky 1984). Sun leaves may be preferred for oviposition because larvae may be able to develop faster in warmer temperatures. In addition, sun leaves are thicker (Kozlowski et al. 1991) therefore, they may provide more tissue per unit area for consumption by larvae. However, oviposition in relation to light intensity was not quantified in this study.

CHAPTER TWO

INTERSPECIFIC VARIATION IN VOLTINISM PATTERNS RESULTS FROM INTERSPECIFIC DIFFERENCES IN HOST PHENOLOGY

Feeny (1970) reported that the number of spring feeding Lepidoptera on oak trees, such as the winter moth (*Operophtera brumata* L.), exceeded the number of late season feeders. Feeny proposed that this difference was due to seasonal changes in the texture and chemical composition of the leaves. Immature leaves are less tough, and contain lower tannin concentrations than older leaves (Feeny 1970). Furthermore, concentrations of nitrogen, a limiting nutrient to herbivores, is highest in young tissues (Mattson 1980). Seasonal changes in leaves may result in mature leaves becoming a less suitable food resource for larvae, and may select for early season feeding (Feeny 1970).

Differences in leaf characteristics of mature and immature leaves may also play a role in the voltinism patterns of leafminers. Harbaugh et al. (1983) reported that damaged caused by *Liriomyza trifolii* Burgess, a leafminer on chrysanthemum, increased linearly as leaf nitrogen increased from 2.2 to 4.0%. Mature leaves may also posses noxious or digestibility reducing compounds such as alkaloids and tannins (Faeth et al. 1981). Ayres and MacLean (1987) identified several leaf characteristics that change with age, which affect the growth of a geometrid larvae (*Epirrita autumata*). As leaves aged, water, nitrogen and phosphorous concentrations declined, toughness and specific mass increased and larval performance decreased. They proposed that phenological constraints imposed on the larvae by leaf maturation should influence the evolution of larval phenology, selecting for early season development. A similar relationship between leaf and insect phenologies was documented by Merle (1988) for the green oak leafroller, *Tortrix viridana* (Lepidoptera: Tortricidae) and various species of oak.

Such phenological constraints have also been reported by Auerbach and Simberloff (1984) for two leafmining species, *Eriocraniella* sp. and *Dyseriocranina* sp. (Lepidoptera: Eriocraniidae). Both are phenologically restricted, feeding only on first-flush leaves. In addition, Faeth et al. (1981) stated that the densities of active mines of several leafmining species vary seasonally because of their voltinism patterns and because feeding is restricted to a particular leaf stage. The density of two other leafmining species, *Acrocercops* sp. and *Neurobathra strigifinitelia*, were highly correlated with new leaf production (Auerbach and Simberloff 1984). They suggested that the abundance of both species is often limited by the availability of immature leaves. Faeth et al. (1981) suggested that both the number of phytophagous species present and their density would decreases seasonally due to an increase in leaf toughness and tannins, and a decrease in nitrogen.

Since alder, birch, and hawthorn leafminer females prefer immature leaves as oviposition sites (Parrott & Fulton 1915, Friend 1933, Wilson et al. 1982, Hart et al. 1991) the evolution of their voltinism patterns may have been influenced by the phenological pattern of new leaf production by their respective host plants. Alder and birch trees display indeterminate growth, producing new foliage throughout the summer, while hawthorn trees do not produce new foliage after the initial flush at bud-break, and thus display determinate growth (personal observation). The continued production of immature leaves, throughout the season may have allowed alder and birch leafminer to evolve a multivoltine life cycle. The lack of immature leaves after bud break may have constrained the hawthorn leafminer to one generation.

OBJECTIVES

This research tested the following predictions relating host phenology (availability and quality of immature foliage) to the evolution of voltinism patterns: (1) adults oviposit only in immature leaves, (2) immature leaves are less tough and more nutritous than mature leaves, and (3) age specific larval mortality increases as leave mature.

METHODS AND MATERIALS

Leaf Age Influences Oviposition Site Selection of Second and Third Generation Alder and Birch Leafminer

Birch Leafminer

Infested leaves containing fifth instars were collected and brought to the laboratory. These leaves were placed in a petri-dish with a plaster filled base saturated with water to maintain humidity. They were then placed in an incubator at 25 °C (18:6 light:dark) until adults emerged. Ten female and five male adults were confined to a potted birch seedling within a mesh bag, where females were allowed to oviposit freely. The availability of immature and mature leaves was manipulated manually by removing either mature or immature leaves resulting in the following three treatments: (1) seedlings with only mature leaves, (2) seedlings with only immature leaves, and (3) unmanipulated seedlings (mature and immature leaves present). The use of these three treatments resulted in two oviposition preference experiments. Comparison of treatments 1 and 2 represented a no-choice experiment. Comparison of the number of eggs laid in mature and immature foliage in treatment 3 represented a choice experiment.

Seedlings were arranged in a randomized complete block design with four blocks, each containing one seedling from each treatment. The number of eggs per leaf, and the number of immature and mature leaves per seedling were quantified. For each experiment (no-choice and choice) the total number of eggs laid on mature and immature leaves were compared by ANOVA. Data were square root transformed (Y + ½) because eggs were distributed non-randomly among leaves (Little and Hills 1978). Non-transformed data are presented in all figures. SAS was used for all analyses.

Alder Leafminer

Second generation adults were obtained as above. Ten adult females were confined to a branch inside a mesh bag. Three branches were selected from each of five tress. Branches similar in size and in the number of immature and mature leaves were selected for this experiment. The availability of immature and mature leaves were manually manipulated by removing either mature or immature leaves, resulting in three treatments: 1) branches with mature leaves only, (2) immature leaves only, and (3) both immature and mature leaves (unmanipulated branches).

The experiment was designed as a randomized complete block with trees serving as blocks. The number of eggs per leaf, and the number of leaves per branch were quanitified. The selection of these treatments resulted in both a choice and a no-choice oviposition preference experiment as described for the birch leafminer. Data were analyzed as described for birch leafminer.

Number of Eggs Per Leaf in Relation to Physical Leaf Characteristics

For each species, infested leaves were randomly sampled on a weekly basis until eggs hatched. Ten infested leaves per tree were sampled from eight trees of all three species. While in the field, leaves were placed in zip-lock plastic bags and stored on ice in a cooler. Leaves were promptly transported to the laboratory.

The following variables were measured for all leaves: (1) number of eggs per leaf were counted using a disecting microscope, (2) leaf toughness (g) using a penetrometer, (3) leaf area (cm^2) using a image analyzer, and (4) specific leaf mass (g/m^2). The relationships between the number of eggs per leaf and (1) leaf toughness, (2) leaf area and (3) specific leaf mass were quantified using correlation analysis.

Physical and Chemical Comparisons of Mature and Immature Leaves

In 1992 and 1993, during the first generation, fully expanded immature and mature leaves (ten of each per tree) were randomly sampled from each of eight alder (six in 1993), eight birch, and eight hawthorn trees for physical and chemical analysis. Mature leaves were distinguished from immature leaves by their position on a branch, size, color (darker green), and texture (tougher, thicker). Corresponding with the period of peak oviposition of each generation, foliage was sampled during mid-May, mid-July, and mid-August in order to determine the difference in immature and mature leaves available to ovipositing females. The first sample contained only immature leaves. For birch and alder, the second and third samples contained both immature and mature leaves (except on the third sample date in 1992 when only mature leaves were available). Because its determinate growth pattern, on the last two sample dates only mature hawthorn leaves were available for sampling.

While in the field, sampled leaves were placed in zip-lock plastic bags and stored on ice in a cooler. Leaves were promptly transported to the laboratory. For all leaves, the following variables were measured using methods described in Chapter One: (1) toughness, (2) leaf area, (3) dry weight, and (4) nitrogen and phosphorous concentration. Results for each sample date were analyzed separately. Means for mature and immature leaves were compared using a t-test.

Age Specific Larval Mortality In Relation to Leaf Maturity

Ten infested leaves were sampled weekly from eight trees of each species throughout the first and second generation. The following variables were measured: (1) number of larvae (or eggs) per leaf, (2) head capsule width of each insect to determine instar, (3) larval status (dead or alive), (4) number of mature larvae previously emerged from the leaf (determined by exit holes), (5) leaf toughness, (6) leaf area and (7) area of leaf mined.

Data from all leaves were combined to determine percent mortality by instar for both the first and second generations. Mortality rates in relation to leaf toughness were examined quantitatively.

RESULTS

Leaf Age Influences Oviposition Site Selection During the Second and Third Generations

Both alder and birch leafminer preferred to oviposit in immature leaves. In both the no-choice and choice experiments, alder leafminers oviposited exclusively in immature leaves ($F = 56.63$; *d.f.* = 1,9; $P < 0.01$ and $F = 2.63$; *d.f.* = 1,9; $P = 0.18$, respectively) (Figure 16). Birch leafminer females also laid substantially more eggs in immature than mature leaves in the no-choice experiment. In the choice experiment, females oviposited exclusively in immature leaves ($F = 13.39$; *d.f.* = 1,7; $P = 0.04$ and $F = 8.05$; *d.f.* = 1,7; $P = 0.07$, respectively) (Figure 17).

Alder and birch leafminer larval development in immature and mature leaves could not be compared because females did not oviposit in mature leaves, even when offered only mature leaves.

Number of Eggs Per Leaf In Relation to Physical Leaf Characteristics

For all three species, there was no relationship between the number of eggs laid per leaf and leaf toughness (Figure 18) and leaf area (Figure 19).

For the alder leafminer, there was a significant positive, but weak, correlation between the number of eggs per leaf and specific leaf mass ($n = 60$; $Y = 0.33e^{520X}$; $r^2 = 0.18$; $P < 0.01$) (Figure 20A). For the birch and hawthorn leafminers, there was no correlation between the number of eggs per leaf and specific leaf mass (Figure 20B and 20C).

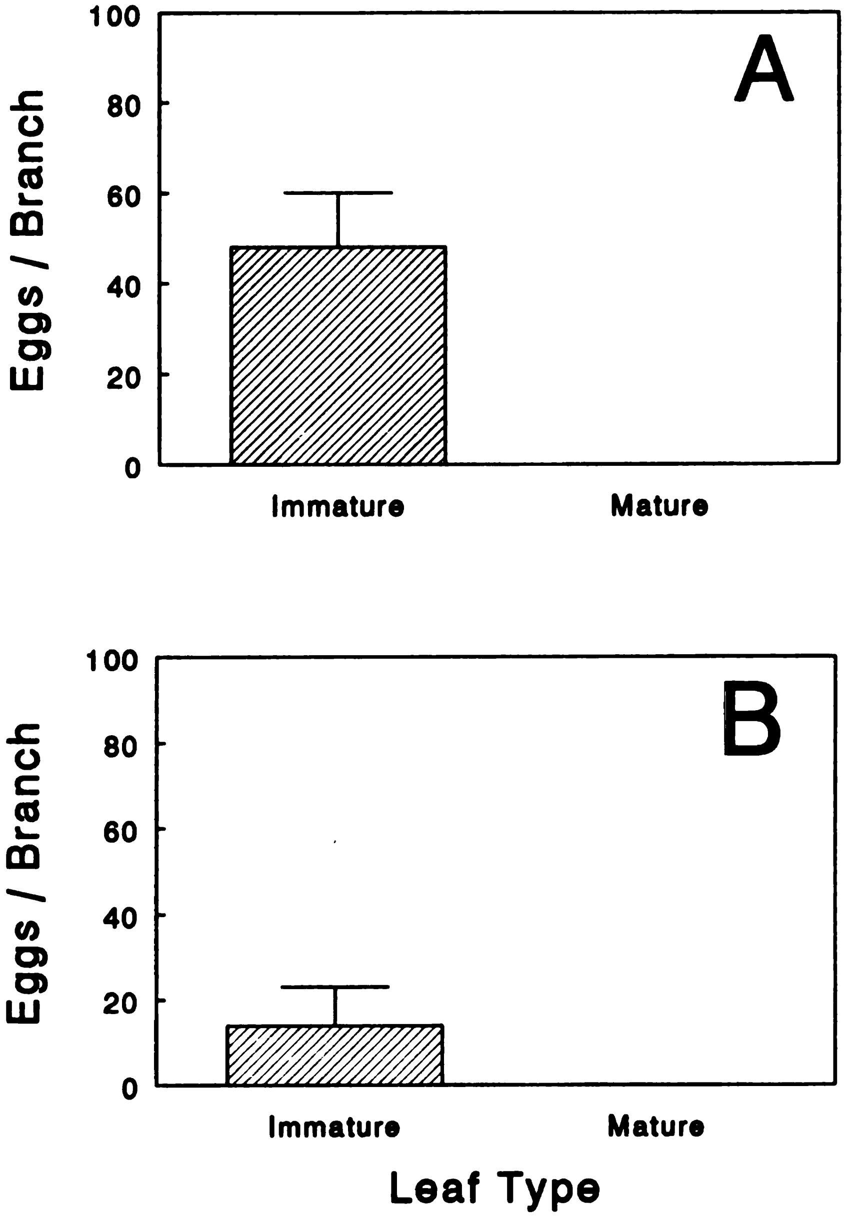

Figure 16: Comparison of immature and mature European alder leaves as oviposition sites by alder leafminer: (A) no-choice experiment, (B) choice experiment.

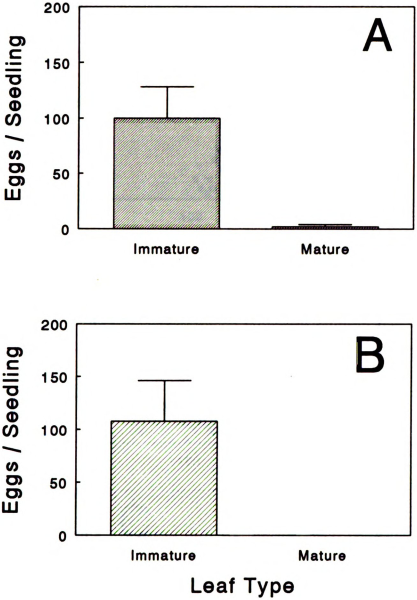

Figure 17: Comparison of immature and mature paper birch leaves as oviposition sites by birch leafminer: (A) no-choice experiment, (B) choice experiment.

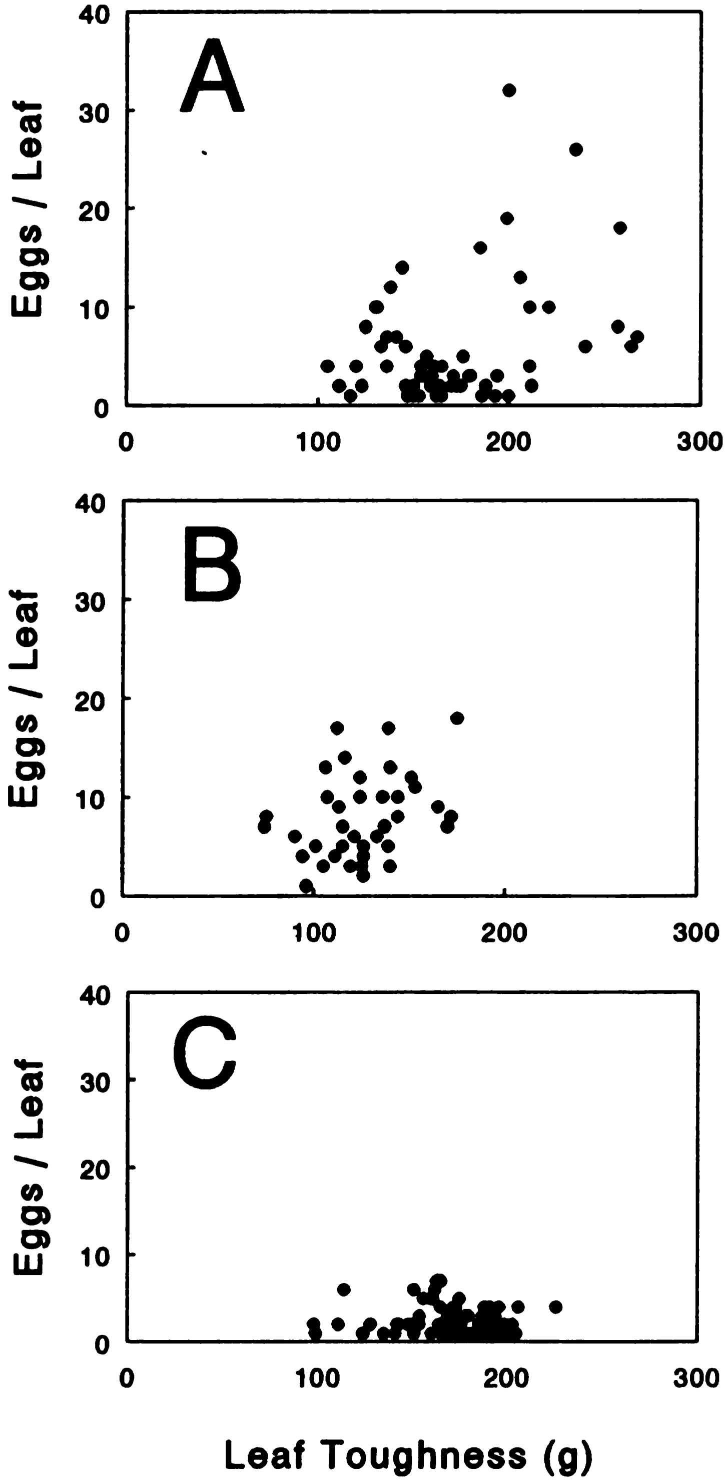

Figure 18: Relationship between leaf toughness and the number of eggs per leaf: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition of first generation alder (A), birch (B), and hawthorn (C) leafminer in 1993.

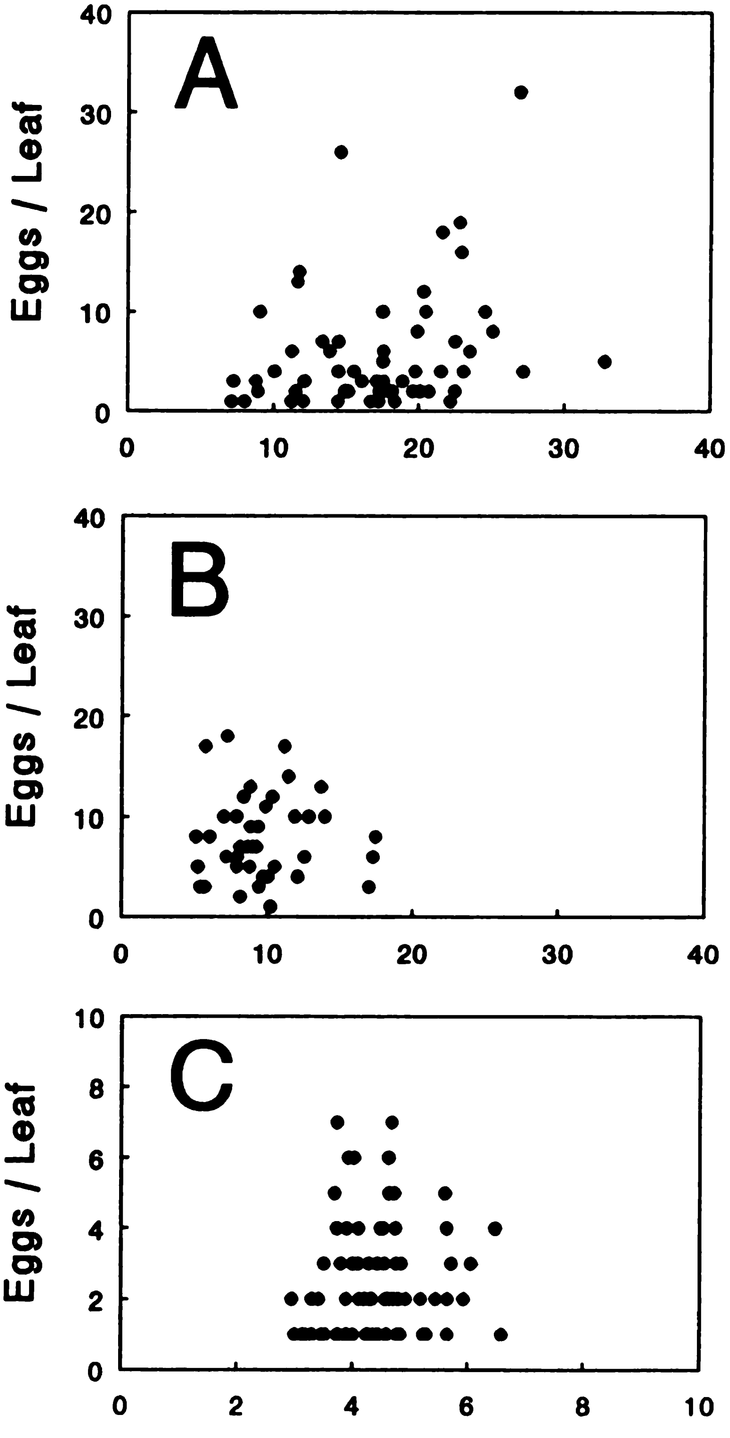

Figure 19: Relationship of leaf area and the number of eggs per leaf: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition of first generation alder (A), birch (B), and hawthorn (C) leafminer in 1993.

