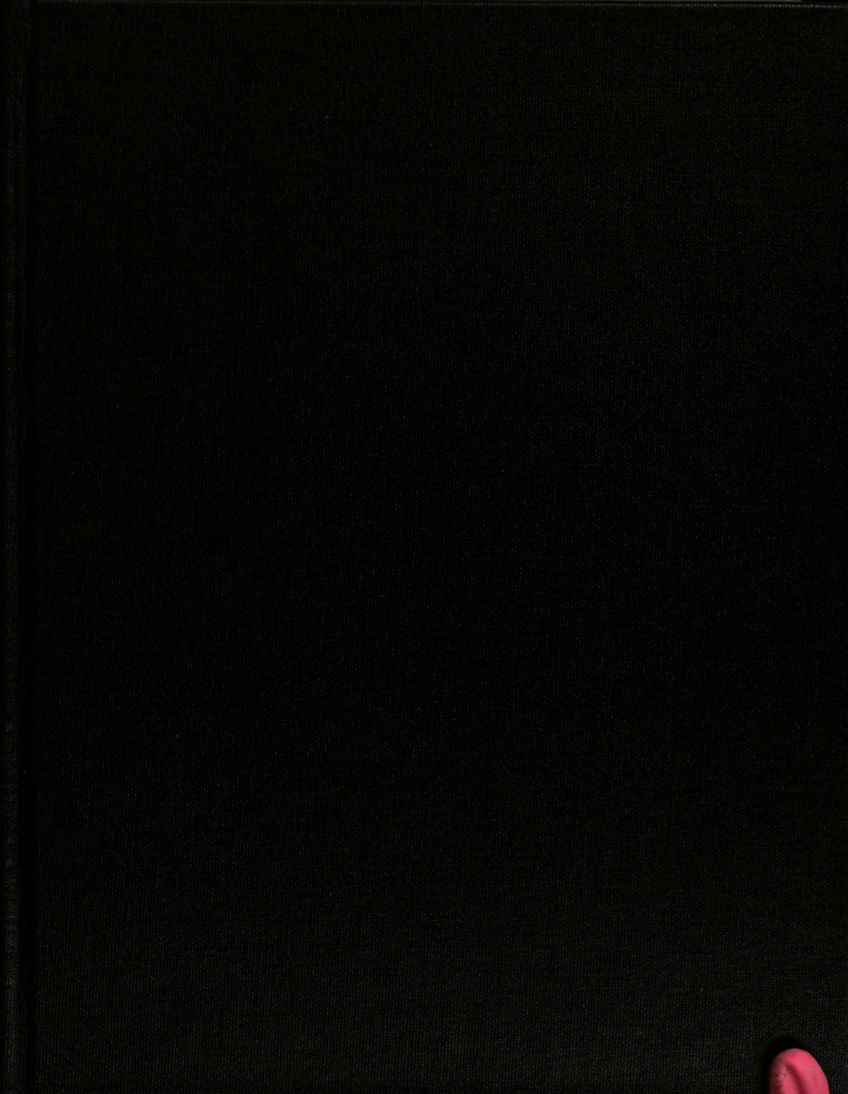




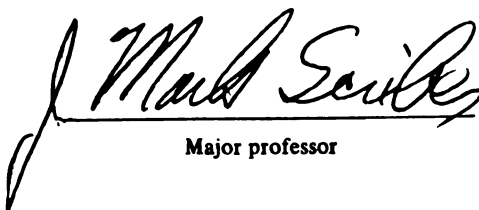


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LIGHT, NUTRIENT AVAILABILITY, AND DEFOLIATION EFFECTS
ON RESOURCE ACQUISITION, RESOURCE ALLOCATION,
AND HERBIVORE RESISTANCE OF PAPER BIRCH
AND SUGAR MAPLE
presented by