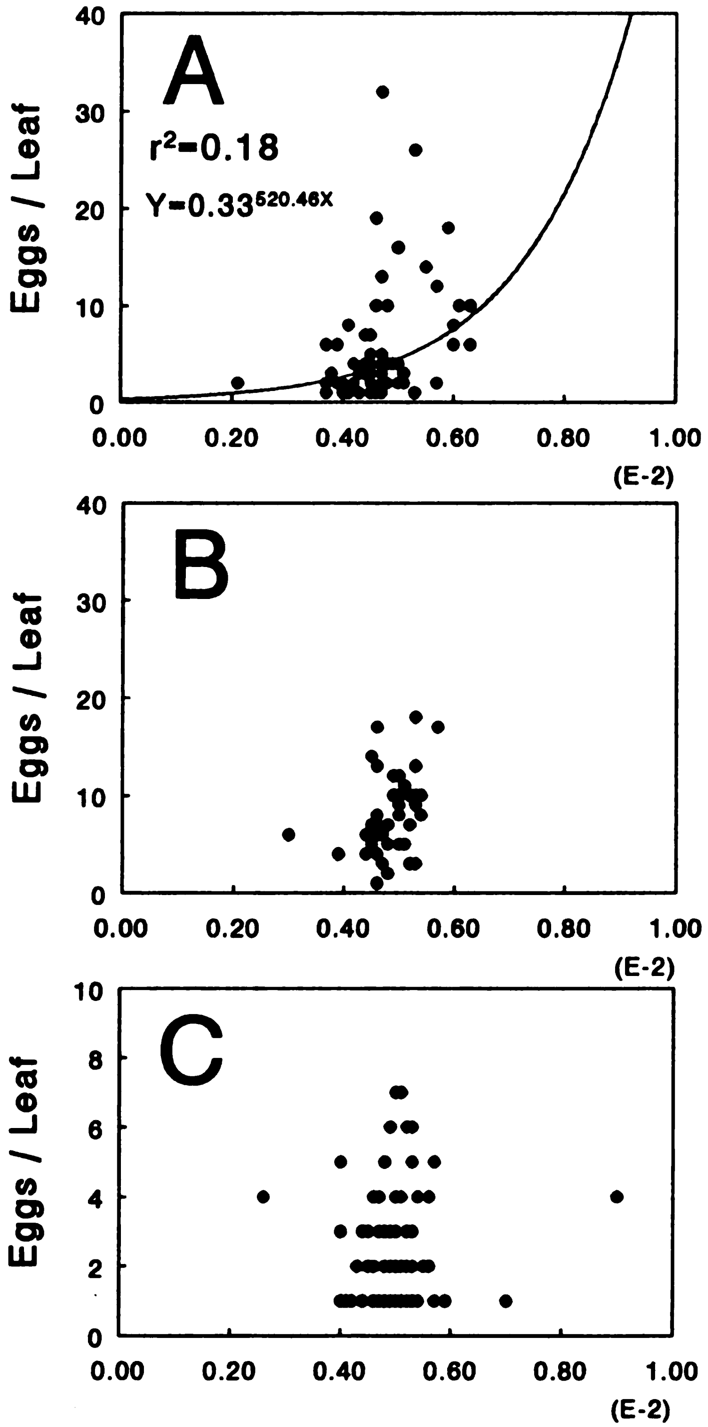

Figure 20: Relationship between specific leaf mass and the number of eggs laid per leaf: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition of first generation alder (A), birch (B), and hawthorn (C) leafminer in 1993.

Physical and Chemical Comparisons of Mature and Immature Leaves

Leaf Toughness

Alder: Mature leaves were always substantially tougher than immature leaves. During peak oviposition of first generation females (22 May 1992), when only immature leaves were available, leaf toughness was 187±9 g (Figure 21A). During the same period in 1993 (12 May), toughness of immature leaves was 173±6 g (Figure 22A).

In 1992 (20 July) and 1993 (14 July), when second generation leafminers were ovipositing, mature leaves were tougher than immature leaves: 253±8 and 138±7 g, respectively in 1992 ($t = -10.46$; *d.f.* =7,7; $P < 0.001$) (Figure 21A), and 246±11 and 141±9 g, respectively in 1993 ($t = -7.41$; *d.f.* =5,5; $P < 0.001$) (Figure 22A).

In 1992, the third sample was taken after leaf production had ceased (26 September), therefore only mature leaves were sampled. Mean toughness of these leaves was 244±11 g (Figure 21A). On 18 August 1993, which corresponded with the third generation of the alder leafminer, mature leaves were much tougher than immature leaves (241±11 and 150±8 g, respectively) ($t = -6.83$; *d.f.* =5,5; $P < 0.001$) (Figure 22A).

Birch: During the first generation when only immature leaves were available for ovipositing females, leaf toughness of immature leaves was 134±7 g in 1992 (22 May) (Figure 21B), and 136±6 g in 1993 (13 May) (Figure 22B).

During the second generation, immature leaves were much less tough than mature leaves: 160±3 and 256±8 g, respectively on 21 July 1992 ($t = -11.13$; *d.f.* =7,7;

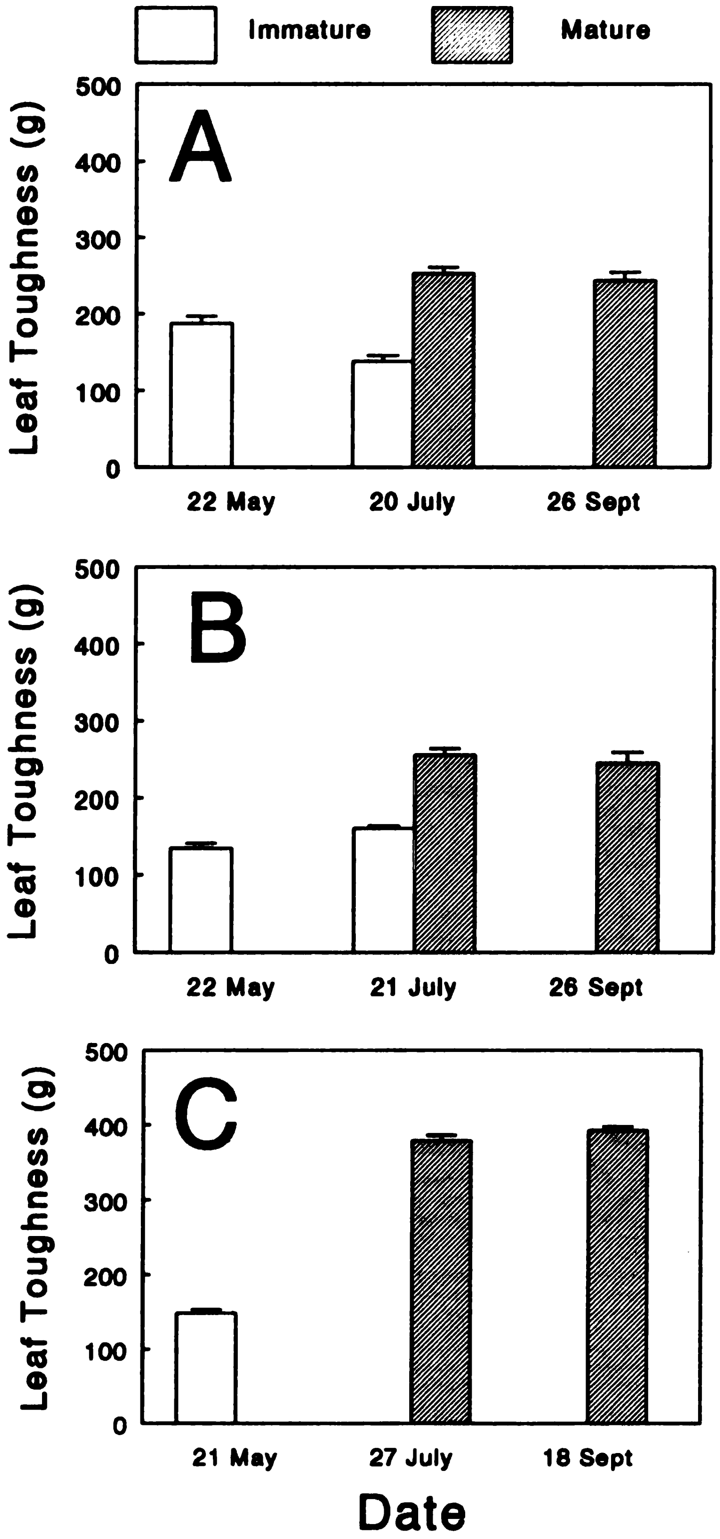

Figure 21: Toughness of immature and mature leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

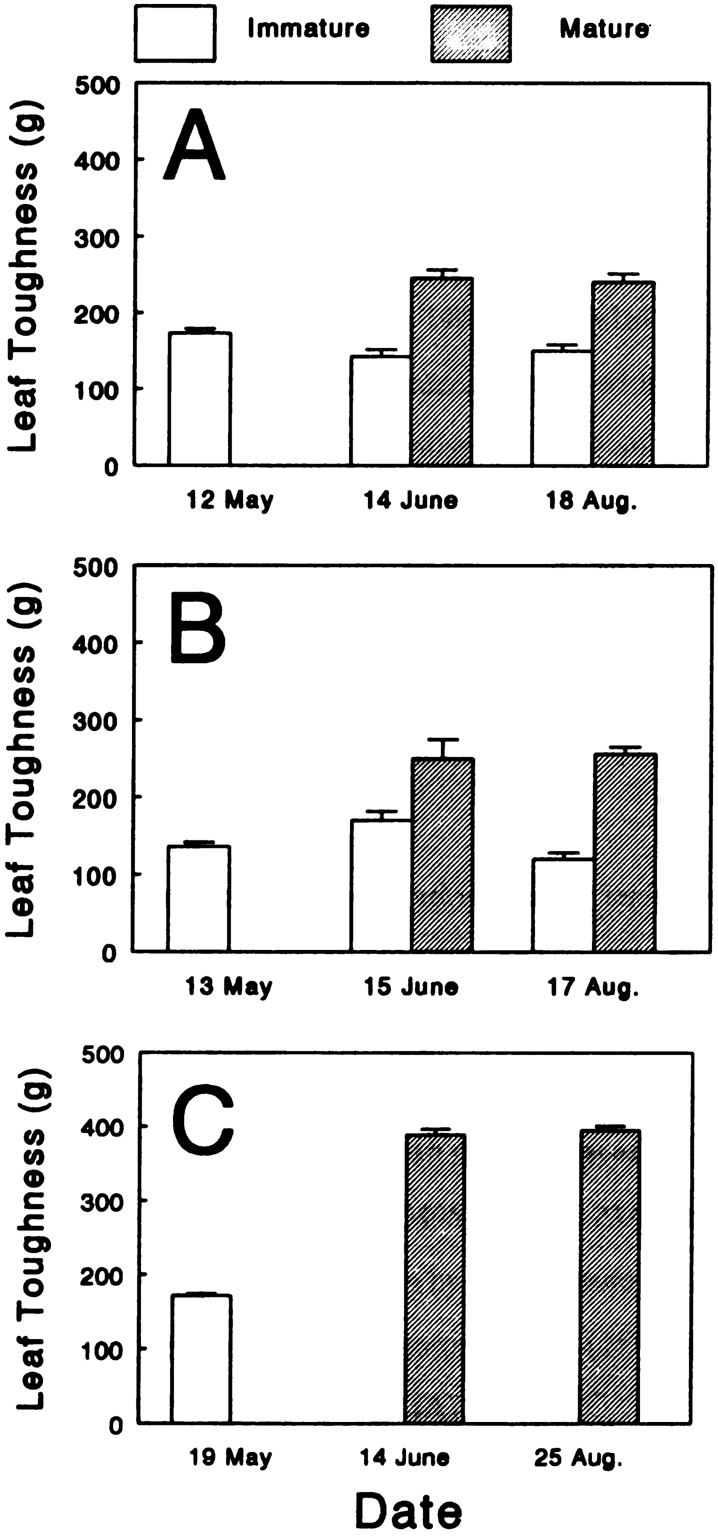

Figure 22: Toughness of immature and mature leaves during 1993: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

$P < 0.001$) (Figure 21B), and 170 ± 12 g and 250 ± 25 g, respectively, on 15 July 1993 ($t = -2.97$; *d.f.* = 6,6; $P < 0.05$) (Figure 22B).

Hawthorn: Immature leaves were first sampled when females were ovipositing (21 May 1992 and 19 May 1993). These leaves were compared to mature leaves sampled at times that corresponded with the presence of second and third generation alder and birch leafminer (27 June and 18 September in 1992 and 14 June and 25 August in 1993, respectively). The toughness of immature leaves was 148 ± 5 g in 1992 (Figure 21C) and 171 ± 3 g in 1993 (Figure 22C). These differences were highly significant.

Leaf Area

Alder: During the first generation of alder leafminer in 1992 (22 May) and 1993 (15 May), respective means of immature leaves were 17 ± 1 (Figure 23A) and 13 ± 1 cm^2 (Figure 24A). On the second sample date in 1992 (20 July), which corresponded with peak oviposition of second generation alder leafminer, immature leaves were not significantly larger than mature leaves (31 ± 2 and 28 ± 2 cm^2, respectively) ($t = 0.97$, *d.f.* = 7,7; $P = 0.3496$) (Figure 23A). However, when leaves were sampled during peak oviposition by second generation adults in 1993 (14 July), immature leaves were smaller than mature leaves (25 ± 2 and 30 ± 1 g, respectively) ($t = -2.22$; *d.f.* = 5,5; $P = 0.05$) (Figure 24A).

On the third sample date in 1992 (26 September) mature leaves (the only leaf type available for sampling) had a mean area of 29 ± 2 cm^2 (Figure 23A). On the third sample date in 1993 (18 August), which corresponded to peak oviposition by third generation adults, immature leaves were smaller than mature leaves (18 ± 2 and 37 ± 3 cm^2, respectively) ($t = -4.84$; *d.f.* = 5,5; $P < 0.001$) (Figure 24A).

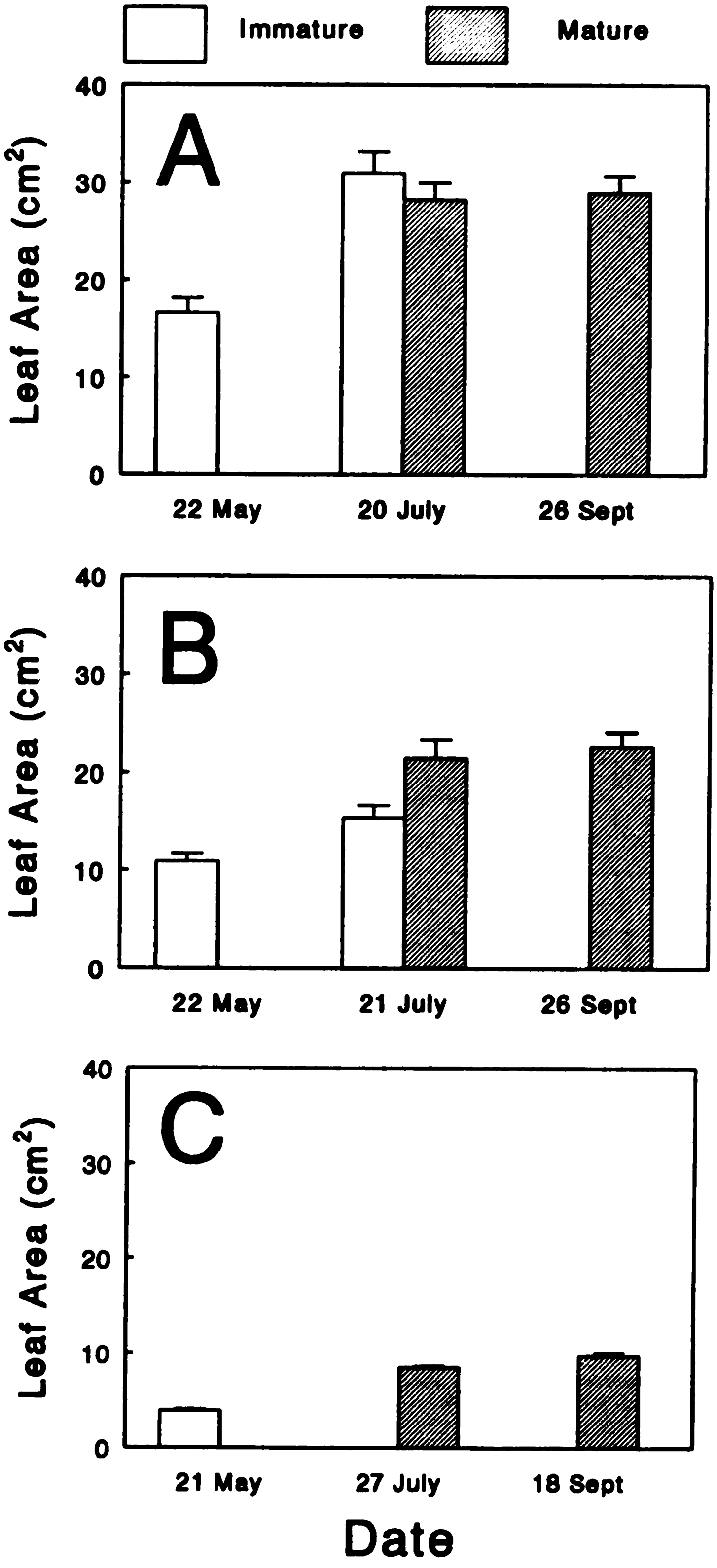

Figure 23: Area of immature and mature leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

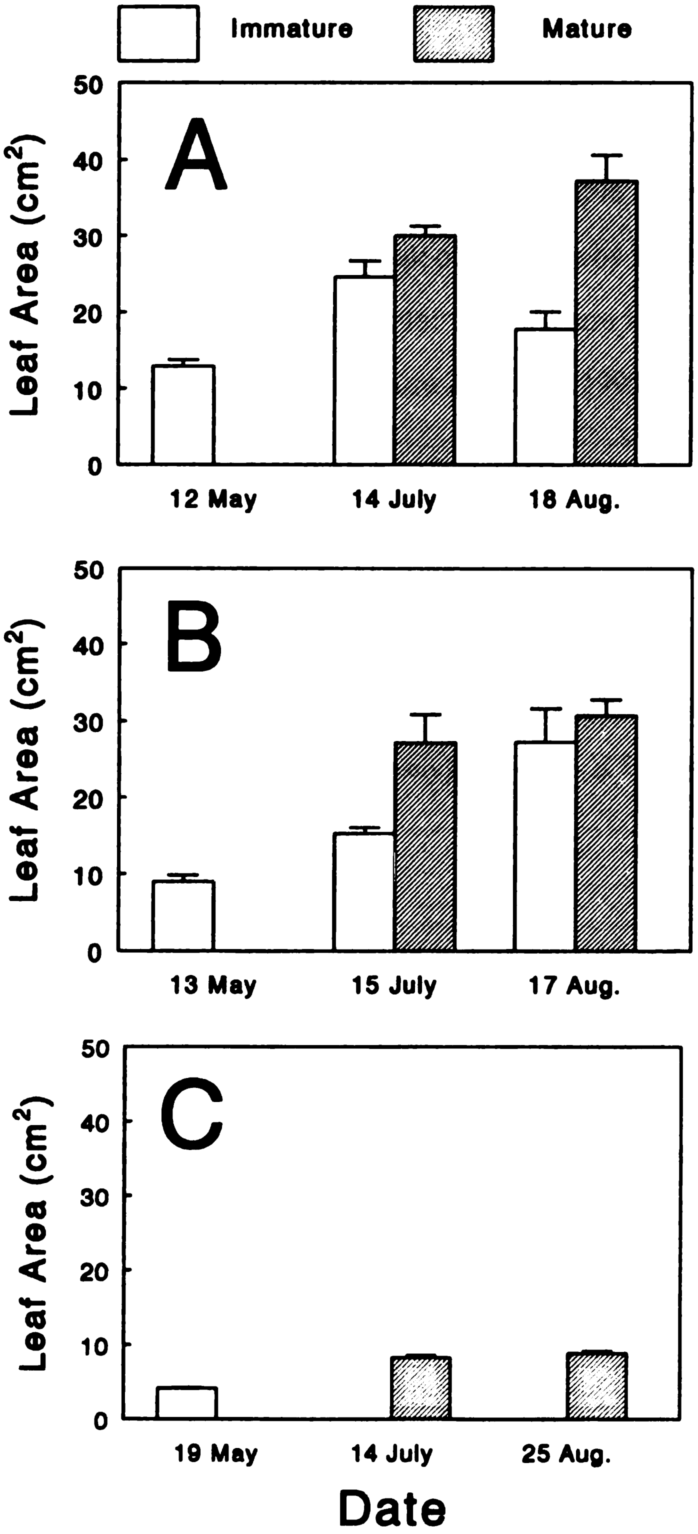

Figure 24: Area of immature and mature leaves during 1993: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

Birch: During the first generation of birch leafminer in 1992 (22 May) and 1993 (13 May), the mean area of immature leaves was 11 ± 1 (Figure 23B) and 9 ± 1 cm^2, respectively (Figure 24B). On the second sample dates (21 July 1992 and 15 July 1993), which corresponded to peak oviposition by second generation birch leafminer, immature leaves were smaller than mature leaves: 1992 (15 ± 1 and 21 ± 2 cm^2) ($t = -2.58$; $d.f. = 7,7$; $P < 0.05$) (Figure 23B) and 1993 (15 ± 1 and 27 ± 4 cm^2) ($t = -3.14$; $d.f. = 6,6$; $P < 0.01$) (Figure 24B).

On the third sample date in 1992 (26 September), only mature leaves were available for sampling, and their area was was 23 ± 1 cm^2 (Figure 23B). On the third sample date in 1993 (17 August), corresponding with third generation birch leafminer oviposition, immature leaves were not significantly smaller than mature leaves (27 ± 4 and 31 ± 2 cm^2, respectively) ($t = -0.70$; $d.f. = 4,4$; $P = 0.5057$) (Figure 24B).

Hawthorn: Immature leaves sampled during the first generation in 1992 (21 May) were smaller in size than mature leaves collected on the second (27 July) and third (18 September) sample dates ($t = -20.27$; $d.f. = 7,7$; $P < 0.001$ and $t = -14.78$; $d.f. = 7,7$; $P < 0.001$, respectively) (Figure 23C). The leaf area increased from 4 ± 1 cm^2 to 10 ± 1 cm^2 as leaves matured (Figure 23C). The same patterns was observed in 1993 (Figure 24C).

Specific Leaf Mass

Alder: On the first sample date in 1992 (22 May) and 1993 (12 May), when only immature leaves were available, their respective specific masses were 46 ± 03 g/m^2 (Figure 25A) and 46 ± 02 g/cm^2 (Figure 26A). On the second sample date in 1992 (27 July) and 1993 (14 July), which corresponded with peak oviposition by

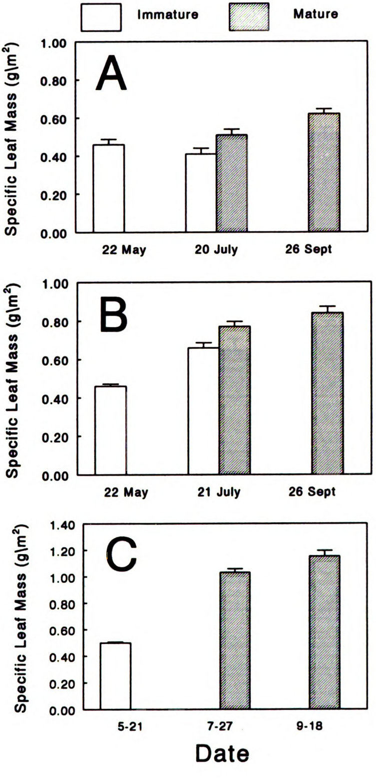

Figure 25: Specific leaf mass of immature and mature leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

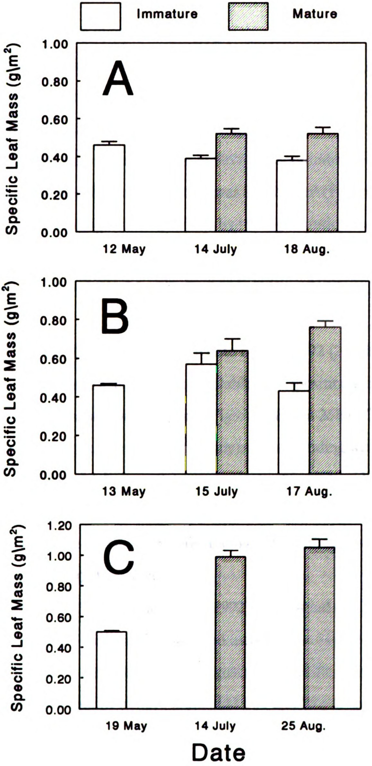

Figure 26: Specific leaf mass of immature and mature leaves during 1993: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

second generation alder leafminer, specific mass of immature leaves was less than that of mature leaves: 41 ± 03 and 51 ± 03 g/m^2, respectively in 1992 ($t = -2.27$; $d.f. = 7,7$; $P < 0.05$) (Figure 25A), and 39 ± 02 and 52 ± 03 g/m^2, respectively in 1993 ($t = -4.16$; $d.f. = 5,5$; $P < 0.01$) (Figure 26A).

On the third sample date in 1992 (26 September) only mature leaves were available, the specific mass of which was 61 ± 2 g/m^2 (Figure 25A). On 17 August 1993, corresponding with oviposition by third generation females, specific mass of immature leaves was less than that of mature leaves (38 ± 02 and 52 ± 03 g/m^2, respectively) ($t = -3.70$; $d.f. = 5,5$; $P < 0.005$) (Figure 26A).

Birch: On the first sample date in both 1992 (22 May) and 1993 (13 May), when only immature leaves were available to ovipositing females, their respective specific leaf mass was 46 ± 01 g/m^2 (Figure 25B and 26B). On the second sample date in 1992 (21 July) and 1993 (15 July), corresponding with oviposition by second generation birch leafminer, the specific mass of immature leaves was less than that of mature leaves: 66 ± 03 and 77 ± 03 g/m^2, respectively in 1992 ($t = -2.98$; $d.f. = 7,7$; $P < 0.01$) (Figure 25B), and 57 ± 06 and 64 ± 06 g/m^2, respectively in 1993 ($t = -0.87$; $d.f. = 6,6$; $P = 0.40$) (Figure 26B).

On the third sample date in 1992 (26 September), when only mature leaves were available, their specific mass had increased to 84 ± 03 g/m^2 (Figure 25B). On the third sample date in 1993 (17 August), the specific mass of immature leaves was again less than that of mature leaves (43 ± 04 and 75 ± 03 g/m^2, respectively) ($t = -6.16$; $d.f. = 4,4$; $P < 0.001$) (Figure 26B).

Hawthorn: In 1992, immature leaves collected during peak oviposition (21 May) had a lower specific mass than mature leaves collected on the second (27 July) and third (18 September) sampling dates, which corresponded to presence of second and third generation alder and birch leafminers ($t=-18.63$; *d.f.* =7,7; $P<0.001$ and $t=-14.55$; *d.f.* =7,7; $P<0.001$, respectively) (Figure 25C). Specific mass of immature leaves was 50±01 g/m^2, while that of mature leaves was 103±03 (27 July) and 115±04 g/m^2 (18 September) (Figure 25C).

The same trend was observed in 1993, with immature leaves sampled on 19 May being lower in specific mass than mature leaves sampled on 14 July and 25 August ($t=-11.57$; *d.f.* =7,7; $P<0.001$ and $t=-9.82$; *d.f.* =7,7; $P<0.001$, respectively) (Figure 26C). The specific mass of immature leaves was 50±01 g/m^2, increasing to 99±04 g/m^2 on 14 July, and 105±05 g/m^2 on 25 August (Figure 25C).

Nitrogen

Alder: In 1992, on the first sample date (22 May) which corresponded with peak oviposition of alder leafminer, only immature leaves were available for analysis, and their nitrogen concentration was 3.29±0.01% (Figure 27A). On the second sample date (20 July), which corresponded with the second generation leafminers, immature leaves were higher in percent nitrogen than mature leaves (3.18±0.09 and 2.91±0.06%, respectively) ($t=-2.36$; *d.f.* =15,15; $P<0.05$) (Figure 27A). On the third sample date (26 September), only mature leaves were available for analysis and their nitrogen concentration was 2.91±0.06% (Figure 27A).

Birch: On the first sample date in 1992 (22 May), which corresponded with first generation birch leafminer, only immature leaves were available for analysis. The nitrogen concentration of these leaves was 3.60±0.09% (Figure 27B). Immature leaves were higher in nitrogen than mature leaves on the second sample date (21 July) (1.94±0.08 and 1.82±0.04%, respectively) ($t=-1.41$; *d.f.* =14,15; $P=0.1700$) (Figure 27B). On the third sample date in 1992 (26 Sept.), only mature

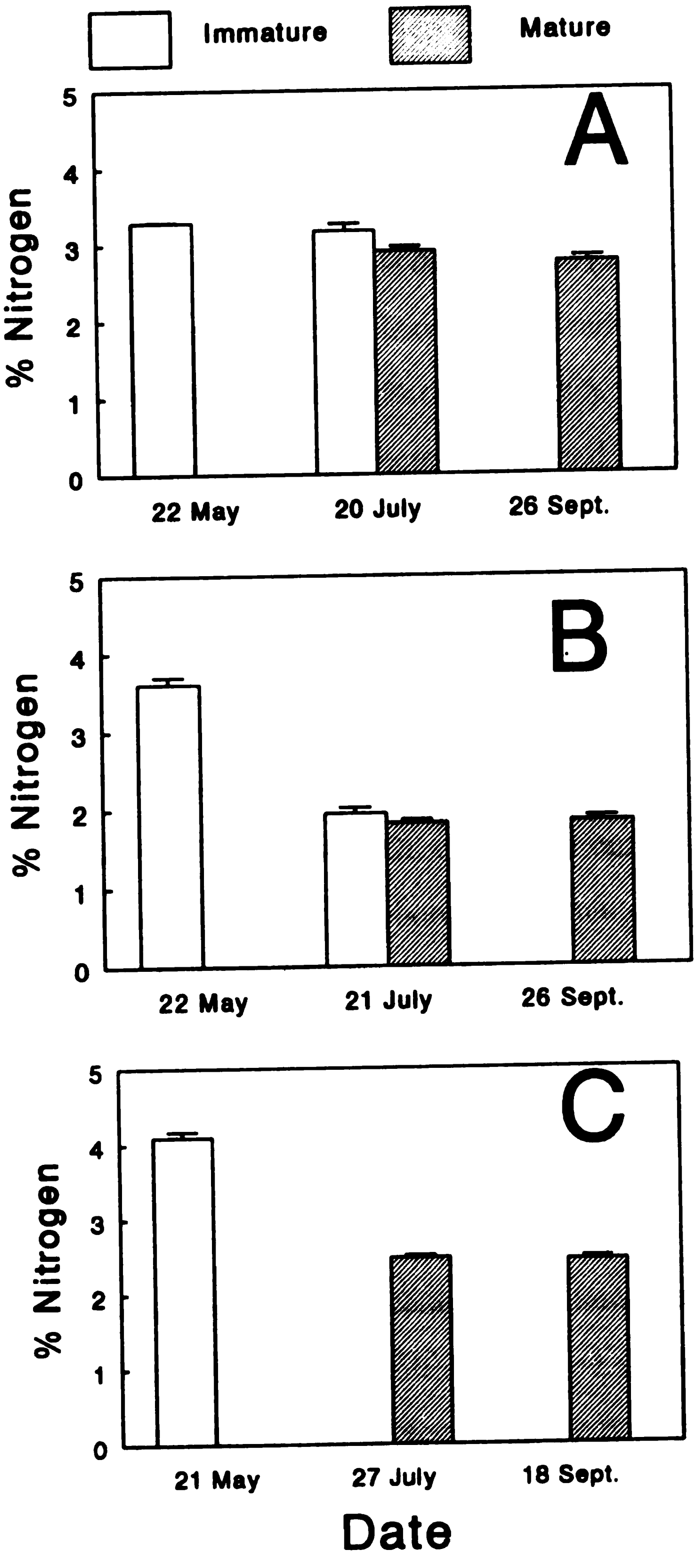

Figure 27: Percent nitrogen concentration of immature and mature leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

leaves were available for analysis, their nitrogen concentration had declined to 1.84 ± 0.06% (Figure 27B).

Hawthorn: On the first sample date in 1992 (21 May), which corresponded with peak oviposition, only immature leaves were available for analysis, and their nitrogen concentration was 4.09 ± 0.01% (Figure 27C). Their nitrogen concentration declined to 2.47 ± 0.04% by 27 July, where it remained unchanged (Figure 27C).

Phosphorous

Alder: The phosphorous concentration of immature leaves sampled on 22 May was 0.24 ± 0.01% (Figure 28A). On the second sample date (20 July), immature leaves were higher in phosphorous than mature leaves (0.22 ± 0.01 and 0.14 ± 0.01%, respectively) ($t = -6.68$; *d.f.* = 15,15; $P < 0.001$) (Figure 28A). On the third sample date in 1992 (26 September), only mature leaves were available for analysis, and their phosphorous concentration had declined to 0.14 ± 0.01% (Figure 28A).

Birch: The phosphorous concentration of immature birch leaves sampled on 22 May was 0.35 ± 0.01% (Figure 28B). Immature and mature leaves were equal in phosphorous concentration on the second sample date (21 July) (0.22 ± 0.01%) ($t = 0.19$; *d.f.* = 14,15; $P = 0.8493$) (Figure 28B). On the third sample date in 1992 (26 September), only mature leaves were available for analysis, the phosphorous concentration of which increased to 0.32 ± 0.02% (Figure 28B).

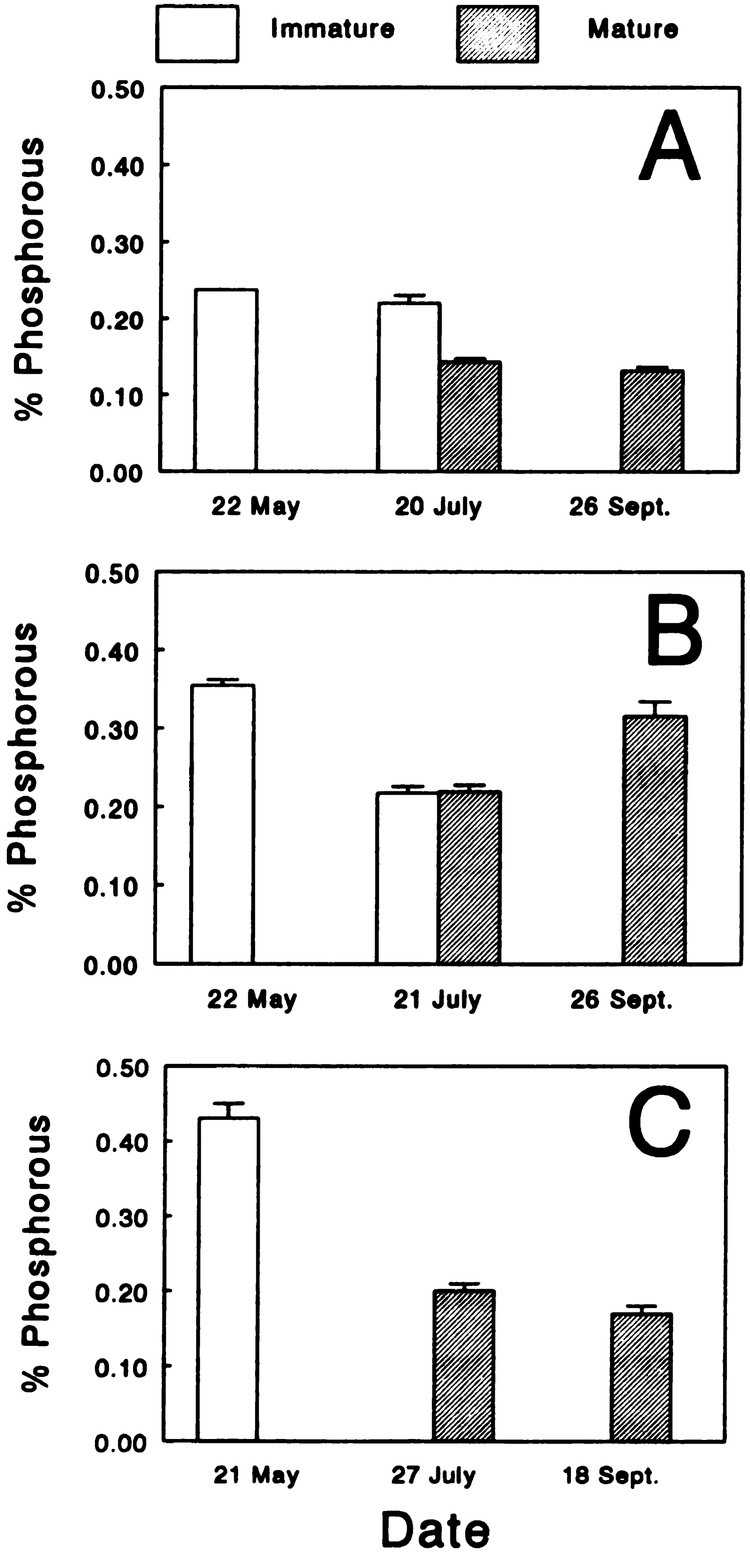

Figure 28: Percent phosphorous concentration of immature and mature leaves during 1992: (A) European alder, (B) paper birch, and (C) hawthorn. Sampling dates correspond with peak oviposition and larval development of each generation of alder (A), birch (B), and hawthorn (C) leafminer.

Hawthorn: The phosphorous concentration of immature hawthorn leaves sampled on 21 May was 0.43 ± 0.01% (Figure 28C). On the second sample date (27 July) only mature leaves were available for analysis, and these leaves were compared to immature leaves collected on the first sample date. The immature leaves had a phosphorous concentration twice that of mature leaves (0.44 ± 0.02 and 0.20 ± 0.01%, respectively) ($t = -13.26$; *d.f.* = 15,15; $P < 0.001$) (Figure 28C). On 18 September, the phosphorous concentration of mature leaves was also substantially less than that of immature leaves (0.44 ± 0.02 to 0.17 ± 0.01%) ($t = -15.85$; *d.f.* = 15,15; $P < 0.001$) (Figure 28C).

Age Specific Larval Mortality In Relation to Leaf Maturity

Leaf toughness

Alder leafminer: For first generation alder leafminer in 1992, first instar mortality was 36% in leaves with a toughness of 200 g or less, while mortality was 40% in leaves greater than 200 g (Figure 29A). No fourth and fifth instar mortality was observed. However, these conclusions are limited by the number of observations. In 1993, first generation first instar mortality increased with leaf toughness. Mortality was 24% when leaf toughness was less than 200 g, and increased to 53% for leaves within the toughness range of 250-300 g (Figure 29B). However, mortality of second and third instars decreased as toughness increased and leaves matured (Figure 29B).

For the second generation in 1992 and 1993, first and second instar mortality tended to increase as leaf toughness increased. In 1993, first and second instar mortality was 46% and 22%, respectively, when leaf toughness was less than

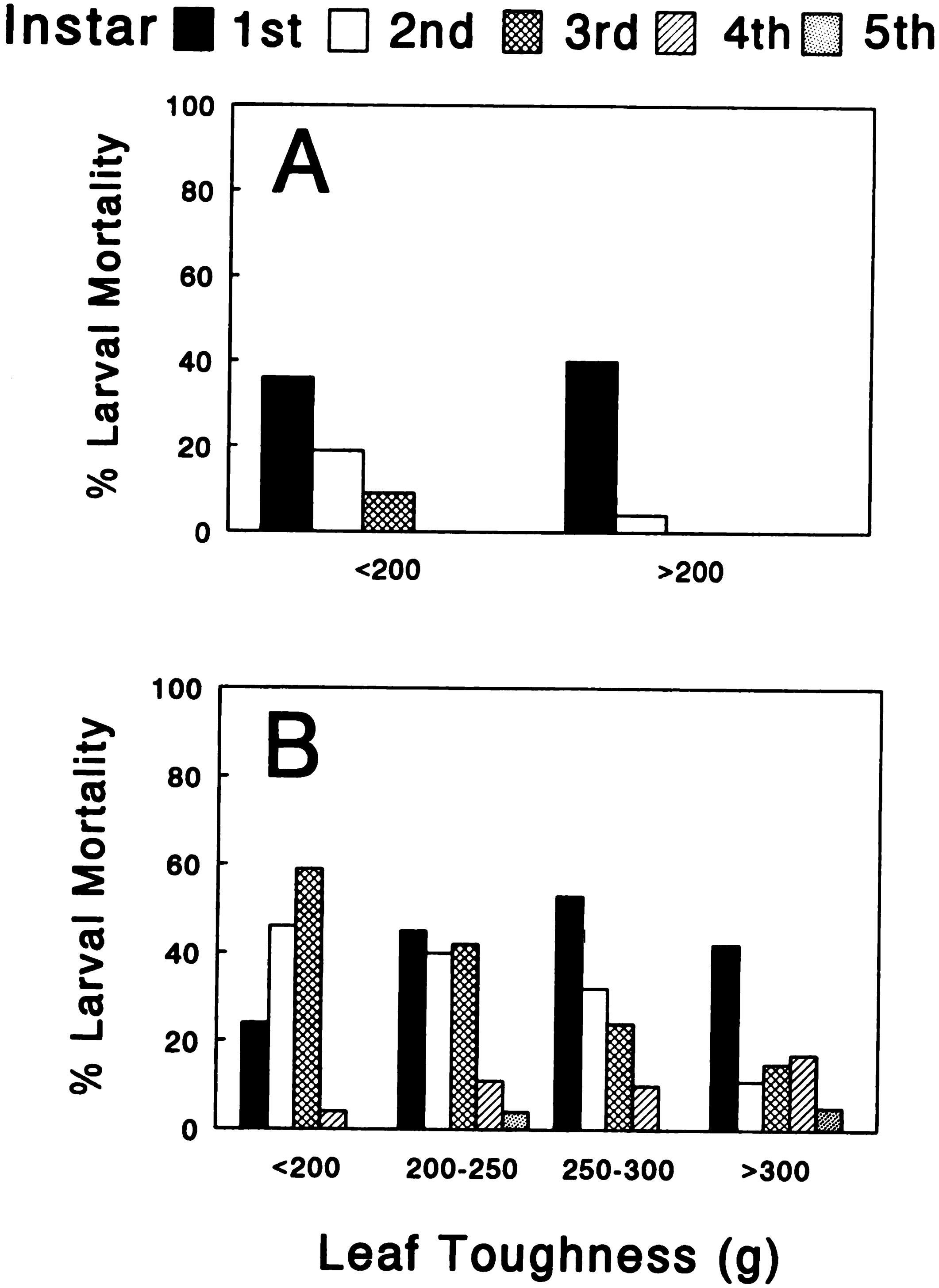

Figure 29: The relationship between leaf toughness and instar-specific larval mortality of first generation alder leafminer in: 1992 (A) and 1993 (B).

200 g and increased to 92% and 80%, respectively, when leaf toughness exceeded 250 g (Figure 30B).

Birch leafminer: In 1992 and 1993, first generation mortality of first and second instar birch leafminer increased as leaf toughness increased (Figure 31A). In 1992, first and second instar mortality was 21% and 14%, respectively, when leaf toughness was less than 150 g, and increased to 50% and 29%, respectively, when leaf toughness exceeded 200 g (Figure 31A). In 1993, when leaf toughness was less than 150 g, first and second instar mortality was 5% and 0%, respectively, and increased to 67% and 60%, respectively, when leaf toughness exceeded 250 g (Figure 31B).

Second generation morality was not analyzed in 1992 due to limited observations. However, in 1993 first instar mortality increased from 58%, when leaf toughness was less than 150 g, to 80% when leaf toughness exceed 200 g (Figure 32).

Hawthorn leafminer: In 1992, first, second and third instar hawthorn leafminer mortality increased from 50%, 6%, and 0%, respectively, when leaf toughness was less than 150 g to 67%, 11%, and 8%, respectively, when leaf toughness exceeded 200 g (Figure 33A). To few observations were recorded for fourth and fifth instars to draw conclusions.

In 1993, second and third instars and fourth and fifth instars were grouped to increase the number of observations. For each grouping (1st, 2nd+3rd, and 4th+5th instars), mortality increased as leaf toughness increased. First instar mortality ranged from 34% to 99% and second instar mortality ranged from 16 to 75% for leaves with toughness less than 200 and greater than 250 g, respectively (Figure 33B).

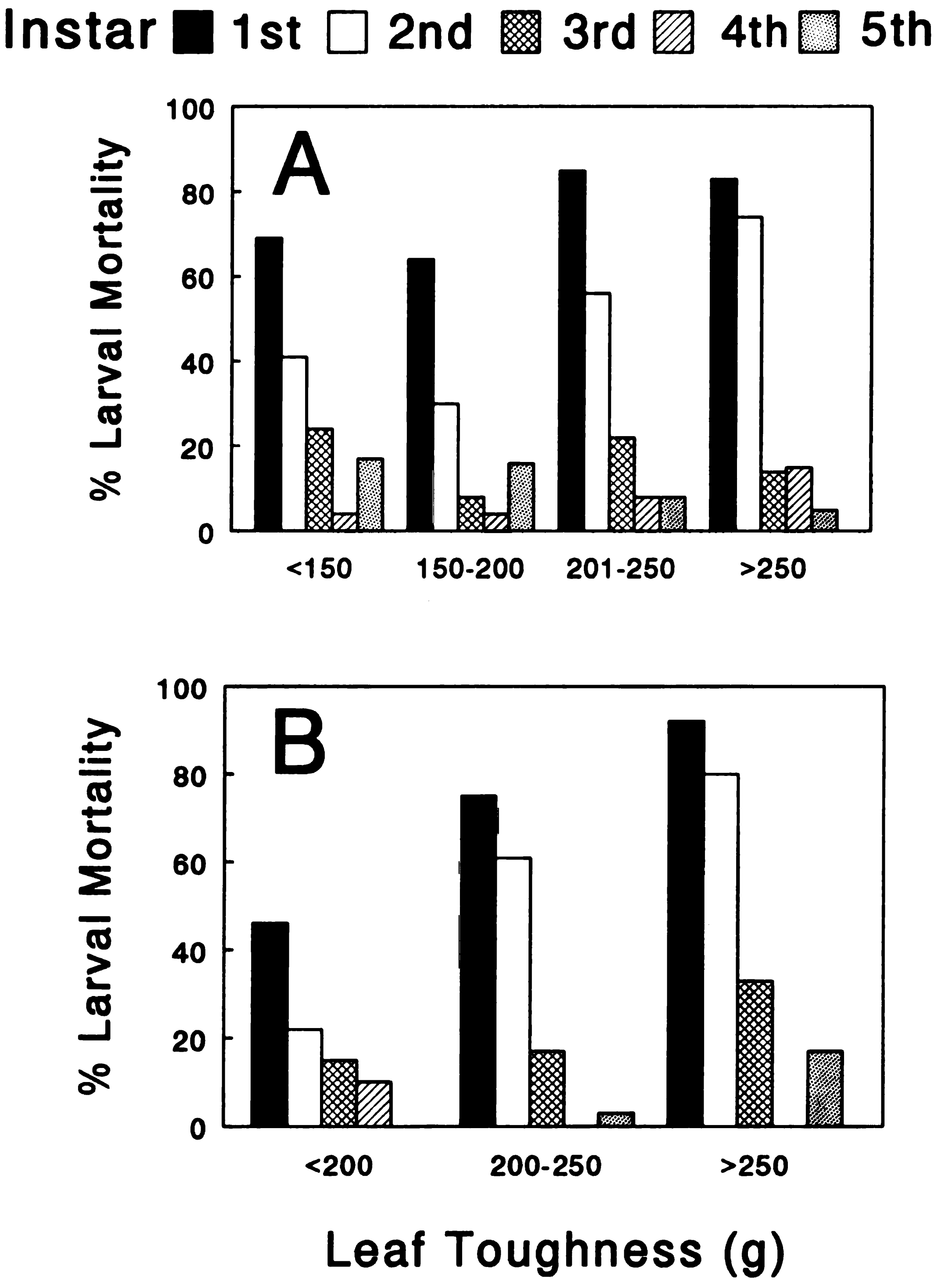

Figure 30: The relationship between leaf toughness and instar-specific larval mortality of second generation alder leafminer in 1992 (A) and 1993 (B).

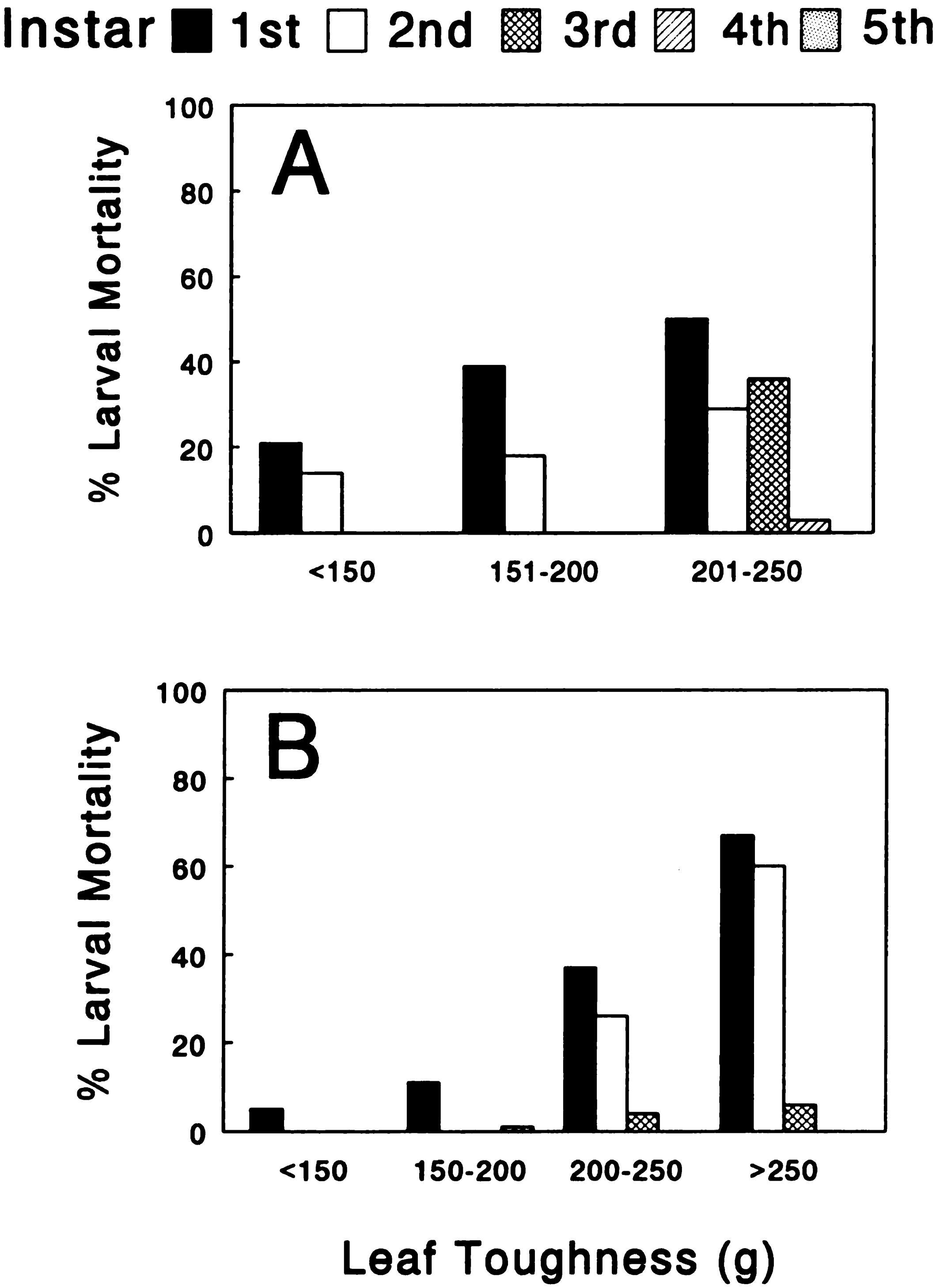

Figure 31: Relationship between leaf toughness and instar-specific larval mortality of first generation birch leafminer in 1992 (A) and 1993 (B).

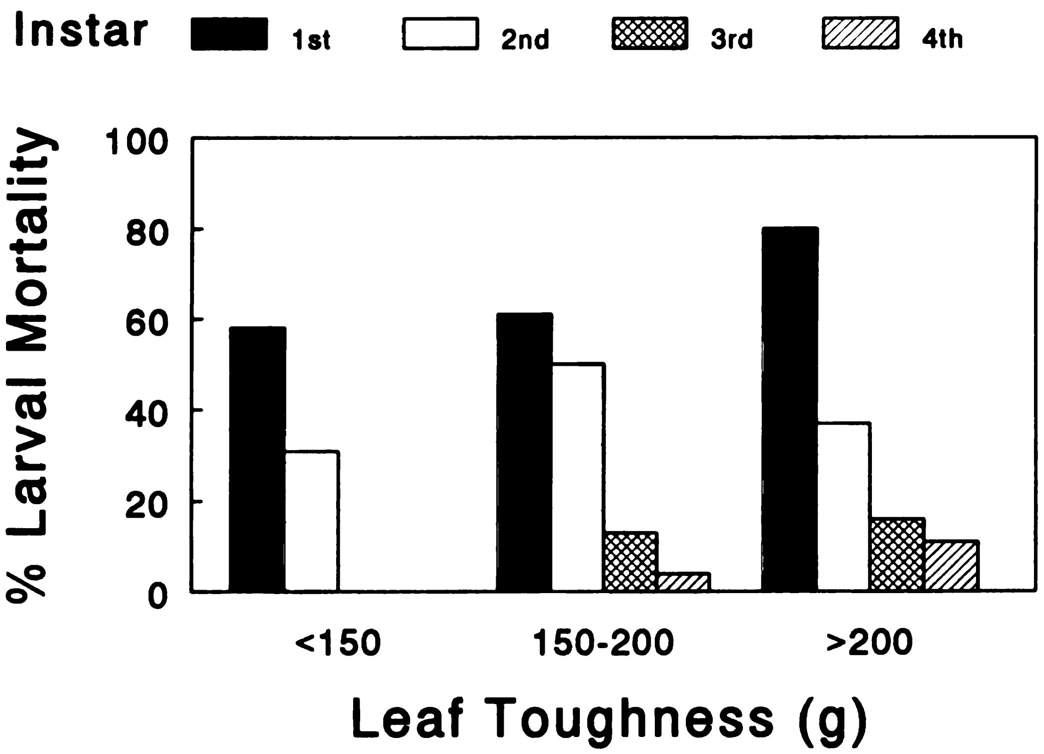

Figure 32: Relationship between leaf toughness and instar-specific larval mortality of second generation birch leafminer in 1993.

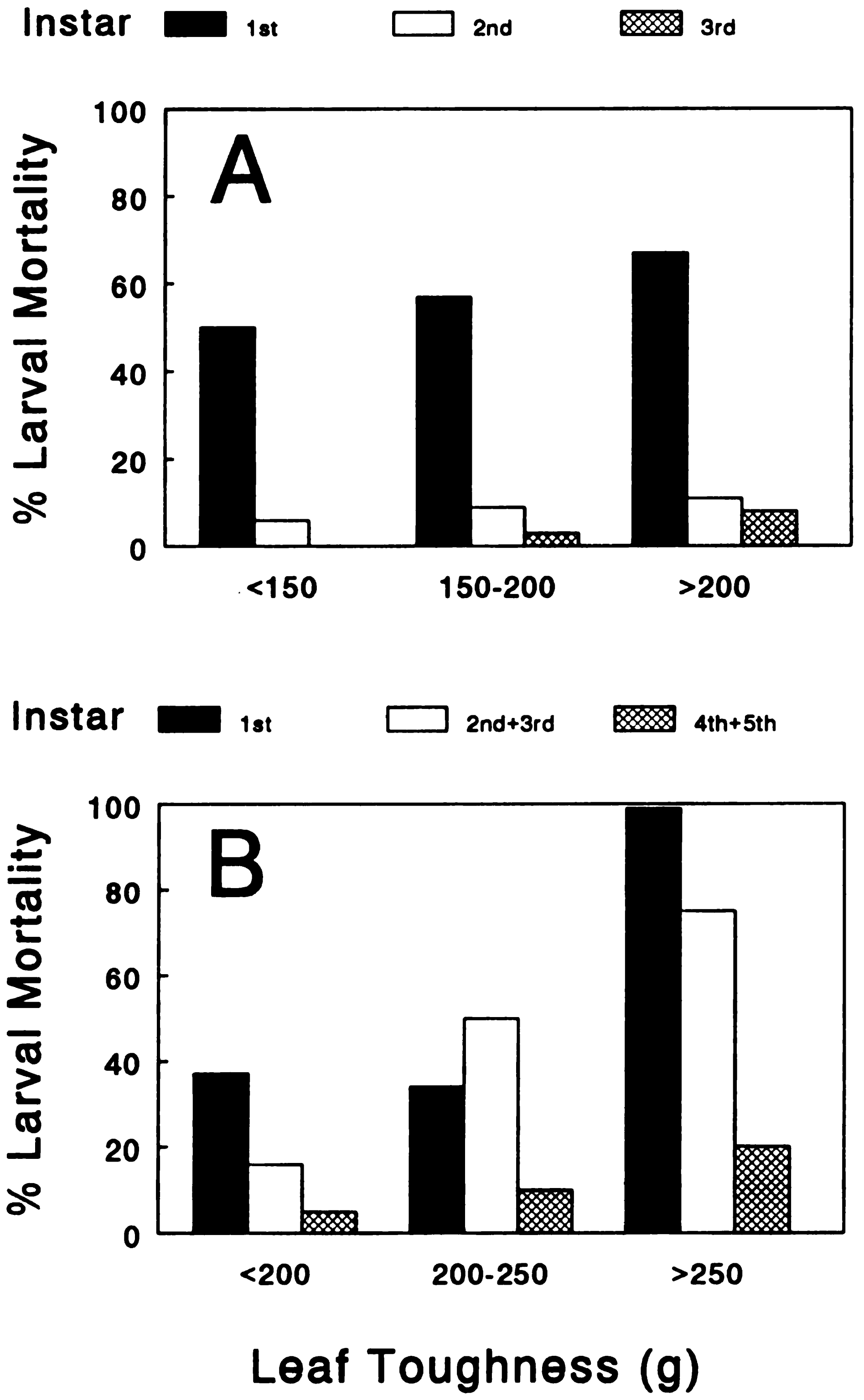

Figure 33: Relationship between leaf toughness and instar-specific larval mortality of hawthorn leafminer 1992 (A) and 1993 (B).

DISCUSSION

Alder leafminers oviposited exclusively on immature leaves and birch leafminers highly preferred immature leaves as oviposition sites, even when only mature leaves were available. When birch leafminer females were offered mature leaves for oviposition, only two leaves were selected as oviposition sites in the entire experiment; these two leaves were the youngest leaves on a single seedling and received a total of 8 eggs. This provides strong evidence that immature leaves are the only leaves utilized by alder and birch leafminers as oviposition sites. Friend (1931) reported that birch coppice growth, which produces new leaves in abundance, were heavily infested by birch leafminer compared to foliage on established shoots. These data, combined with high first instar mortality in mature leaves, strongly suggest these species are dependent on immature leaves for survival. This conclusion is consistent with the hypothesis that the indeterminate growth pattern of European alder and paper birch may have allowed the alder and birch leafminers to evolve a multivoltine life cycle by providing a continuous supply of immature leaves.

Experiments were not conducted to determine if hawthorn leafminers would oviposit in mature leaves. However, given the fact of nearly 100% first instar mortality occurs in leaves with toughness exceeding 250 g, and that mature leaves present in July and August approach 400 g in toughness, it is speculated that early instar larvae would not be able to survive in the tougher mature leaves.

Feeny (1970) found one of the most obvious changes in oak leaves as they mature to be an increase in toughness. He suggested that increased toughness may have a significant negative effect on the ability of larvae to feed. Consistent with this, as alder, birch, and hawthorn leaves matured, their toughness increased and early instar survival declined. However, as larvae matured, leaf toughness had a much less pronounced effect on mortality. Tanton (1962) reported that leaf toughness retarded feeding and growth of *Phaedon cochleariae* mustard beetle) larvae. As toughness increased the area consumed by larvae decreased, larval and pupal mortality increased, and growth rate decreased. As observed in this research with late instars, Tanton found third instar larvae to be less influenced by toughness. He proposed that this was due to an increase in mandibular strength.

Physical and chemcial differences between immature and mature alder and birch leaves available to the second and third generations were large. Differences between immature hawthorn leaves sampled during peak emergence of the hawthorn leafminer, and mature leaves sampled in correspondence with the second generation of alder and birch leafminer were also large. For each species, mature leaves were tougher, larger, higher in specific leaf mass, and were lower in phosphorous and nitrogen concentrations, which may have a negative influence on developing larvae.

Feeny (1968) reported that tannin concentrations of oak leaves increased with leaf age, and that larval growth and pupal weight decreased as tannin concentrations increased. Feeny suggested that this was due to a reduction in the availability of protein. In addtion, Feeny suggested that the seasonal increase in tannins concentration of oak leaves may be a factor contributing to the selection for early feeding larvae on oak.

All of these characteristics of mature leaves may help to explain why all three species oviposit in immature leaves, and first instar larval mortality of all three species increases as leaves mature. These results are consistent with the hypothesis that the hawthorn leafminer is constrained to a univoltine life-history by the determinate growth habit of it host plant, while the indeterminate growth habit of birch and alder has permitted the evolution of a multivoltine life history of the birch and alder leafminer.

CHAPTER THREE

HOST PHENOLOGY, INTRASPECIFIC COMPETITION, CLIMATIC VARIATION AND THE EVOLUTION OF INTRASPECIFIC VARIATION IN VOLTINISM PATTERNS

Although alder and birch trees display indeterminate growth, the number of immature leaves present at any one time may decrease rapidly after the first flush. Consequently, the quantity of immature leaves available to second and third generation alder and birch leafminers may only be a fraction of that available to the first generation. Corresponding to this pattern of resource availability, numbers of first generation adult birch leafminers (overwintering individuals) always exceed numbers of second and third generation adults (Herms, unpublished). In the case of alder leafminer, large first and second generations are followed by a small third generation (Hart et al. 1991). In addition, decreased availability of immature leaves available for females to oviposit in could create the potential for competition among larvae as larvae are concentrated within the few available leaves.

Intraspecific competition has been documented in several leafminer species. Tuomi et al. (1981) documented intraspecific competition in *Scolioneura betuleti*, a hymenopteran leafminer infesting birch leaves. Intraspecific competition occurred as the population density increased, decreasing larval survival. They also found a negative correlation between mean larval weight and larval density. Furthermore, mean larval weight was positively correlated with leaf area available per larva. These findings coincide with those of Quiring and McNeil (1984a), who documented competition in alfalfa blotch leafminer (*Agromyza frontella*) populations. Stiling

(1984) reported that larval mortality of *Hydrellia valida*, on salt marsh cordgrass, increased when two or more mines coalesced, and that intraspecific competition accounted for 11.4% of total mortality. Intraspecific competition has also been documented in populations of leafminers on oak, aspen, and lima beans (Bultman & Faeth 1986a, Auerbach 1991, and Petitt & Wietlisbach 1992, consecutively). It appears that in leafminers intraspecific competition frequently occurs at high larval densities due to food limitation.

Intraspecific competition may select for density-dependent premature extra-long diapause (*sensu* Hanski 1988), which occurs when some individuals enter diapause, avoiding competition, but missing one or more breeding opportunities experienced by individuals within the population that do not diapause. The pitcher-plant mosquito, *Wyeomyia smithii*, is one of the best documented examples of density-dependent premature extra-long diapause. According to Istock et al. (1975), at high densities some individuals may enter diapause, foregoing breeding opportunities experienced by others, but avoiding competition for limited food.

The observed small population size of the second generation of birch leafminer, relative to the first generation, suggest that most individuals may not emerge from pupation as second generation adults. Instead, these individuals may enter density-dependent premature extra-long diapause to avoid intraspecific competition among offspring in a resource limited habitat. These individuals loose breeding opportunities experienced by non-diapausing individuals, but emerge the following spring when a full flush of immature leaves is once again available. This may represent a bet-hedging strategy (Philippi and Seger 1989). Philippi and Seger (1989) defined bet-hedging as an evolutionary trade-off between mean and fitness of individuals; individuals with a lower mean fitness may have a selective advantage over highly fit (high mean fitness) individuals under certain conditions.

Climatic conditions such as photoperiod, temperature, and resource availability and quality may play a significant role in insect phenology (Tauber and Tauber 1973). Climatic conditions influence the termination date of leaf production and larval development rates (Scriber & Lederhouse 1992), and thus have the potential to select for evolution of a density-independent premature extra-long diapause (*sensu* Hanski 1988). Decreased numbers of adults giving rise to third generation larvae suggest that some second generation birch and alder leafminer individuals may enter density-independent premature extra-long diapause during the pupal stage. The second generation of each species may be "hedging their bet" (Philippi and Seger 1989) as to whether to give rise to third generation individuals or to enter diapause. Third generation individuals may have to "race" to finish development before unfavorable fall climate conditions prevail, and may not be able to complete development in some years. However, diapausing individuals loose a breeding opportunity, but will emerge the following spring. However, they may also face elevated risks of mortality prior to reproduction.

Bet-hedging may also take the form of risk spreading through phenotypic variability in offspring. Offspring from a single female may be phenotypically different to ensure that at least some will be adapted to a uncertain future. Thus, it may be an adaptive strategy that may increase fitness in a temporally variable environment (Bradford & Roff 1993). Bradford and Roff (1993) suggested that a bet-hedging diapause strategy occurs in a partially bivoltine population of cricket (*Allanemobius fasciatus*). As first generation females aged, the proportion of diapausing eggs increased, reflecting a decrease in the probability of second generation offspring being able to complete development before the end of the growing season. Therefore, it is possible that observed variability in voltinism patterns can be controlled by the ovipositing female. A small portion of the offspring of second generation birch and alder leafminer may be conditioned by their mother to emerge as second or third generation adults, while the majority would overwinter.

OBJECTIVES

The objective of this research was to test the following predictions: (1) there is intraspecific seasonal variation in the availability of immature leaves, (2) phenotypic patterns of voltinism within and among the three species correspond to seasonal patterns of resource availability (immature leaves), and (3) intraspecific competition occurs among larvae which may give rise to density dependent premature extra-long diapause.

METHODS AND MATERIALS

There is Seasonal Variation in the Production of Immature Leaves

In 1992, four branches per tree from each of eight trees of all three species, which showed long extensional growth the previous year were flagged at bud break. The following variables were measured three times throughout the summer (late May, late July, and early September): (1) total number of leaves per branch, (2) number of immature leaves per branch, (3) number of leaves on the terminal shoot, and (4) terminal shoot length. Seasonal variation in foliage production on birch trees was documented. However, due to severe deer browsing, insufficient data were collected for alder and hawthorn trees.

In 1993, the following modifications to methods were made: (1) alder (only 6 alder trees was used) and hawthorn branches above the deer browse line were sampled, (2) in order to increase the accuracy of counting the total number of hawthorn leaves per branch, the terminal leaf on each shoot was tagged and (3) measurements were made at the time of peak adult emergence of each generation (early June, mid-July, and mid-August) to estimate the number of immature leaves available for the ovipositing adults. Measurements ceased when terminal bud formation occurred.

Total number of immature leaves, total number of leaves, mean terminal shoot length, and total number of leaves on terminal shoots were compared in relation to Julian date by using Repeated Measures ANOVA. The proportion of immature leaves available to the second and third generations relative to that of the first generation were compared using ANOVA.

Intraspecific Variation in Voltinism Patterns Corresponds to Phenological Patterns of New Leaf Production

The number of individuals emerging as adults (not entering premature extra-long diapause) in relation to the rate of production of new leaves by the host plant (resource availability) was determined. See Chapter One for adult sampling protocols.

The average number of adults per trap at peak emergence, average number of larvae per leaf and percent of leaves infested were correlated with the number of available immature leaves using correlation analysis.

Intraspecific Competition Among Larvae

In 1992, leaves with second generation birch and alder leafminer larvae, and in 1993, leaves with first and second generation alder and birch larvae were collected within two days of emergence of mature larvae and brought into the laboratory for analysis. Leaf area (cm^2) was recorded, the petiole was inserted into a Water Pik, and each leaf was then placed in a petri-dish with a plaster filled base saturated with water to maintain humidity. The leaves were stored in a incubator (18:6) at 25 ^{O}C until larva(e) completed development. After larvae emerged from the leaf, they were placed individually in a sand filled vial in order to better simulate natural conditions. After all larvae had exited the leaf, total mined area (cm^2) of the leaf was determined. After the larvae pupated, each pupa was removed from its case and weighed (mg). After weighing, each individual was returned to its designated vial. Each vial was examined daily to determine if adult emergence occurred. Vials were examined for two weeks after the last adult emerged. Individuals that did not emerge as an adult were examined to determine whether they remained as pupae in diapause or if they died.

The relationship between larval density and (1) pupal mass, (2) percent emerging as adults, (3) percent diapausing and (4) percent survival (percent emerging as adults + percent diapausing) were quantified using correlation analysis.

RESULTS

Seasonal Variation in the Production of Immature Leaves

Alder

The production of immature leaves by alder decreased over the course of the season in 1993 ($P<0.001$) (Figure 34A). During peak emergence of first generation alder leafminer (1 June), there were 74 ± 9 immature leaves per branch. By 29 June, the number of immature leaves per branch had decreased to 42 ± 5. By 13 July, during peak oviposition by second generation alder leafminer, the number of leaves per branch had decreased to 20 ± 3. Even fewer immature leaves were available to third generation females (20 August), when each branch had only 7 ± 1 immature leaves per branch (Figure 34A).

Birch

Seasonal production of immature leaves by birch trees also decreased in 1993 ($P=0.01$) (Figure 34B). For first generation (1 June) birch leafminer, when all leaves were immature, there were 49 ± 3 leaves per branch. By the second generation (15 July), there was a seven-fold decrease in leaf production to 7 ± 1 immature leaves per branch. Finally, during the third generation (12 August), there were only 0.1 ± 1 immature leaves per branch (Figure 34B).

Hawthorn

Immature leaves were present on hawthorn only during the first generation of hawthorn leafminer (at bud break). No leaf production occurred after the initial flush at bud break (Figure 34C).

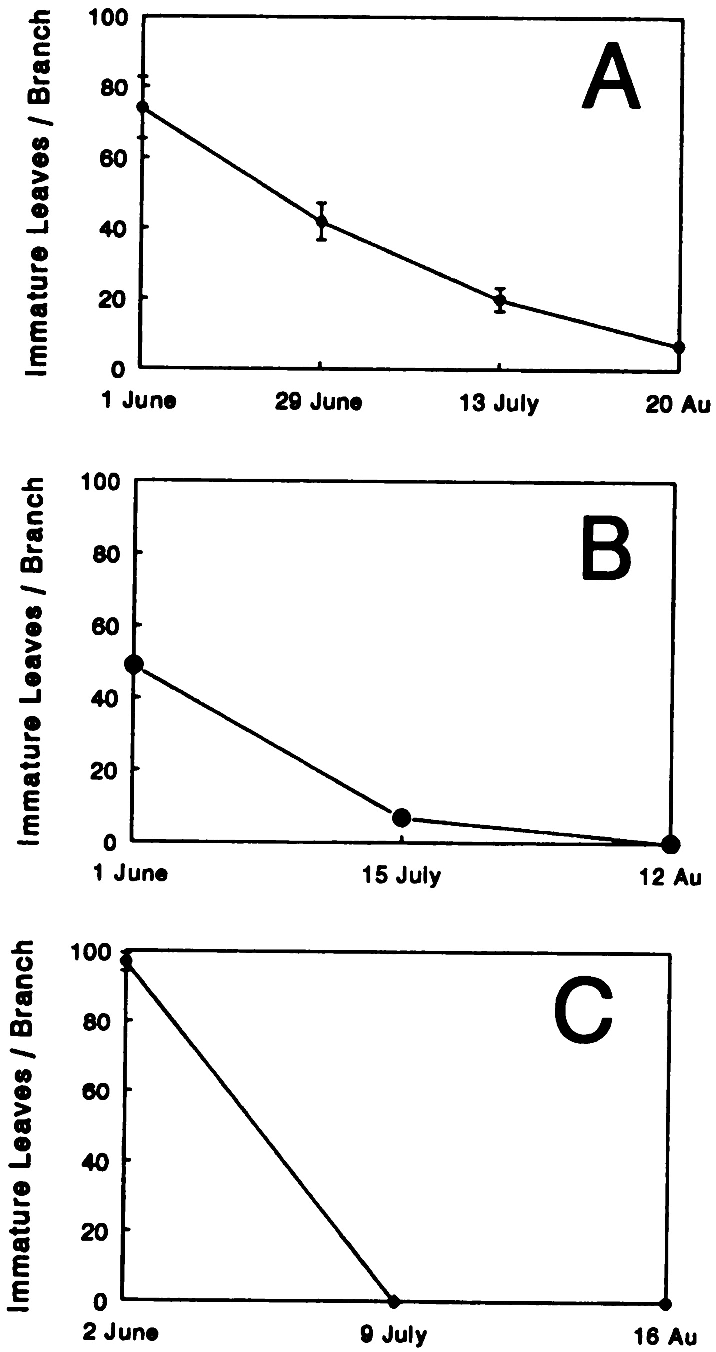

Figure 34: Seasonal production of immature leaves by European alder (A), paper birch (B), and hawthorn (C).

Intraspecific Variation in Voltinism Patterns Corresponds to Phenological Patterns of New Leaf Production

Alder Leafminer

The relative population size of each generation of adult alder leafminer did not correspond with leaf production in 1993 (Figure 35A). The second generation was only eight percent smaller than the first generation, even though the number of immature leaves available to the second generation had declined to 84 percent. The availability of leaves to the third generation was only five percent of that available to the first generation, corresponding with the size of the third generation of alder leafminer relative to that of the first (Figure 35A).

Birch leafminer

Changes in adult population size from one generation to the next was highly correlated with changes in immature leaf production. The second generation of adults was 76% smaller than the first generation, and the third was 98% smaller. Meanwhile, the number of immature leaves available to the second and third generations was 86% and 99% less than that available to the first generation, respectively (Figure 35B).

Hawthorn leafminer

After the first generation was completed no adults emerged for a second generation (univoltine) and no leaf production occurred (Figure 35C).

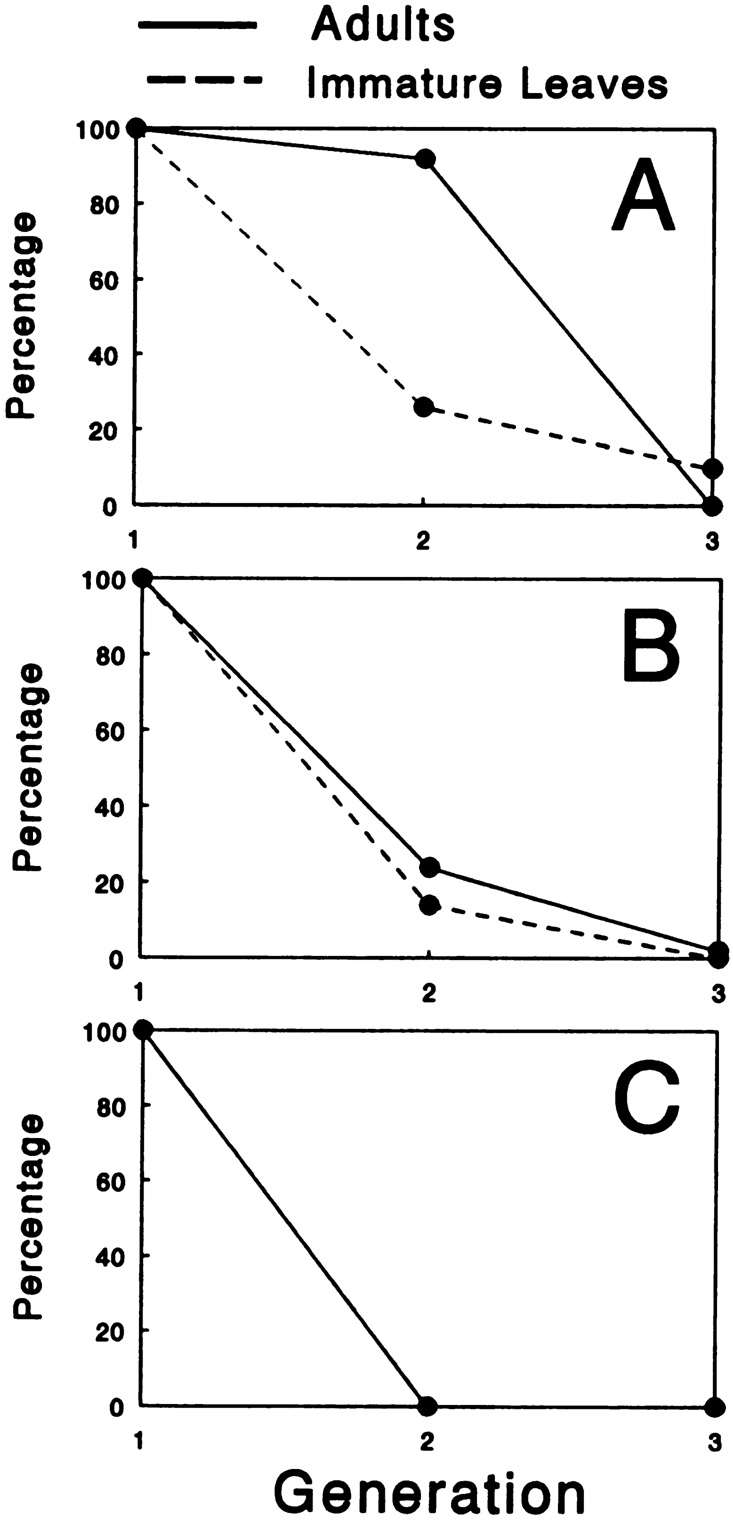

Figure 35: Relationship between the percent of leaves that are immature and the size of each generation of adult leafminers, relative to the first generation: alder (A), birch (B), and hawthorn (C) leafminer (1993).

Intraspecific Competition Occurs Among Larvae

Relationship Between Larval Density and Pupal Mass

Alder leafminer: During the first generation in 1993, pupal mass decreased as larval density increased ($Y=0.0041^{-0.46X}$; $r^2=-0.41$; $P<0.01$) (Figure 36A). This trend was also observed during the second generation ($Y=0.0047^{-0.64X}$; $r^2=-0.40$; $P<0.01$) (Figure 37A).

Birch leafminer: During the first generation, pupal mass decreased exponentially as larval density increased ($Y=0.0027^{-0.86X}$; $r^2=-0.22$; $P<0.01$) (Figure 36B). Pupal mass also decreased as larval density increased during the second generation ($Y=0.0035^{-0.57X}$; $r^2=-0.52$; $P<0.01$) (Figure 37B).

Hawthorn leafminer: There was not a significant relationship between larval density and pupal mass.

Relationship Between Larval Density and Survival, Adult Emergence, and Diapause

Alder leafminer: For first generation alder leafminer, percent of individuals per leaf surviving, emerging as adults, and diapausing decreased as larval density increased (Figure 38) ($Y=105.50e^{-2.45X}$; $r^2=-0.77$; $P<0.01$, $Y=94.19^{-2.33X}$; $r^2=-0.69$; $P<0.01$ and $Y=22.18^{-2.14X}$; $r^2=-0.88$; $P<0.01$, respectively). The percentage of individuals surviving, emerging as adults, and diapausing was 52, 49, and 2%, respectively.

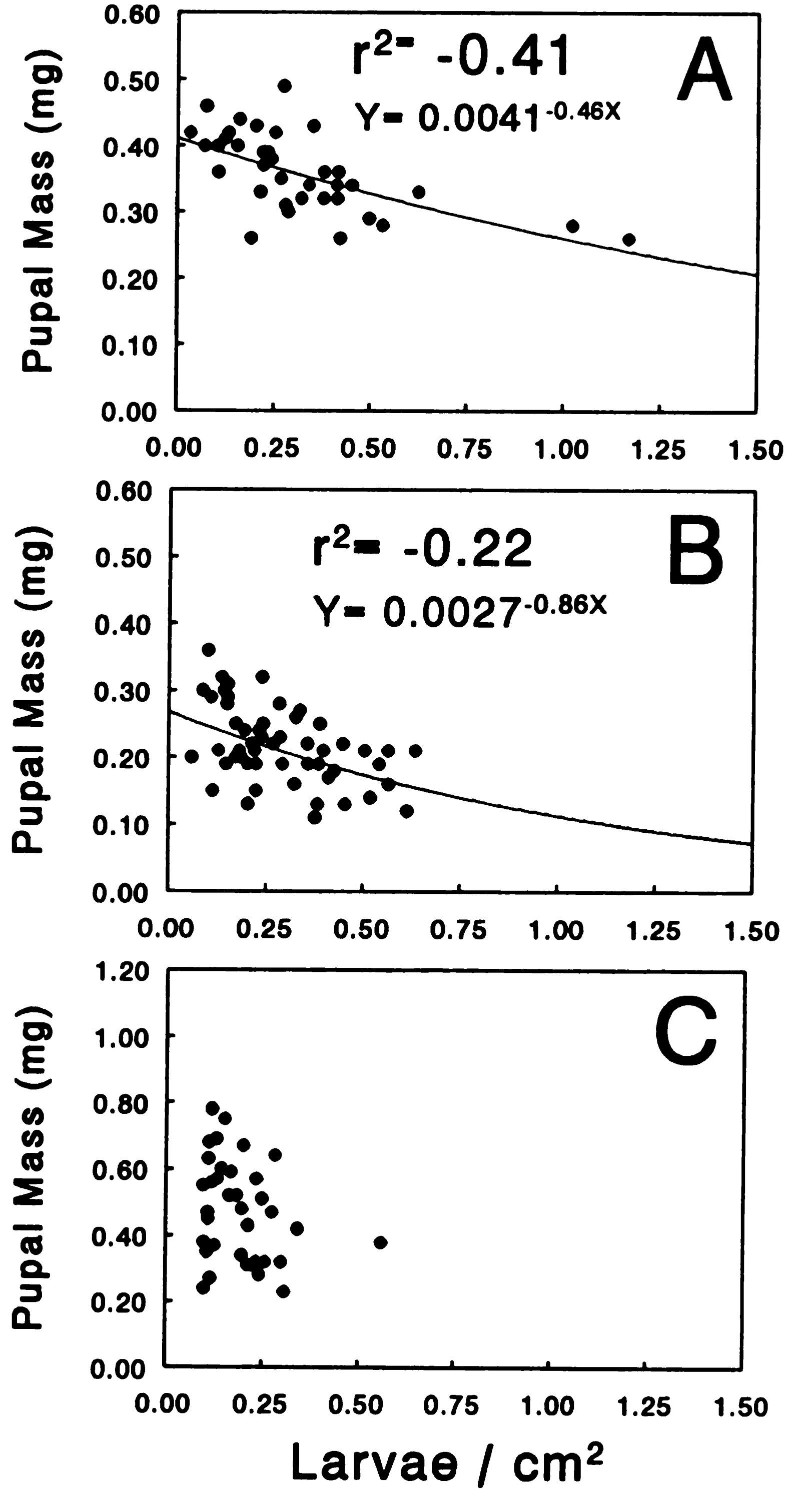

Figure 36: Relationship between first generation larval density and pupal mass in 1993: (A) alder leafminer, (B) birch leafminer, and (C) hawthorn leafminer.

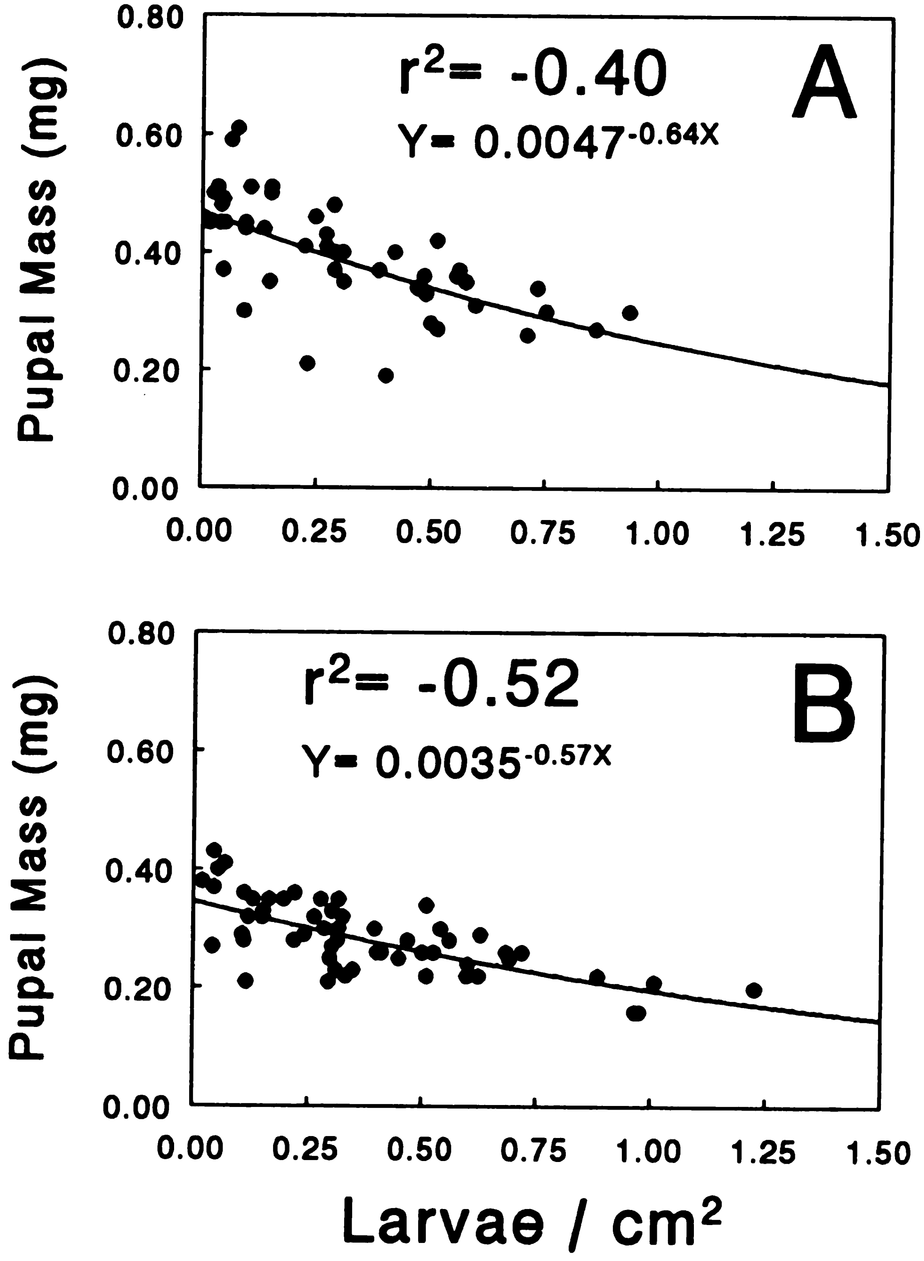

Figure 37: Relationship between second generation larval density and pupal mass in 1993: (A) alder leafminer and (B) birch leafminer.

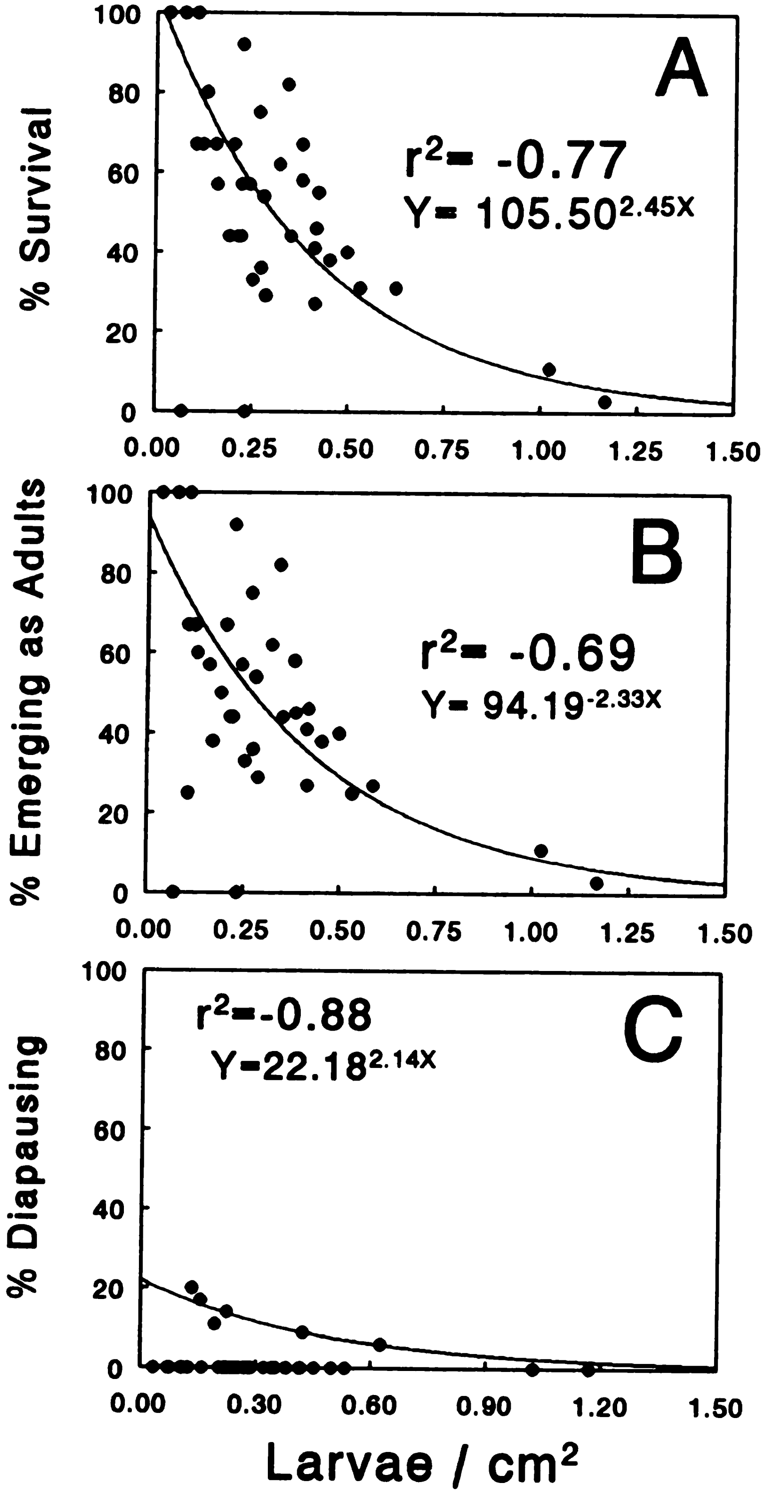

Figure 38: Relationship between first generation alder leafminer larval density and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

The same trend occurred in the second generation, with percent survival, percent emerging as adults, and percent diapausing decreasing as larval density increased (Figure 39) ($Y=93.40^{-2.09X}$; $r^2=-0.61$; $P<0.01$, $Y=96.96^{-2.53X}$; $r^2=-0.61$; $P<0.01$ and $Y=37.91^{-1.79X}$; $r^2=-0.50$; $P<0.01$, respectively).

Birch leafminer: During the first generation, there was no correlation between larval density and percent survival ($P>0.10$), percent emerging as adults ($P>0.10$), and percent of individuals diapausing ($P>0.10$) (Figure 40C). The percent surviving, emerging, and diapausing were 58, 39, and 19%, respectively.

During the second generation of birch leafminer, there was no correlation between percent survival and larval density ($n=56$; $P>0.10$) (Figure 41A). However, as larval density increased, percent emerging as adults and percent diapausing decreased ($Y=61.70^{-0.58X}$; $r^2=-0.14$; $P<0.05$ and $Y=26.63^{-0.94X}$; $r^2=-0.13$; $P<0.05$, respectively) (Figure 41B&C, respectively). As was the case for alder leafminer, the percentage surviving and emerging increased from the first generation to 67 and 51%, respectively. Meanwhile, the percent diapausing decreased slightly to 16%.

Relationship Between Pupal Mass and Survival, Adult Emergence, and Diapause

Alder leafminer: For first generation alder leafminer, percent of individuals per leaf surviving (individuals emerging as an adult + diapausing individuals), emerging as adults, and diapausing increased as pupal mass increased (Figure 42) ($Y=7.06^{529X}$; $r^2=0.23$; $P<0.01$, $Y=6.72^{518X}$; $r^2=0.22$; $P<0.05$ and $Y=2.72^{434X}$; $r^2=0.46$, respectively).

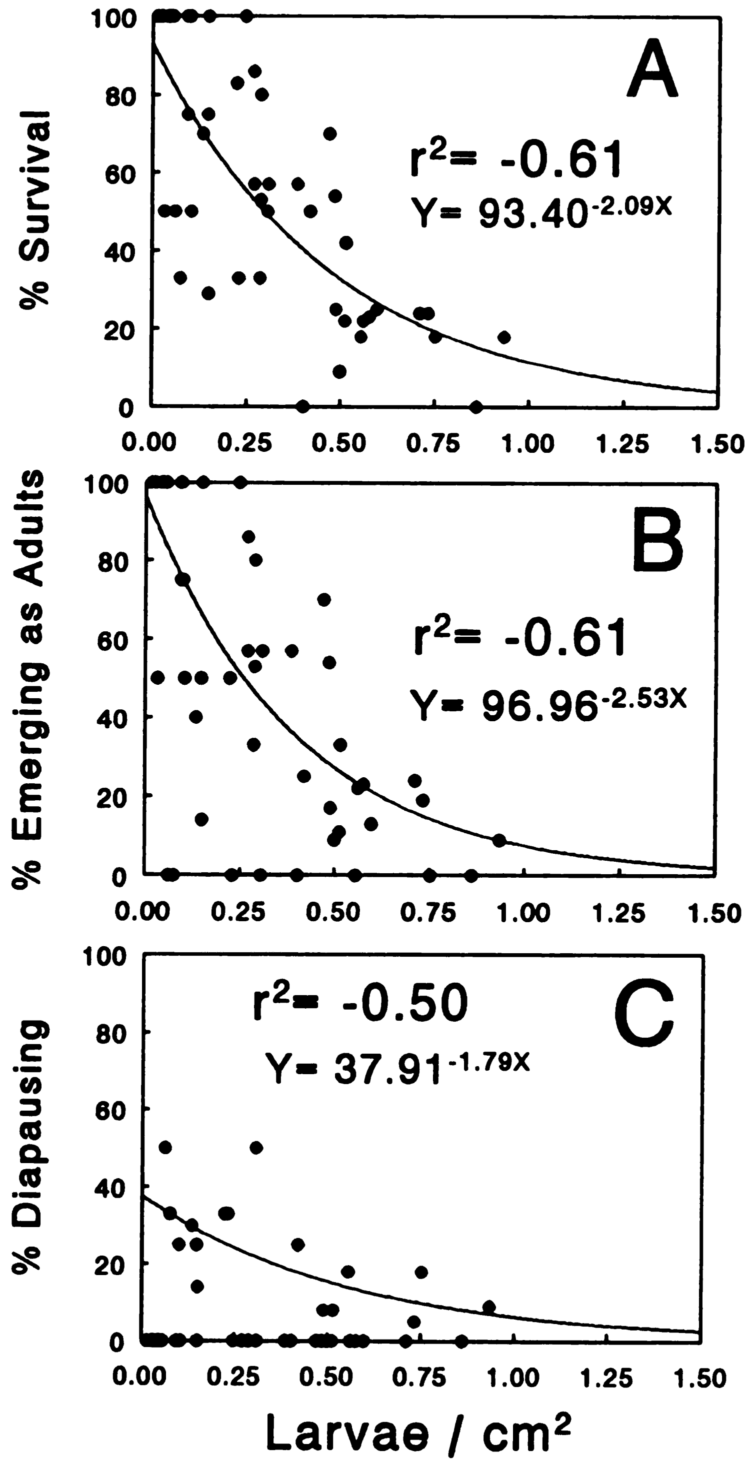

Figure 39: Relationship between second generation alder leafminer larval density and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

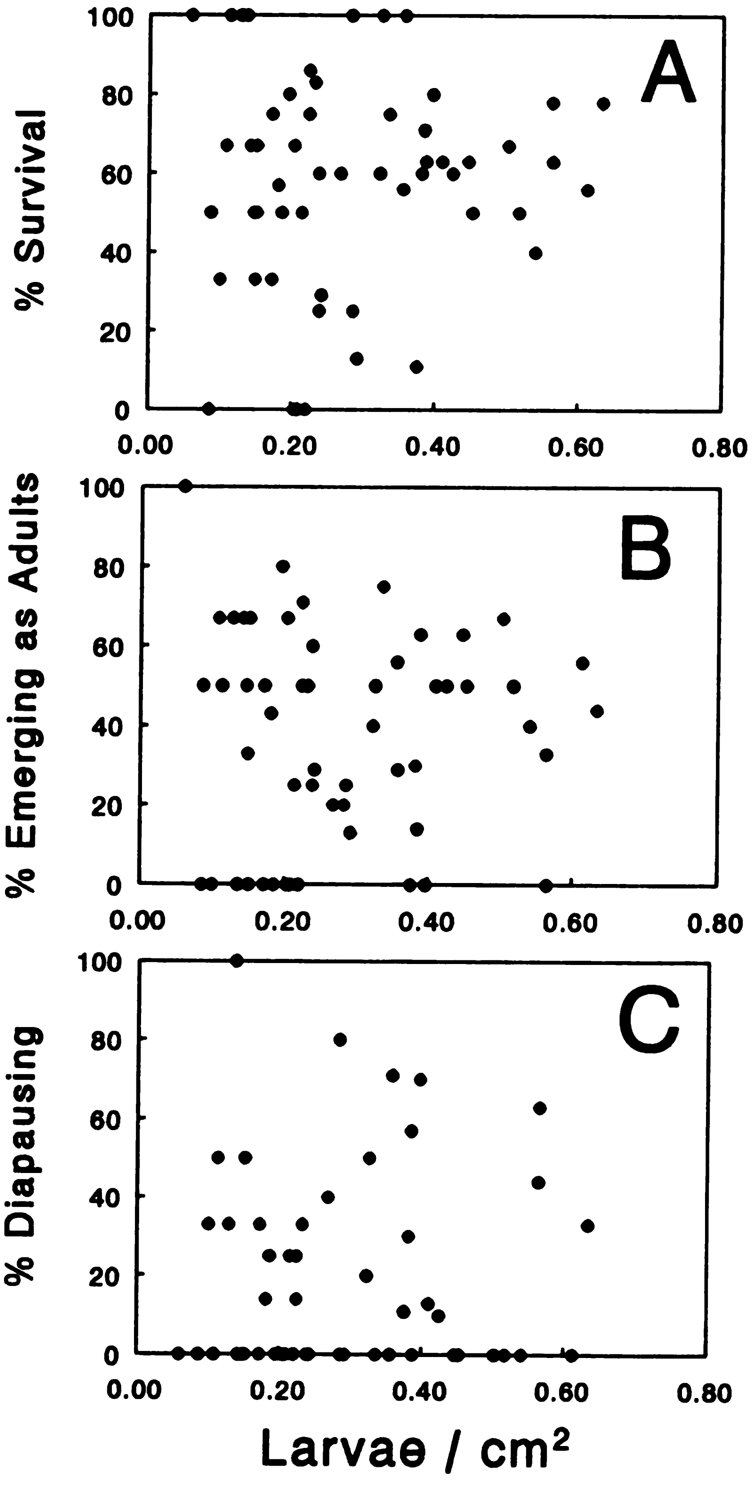

Figure 40: Relationship between first generation birch leafminer larval density and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

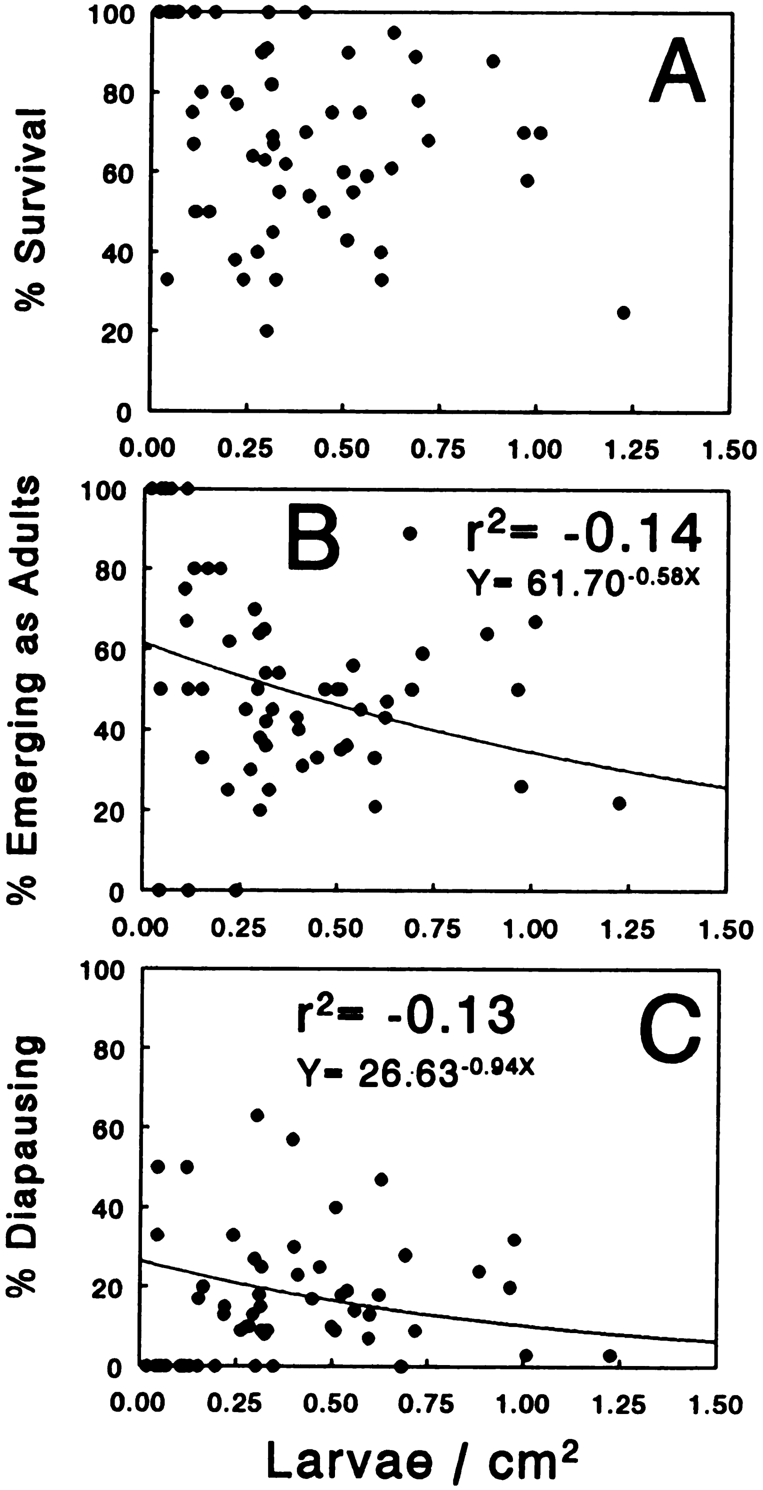

Figure 41: Relationship between second generation birch leafminer larval density and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

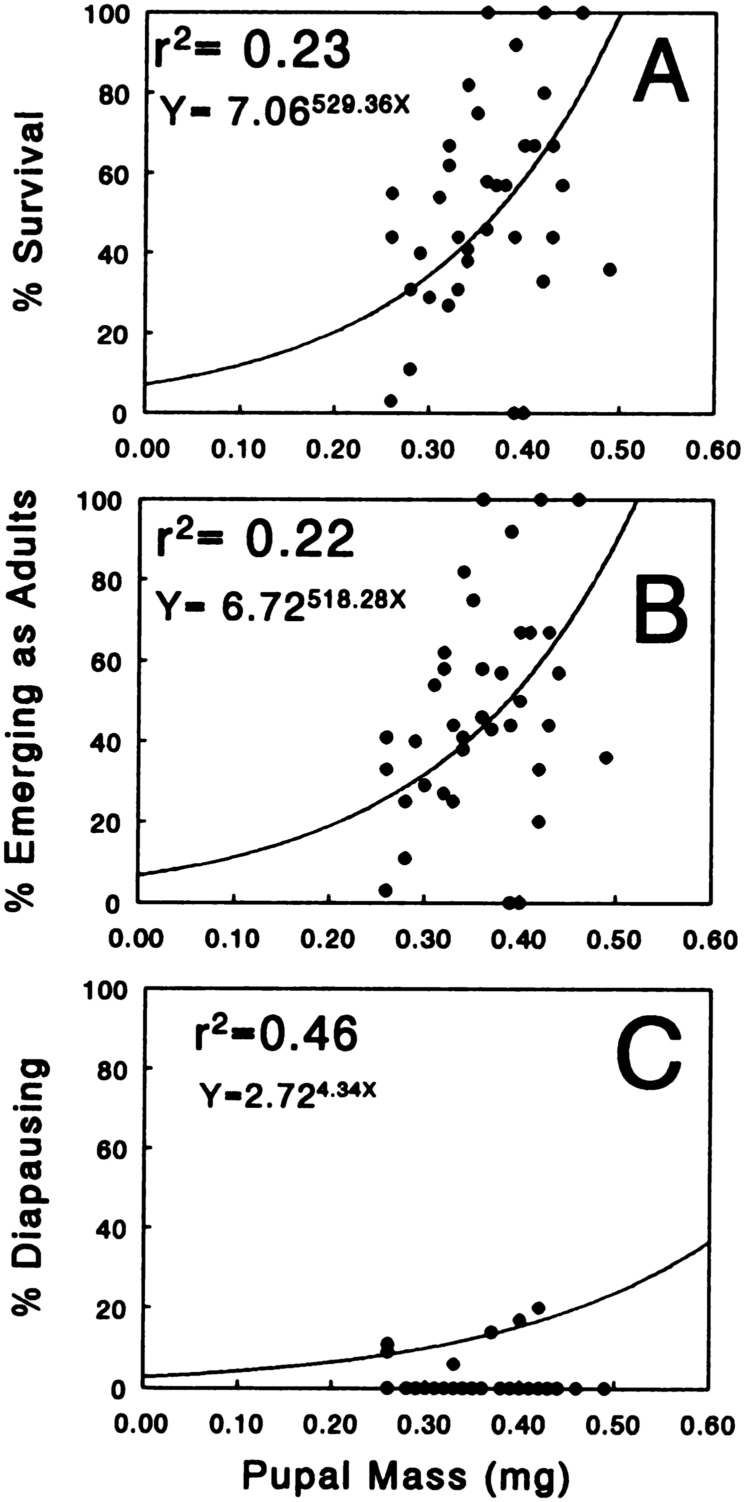

Figure 42: Relationship between first generation alder leafminer pupal mass and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

For second generation alder leafminer, the percent of individuals surviving, emerging as adults, or diapausing increased as pupal mass increased (Figure 43) ($Y = 13.98^{312X}$; $r^2 = 0.18$; $P < 0.05$, $Y = 6.25^{494X}$; $r^2 = 0.22$; $P < 0.01$ and $Y = 6.37^{288X}$; $r^2 = 0.22$; $P < 0.01$, respectively).

Birch leafminer: For first generation birch leafminer pupal mass was not related to the percent of individuals surviving ($P > 0.10$) (Figure 44A) nor the percent of individuals per leaf emerging as adults ($P > 0.10$) (Figure 44B). However, the percent of individuals diapausing per leaf was positively correlated with mean pupal mass (Figure 44C) ($Y = 11.78^{495X}$; $r^2 = 0.24$; $P < 0.01$).

As with the first generation, percent survival per leaf was not related to pupal mass ($n = 56$; $P > 0.10$) for the second generation of birch leafminer. However, the percent emerging as adults and percent diapausing increased as pupal mass increased ($Y = 22.54^{274X}$; $r^2 = 0.14$; $P < 0.05$ and $Y = 5.93^{388X}$; $r^2 = 0.10$; $P < 0.10$, respectively) (Figure 45).

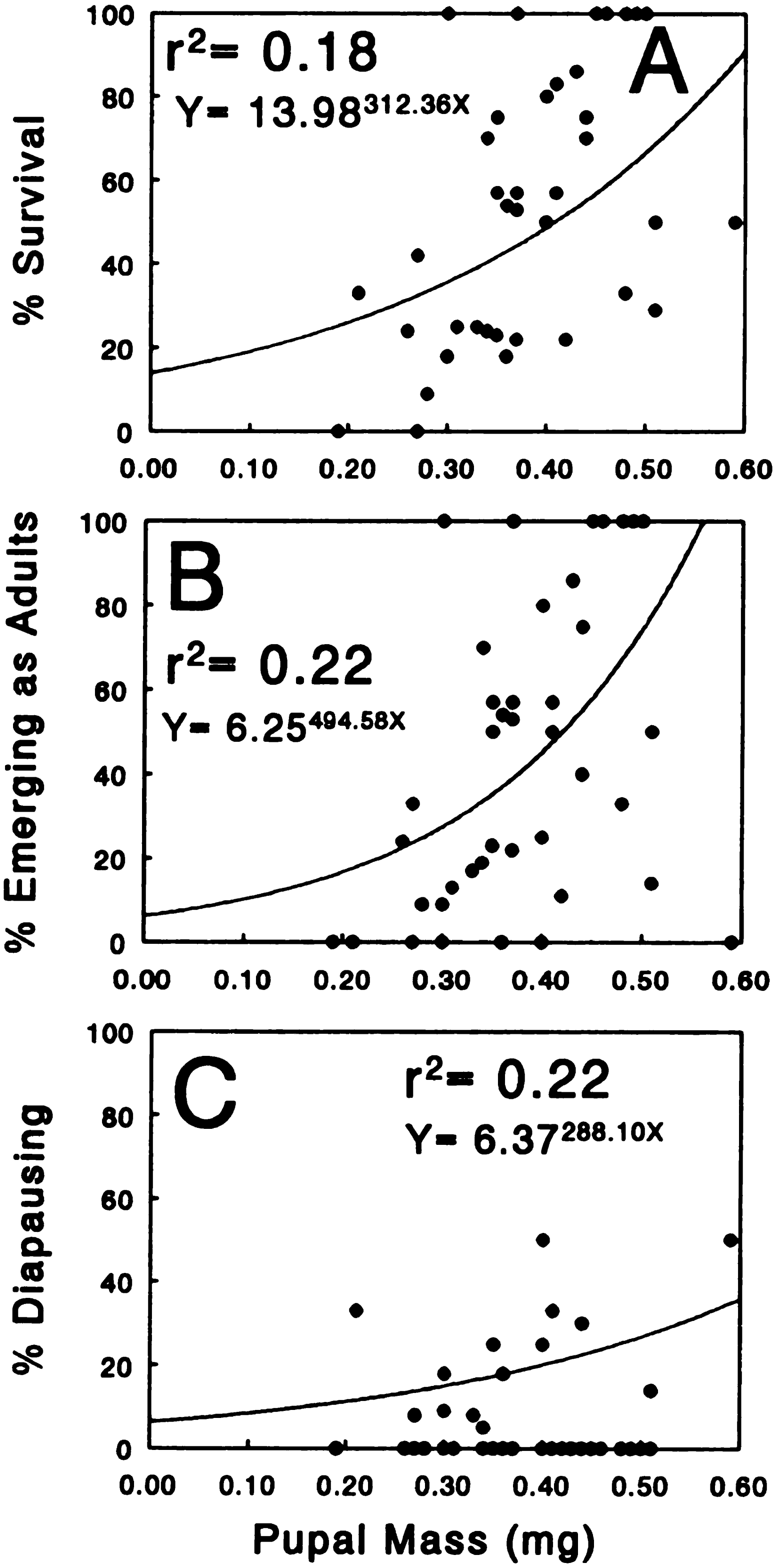

Figure 43: Relationship between second generation alder leafminer pupal mass and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

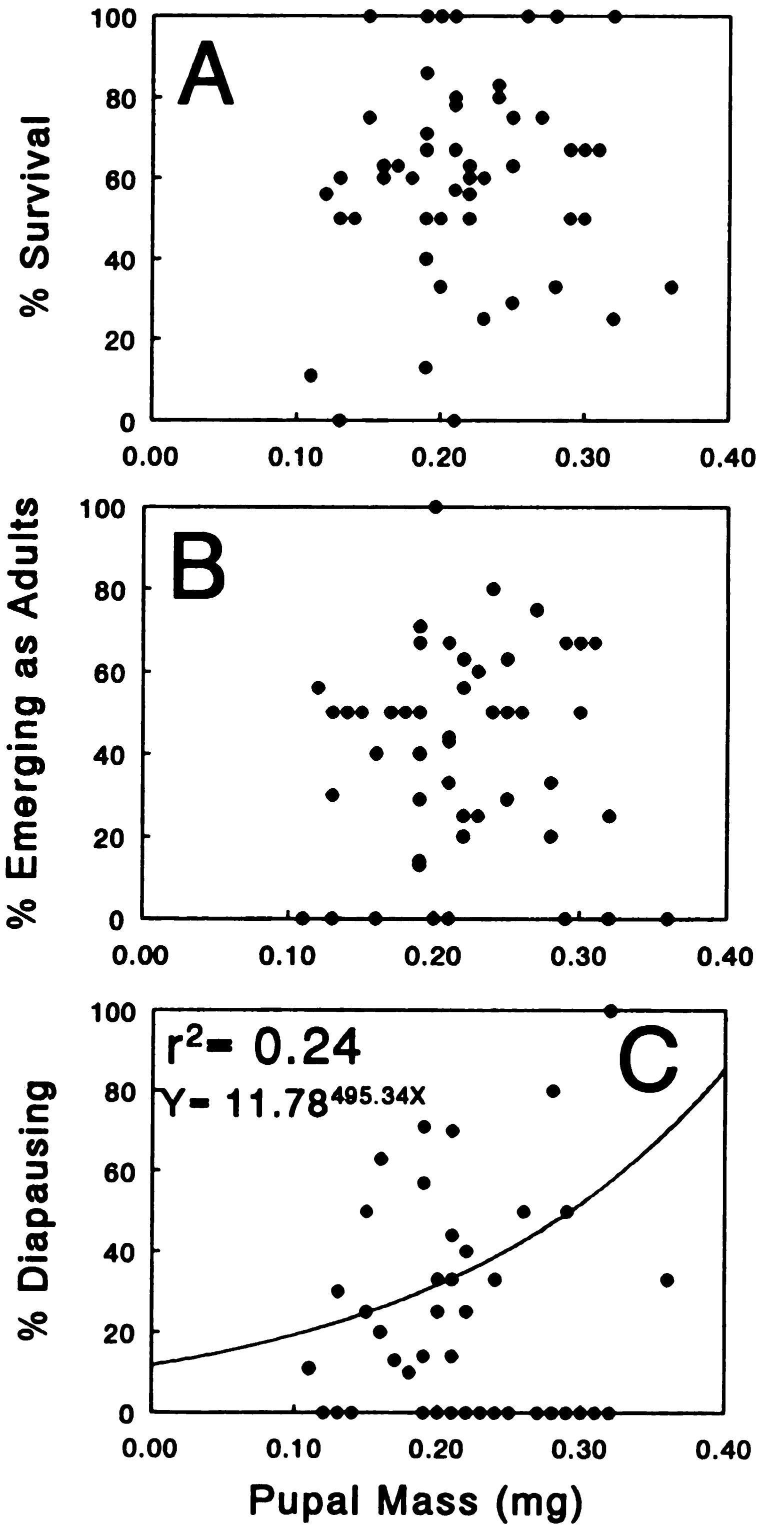

Figure 44: Relationship between first generation birch leafminer pupal mass and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

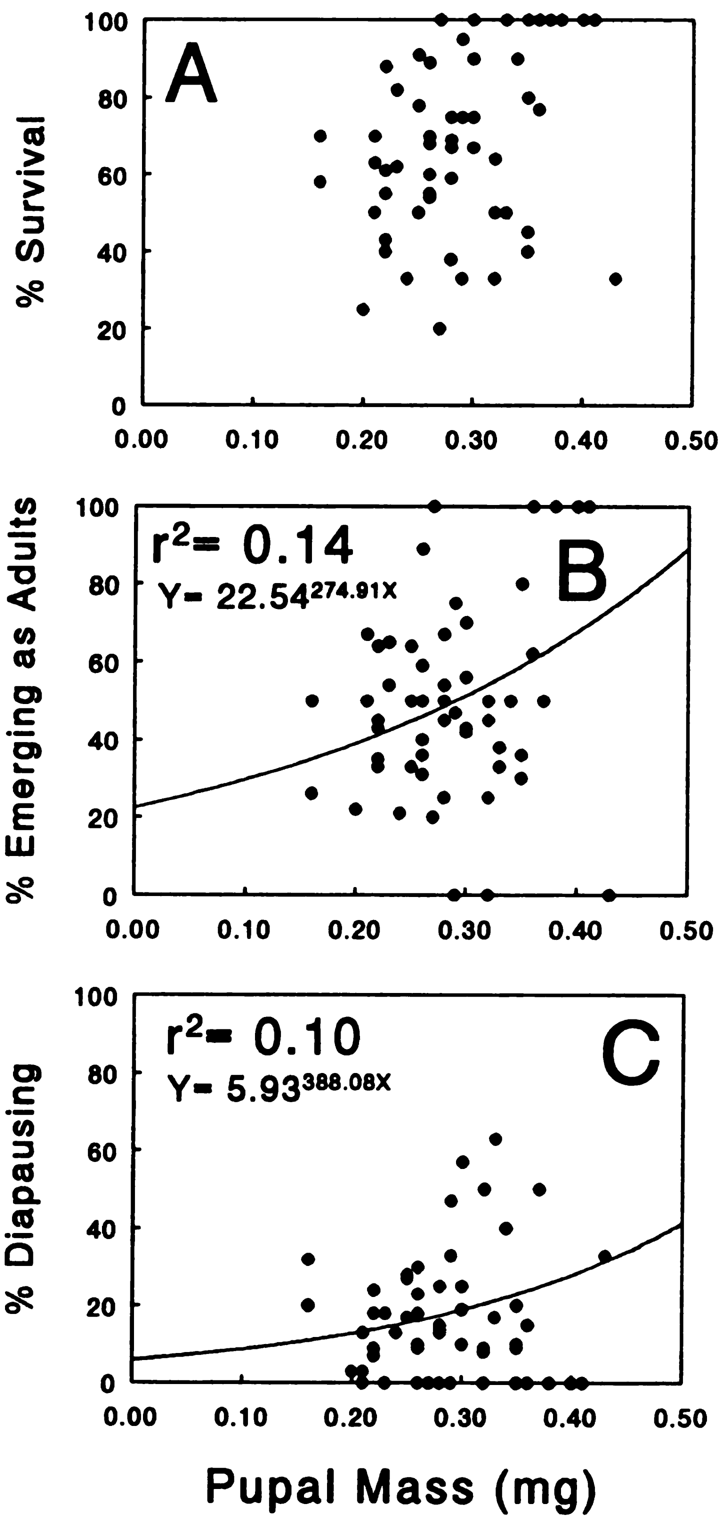

Figure 45: Relationship between second generation birch leafminer pupal mass and (A) percent of individuals surviving, (B) percent of individuals emerging as adults, and (C) percent of individuals diapausing (1993).

DISCUSSION

The results of these studies combined with previous results showing that larvae can develop only in immature foliage (Chapter 2), support the hypothesis that the indeterminate growth of birch and alder has permitted the evolution of multivoltinism in birch and alder leafminers, while the determinate growth pattern of hawthorn has constrained hawthorn leafminer to one generation. Thus, the data are consistent with the idea that the patterns of production of immature leaves by their host plants has directed the evolution of voltinism patterns in the three species and also selected for premature extra-long diapause in birch and alder leafminer.

Alder leafminers oviposit almost exclusively in immature leaves. Furthermore, survival of first and second instars is much greater in immature leaves (Chapter 2). Thus, immature leaves seem to be an obligate resource for alder leafminer. Alder trees continued to produce immature leaves through mid-August. However, the quantity of immature leaves available to each successive generation of alder leafminer decreased greatly as the season progressed, resulting in fewer leaves being available for ovipositing females and less food for larvae. However, sampling showed that the second generation alder leafminer adult population size only declined eight percent compared to the first generation, even though the availability of immature leaves declined dramatically. Therefore, the small change in the second generation adult population size, relative to the first, did not correspond to the dramatic decrease in the availability of immature leaves over the same period. However, the size of the third generation was greatly reduced relative to the first generation. This suggests that a large proportion of the second generation population entered premature extra-long diapause, corresponding with the large reduction in the availability of immature leaves. Laboratory experiments also confirmed the presence of extra-long diapause in first and second generation individuals.

The clumped distribution of alder leafminer larvae (Chapter 1), coupled with the fact that larvae are restricted for life to a single leaf, creates the potential for intraspecific competition for the available food resource. This seems to be especially true during the second generation when the availability of immature leaves is greatly limited, relative to the population size. In fact, as second generation alder leafminer larval density increased, survival and pupal mass decreased. The greatly reduced population size of third generation alder leafminer could be the result of selection for a bet-hedging strategy based on extra-long diapause. Competition among second generation larvae may have selected for density-dependent extra-long diapause (weak competition among first generation larvae would help explain the lack of premature extra-long diapause in first generation individuals). Individuals entering diapause would fore-go a breeding opportunity and avoid placing their offspring in a more competitive environment (relative to that experienced by first generation individuals). They would emerge the following spring as first generation adults, when immature leaves are once again most plentiful.

Alternatively, year-to-year variation in degree-day accumulation could select for density-independent extra-long diapause. Third generation larvae may have to "race" climatic conditions to complete development before leaf maturation and/or abscission occurred. Increased degree-day accumulation during a warm summer would favor individuals emerging since third generation larvae would have an increased probability of completing development (because third generation adults would emerge sooner and/or larvae would be able to complete development faster). Decreased degree-day accumulation in cooler summers would favor diapausing individuals. Individuals attempting a third generation would appear later in the summer (due to slower development of the first and second generation individuals), decreasing the probability of their offspring completing development before cessation of immature leaf production and leaf abscission. The result may be a bet-hedging diapause strategy in which most individuals diapause and some emerge as third generation adults. The relative proportion of each group in the population would be dependent upon the probability of their being sufficient time for their offspring to complete development before unfavorable conditions arrive.

Similar trends were observed for birch trees and birch leafminer. Birch leafminer first and second instar larval mortality increased as leaves matured and became tougher. Previous experiments revealed that females require immature leaves for oviposition (Chapter 2), indicating the importance of immature leaves for leafminer survival. Thus, there was a strong correspondence between the large reduction in immature leaf availability over the course of the season and the large reduction in the number of second and third generation birch leafminer adults, relative to the first generation. Trapping data revealed the size of the second generation adult population was only 24% of the size of the first generation. The rest presumably entered premature extra-long diapause or died. In addition, as leaf production continued to decline, an even smaller proportion (2%) of birch leafminer individuals, relative to the first generation, were trapped during the third generation. The reduction in population size is not the result of mortality because the large population size of the first generation is consistently preceded by a relatively small third generation of the previous year. These data and laboratory experiments confirmed the presence of premature extra-long diapause in this birch leafminer population.

As for alder leafminer, the distribution of birch leafminer larvae was clumped (Chapter 1), resulting in the potential for intraspecific competition among larvae for the available food resource. In accordance, during the second and third generations, when immature leaves are less available, survival and pupal mass decreased, as birch leafminer larval density increased.

These data are consistent with the hypothesis that the premature extra-long diapause is density-dependent, selected for by competition. In other words, the birch leafminer population hedged their collective bet that there would be a sufficient food resource (immature leaves) for their offspring, with the proportion of individuals of each generation entering premature extra-long diapause (thus by-passing a breeding opportunity) corresponding to the relative proportion of immature leaves available to the larvae of that generation.

Interestingly, individuals experiencing higher levels of competition (lower pupal mass) were less likely to enter premature extra-long diapause. Possibly, these individuals are less likely to survive a long diapause period due to relatively small amounts of stored resources. Therefore, these individuals may have no viable choice but to emerge as a second or third generation adults.

The greatly reduced population size of third generation birch leafminer could also be due to a density-independent bet-hedging diapause strategy in the population. Density-independent extra-long diapause could be selected for by year-to-year variation in degree-day accumulation, as discussed above for the alder leafminer.

In contrast to alder and birch, hawthorn displayed determinate growth with immature leaf production being terminated following first leaf flush. Consequently, no immature leaves were present later in the season that would be available to successive generations. This appears to be a fundamental constraint restricting hawthorn leafminer to a univoltine life history. First instar mortality approaches 100% in leaves tougher than 200 g, and leaves reach a toughness of 400 g as they mature. Thus, the dependency upon immature foliage for larval development may have restricted the hawthorn leafminer to a univoltine life cycle.

Compared to alder and birch leafminer, intraspecific competition was less significant among hawthorn leafminer larvae, with no relationship between larval density and pupal mass documented. This may have resulted because larval distribution was less aggregated, possibly due to the smaller leaves of hawthorn, which support one or two larvae versus five to ten commonly observed in birch and alder leaves (Chapter 1).

SUMMARY

Alder, birch, and hawthorn leafminer specialize on tree species within the genera *Alnus*, *Betula*, and *Crataegus*, respectively; and all three species oviposit almost exclusively in immature leaves. Alder and birch trees display a indeterminate growth pattern while hawthorn displays a determinate growth pattern. Alder and birch leafminer are multivoltine with second and third generation adults emerging in early July and mid-August, respectively (DeClerk & Shorthouse 1985; Hart et al. 1991). The hawthorn leafminer is univoltine (Wilson et al. 1992). The population size of first and second generation of alder leafminer is large relative to the third generation, and the size of the first generation of birch leafminer is large relative to its second and third generation, consistent with the presence of premature extra-long diapause in a few first, and many second generation alder leafminer individuals and in most first generation birch leafminer individuals.

The primary objective of this research was to test the theoretical hypothesis that the evolution of the voltinism patterns of alder, birch, and hawthorn leafminer have been directed by the phenological pattern of immature leaf production by their respective host plants. Specifically, the hypothesis states that the determinate growth pattern of hawthorn has constrained the hawthorn leafminer to a univoltine life history, while the indeterminate growth pattern of alder and birch has allowed the evolution of a multivoltine life history by alder and birch leafminer. Furthermore, the hypothesis was tested that the reduced production of immature leaves after bud-break by alder and birch has resulted in a bet-hedging diapause strategy in alder and birch leafminer. This strategy takes the form of density-dependent premature extra-long diapause (*sensu* Hanski 1988) within the population of first and second generation pupae selected for by intraspecific competition for the immature leaf food resource. Thus, individuals emerging as third generation adults take advantage of a breeding opportunity even though they risk placing their offspring in a highly competitive environment.

The following experimental objectives were used to test this theoretical hypothesis: (1) to characterize the seasonal presence and abundance of adults; (2) to characterize the spatial distribution of larvae within and among leaves; (3) to compare physical and chemical traits of colonized and non-colonized leaves; (4) to test the hypothesis that immature leaves are the only suitable resource for larval development and survival by: (a) choice and no-choice experiments comparing oviposition preference for immature relative to mature foliage, (b) comparing chemical and physical differences of immature and mature leaves, and (c) quantifying larval survival in relation to leaf maturity; (5) to quantify seasonal variation in the presence of immature leaves; (6) to test for intraspecific competition among larvae for their immature leaf resource; and (7) to test for correspondence between seasonal variation in the abundance of the three leafminer species and seasonal variation in the production of immature leaves by their respective host plants.

In 1992 and 1993, first generation alder leafminer adult emergence began in early May with peak emergence in mid-May. In 1992 and 1993, distinct generations were difficult to distinguish, but at least two population peaks were observed with the July peak being larger than the May peak. In 1992, no adults were observed after mid-September and in 1993 adults were not observed after late August. During both years, a large first generation of birch leafminer was followed by much smaller second and third generations. Peak emergence of the second and third generations were observed in mid-July and early August, respectively. A single generation of hawthorn leafminer was observed during both years, with peak adult emergence in mid-May.

In 1993, larval abundance and distribution were quantified for each species by counting the number of larvae in sampled leaves. For each generation of each species, larval distribution was found to be non-random as the hypothesis of a Poisson distribution was rejected. Larval distribution also did not fit the negative binomial distribution. The variance-to-mean ratio, which was always greater than one and increased at a faster rate than the mean number of larvae per leaf, as well as the *d*-statistic, supported strongly a highly clumped larval distribution for each species (however, Green's index always suggested that larval distributions were more randomly distributed). None of the measured physical and chemical traits of infested and uninfested leaves (toughness, size, specific mass, nitrogen, and phosphorous) explained the clumped pattern of larvae which resulted from oviposition site selection. Furthermore, for all three species, there was also no relationship between the number of eggs laid per leaf and leaf toughness and size. Neither was there a correlation between the number of eggs per leaf and specific leaf mass for birch and hawthorn leafminers. But, there was a positive correlation between the number of alder leafminer eggs per leaf and specific leaf mass.

Both choice and no-choice oviposition preference experiments did show that leaf age influences oviposition site selection of second and third generation alder and birch leafminer, both of which oviposited almost exclusively in immature leaves.

Leaf maturation also had a negative effect on larval survival. For all three species, first instar mortality increased as leaf toughness increased. Mortality reached 80 to 90% when leaves exceeded 200-250 g. Leaf toughness had little influence on fourth and fifth instar larvae.

As expected, mature leaves of each species were tougher, larger, and higher in specific mass than immature leaves. In addition, mature leaves were lower in percent nitrogen and phosphorous (birch leaves were equal) than immature leaves.

Immature leaves were present on hawthorn only during the single generation of hawthorn leafminer. No leaf production occurred after the initial flush at bud break. The production of immature leaves by alder and birch trees decreased over the course of the season. Therefore, the availability of immature leaves for ovipositing adults also decreased with each generation.

To determine if intraspecific variation in voltinism patterns corresponded to phenological patterns of new leaf production, the number of adults emerging each generation was compared to the relative availability of leaves to each generation. In 1993, adult alder leafminer population size did not correspond with the availability of immature leaves. The second generation was slightly smaller than the first generation, even though the number of immature leaves available to the second generation greatly decreased. However, the greatly reduced third generation was proportional to the greatly reduced availability of immature leaves. Sampling and laboratory experiments suggested that most second generation larvae entered premature extra-long diapause with a few individuals emerging as third generation adults, an observation consistent with the idea that a bet-hedging diapause strategy was utilized by the second generation.

Conversely, changes in birch leafminer adult population size from one generation to the next were highly correlated with changes in immature leaf production. Most first generation birch leafminer larvae entered premature extra-long diapause with only a few individuals emerging as second generation adults, and even fewer for a third generation. This is consistent with the hypothesis that the first generation utilizing a bet-hedging diapause strategy.

For hawthorn leafminer, after the first generation was completed, no adults emerged for a second generation (univoltine) and no leaf production occurred. These results, coupled with high larval mortality in mature leaves, are consistent with the hypothesis that hawthorn leafminer is constrained to a univoltine life history by the determinate growth pattern of its host.

There was strong evidence of intra-specific competition among alder and birch leafminer larvae, but not among hawthorn leafminer larvae. For both first and second generation alder and birch leafminer, pupal mass decreased as larval density increased. Survival decreased as larval density increased for both generations alder leafminer, but not for birch leafminer. For second generation birch leafminer, as larval density and thus competition increased, the percent emerging as adults and the percent diapausing decreased due to increased mortality.

In summary, the results support the hypothesis that larvae of all three species can develop only in immature foliage, therefore the indeterminate growth of birch and alder permitted the evolution of multivoltinism in alder and birch leafminers, while the determinate growth pattern of hawthorn has constrained hawthorn leafminer to one generation. Premature extra-long diapause of birch and alder leafminers may be a bet-hedging strategy; diapausing individuals by-pass a breeding opportunity, but avoid competition for the decreased supply of their obligate food resource (immature leaves). Thus, these data support the theoretical hypothesis that host plant phenology has influenced the voltinism patterns of alder, birch, and hawthorn leafminers, and resulted in bet-hedging diapause strategies in alder and birch leafminers.

APPENDIX

APPENDIX 1

Record of Deposition of Voucher Specimens*

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

Voucher No.: 1994-2

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

Influence of Host Phenology on Voltinism Patterns of Alder, Birch and Hawthorn Leafminer (Hymenoptera: Tenthredinidae), with Evidence for Bet-Hedging Strategies in Birch and Alder Leafminer.

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

Entomology Museum, Michigan State University (MSU)

Other Museums: U.S. National Collection
Systematic Entomology Laboratory, USDA

Investigator's Name (s) (typed)
Joseph Lee chichester
Daniel A. Herms

Date 9-28-94

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

Deposit as follows:

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

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

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Fenusa pusilla Lepeletier	The Dow Gardens Midland Mid. Co., MI 12 May 1993					10	10		MSU
Fenusa dohrnii Tischbein	The Dow Gardens Midland Mid. Co., MI 12 May 1993					10			MSU
Profenusa canadensis Marlett	H.H. Dow estate Midland Mid. Co., MI 12 May 1993					10	10		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Joseph L. Chichester

daniel A. Herms

Date 9-28-94

Voucher No. 1994-2

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

Fred Stehr by M.C. Nielsen 9/30/94

Curator Date

LIST OF REFERENCES

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