Heather L. Govenor

has been accepted towards fulfillment
of the requirements for

M.S. degree in Entomology


Major professor

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LIGHT, NUTRIENT AVAILABILITY, AND DEFOLIATION EFFECTS ON
RESOURCE ACQUISITION, RESOURCE ALLOCATION, AND HERBIVORE
RESISTANCE OF PAPER BIRCH AND SUGAR MAPLE

By

Heather L. Govenor

A THESIS

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ABSTRACT

LIGHT, NUTRIENT AVAILABILITY, AND DEFOLIATION EFFECTS ON RESOURCE ACQUISITION, RESOURCE ALLOCATION, AND HERBIVORE RESISTANCE OF PAPER BIRCH AND SUGAR MAPLE

By

Heather L. Govenor

This research examined light, nutrient, and defoliation effects on resource acquisition, resource allocation, and herbivore resistance in juvenile paper birch and sugar maple. Tree physiological responses were consistent with theories of life history and phenotypic plasticity. Fertilization did not consistently affect resource acquisition or allocation of either tree species, but shade did. Treatment effects on host quality were dependent on insect species. Effects of defoliation on birch physiology mimicked nutrient deficiency. Defoliation of shaded birch elicited rapid induced resistance to lepidopteran larvae. There was no evidence of delayed induced resistance to any herbivore species the year following defoliation.

*This work is dedicated to Grace Agnes Bickel,
the most loving and giving woman I have ever known,*

and

*to the memory of Heather Clair Miller.
All who knew her are better for it.*

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CHAPTER 1:

INTRODUCTION

Forest ecosystems vary in light and nutrient availability across a range of spatial and temporal scales. This variability is driven by both natural and anthropogenic perturbations which alter availability of resources to forest trees. For example, light conditions are altered as a result of gap formation from tree fall, canopy closure over the course of succession, logging, and road construction through forested areas. Global levels of available light may decrease with increased cloud cover as atmospheric carbon dioxide levels increase (Ayres 1993). Nutrient levels in forest soils vary seasonally with shifting input and output of nutrients related to leaf-fall, leaching, and erosion. Tree fall releases nutrients on a local level, while atmospheric nitrogen deposition resulting from industrialization is increasing nutrient levels in soils on a global scale (Vitousek et al. 1997).

Environmental factors can have large impacts on plant physiology, influencing both resource acquisition and resource allocation. A reduction in available light decreases photosynthetic assimilation, thereby limiting carbon available for growth, primary and secondary metabolism, and storage. Plants may acclimate to reduced light by reallocating dry matter, and by changing leaf morphology, leaf chlorophyll, and protein content, decreasing respiration, decreasing enzyme activity, decreasing electron transport activity, altering whole plant architecture (Holt 1995), and increasing,

decreasing, or maintaining photosynthetic rates (Turnbull 1991, Givnish 1988, Holt 1995, Mulkey et al. 1993).

Nitrogen deficiencies can result in reduced levels of foliar nitrogen (Floater 1997), in turn reducing photosynthetic rates (Reich et al. 1991). Fertilization can lead to increased plant growth (Linder and Rook 1984, McDonald et al. 1986, Bryant et al. 1987, Muzika 1993), and increased allocation to above ground biomass production. Stored starch (Wargo et al. 1972), leaf morphology (Linder and Rook 1984), and levels of defensive compounds (Bryant et al. 1992) also are responsive to nutrient availability.

In addition to abiotic stressors such as low levels of light and nutrients, trees respond to biotic stressors such as herbivory. Plant susceptibility to herbivores is influenced by the abiotic environment. Loss of leaf area resulting from herbivory may result in increased photosynthetic rates in remaining foliage (Ovaska et al. 1992, Lovett and Tobiessen 1993), and decreased (Schaffer and Mason 1990, Fox and Morrow 1992), or in some cases compensatory growth (Houle and Simard 1996). The location and severity of herbivory can influence patterns of nitrogen distribution in the plant canopy (Stockhoff 1994). Foliar defenses may increase or decrease following herbivory (Haukioja et al. 1985, Faeth 1992, Bryant et al. 1992) with responses occurring in the season of damage (Haukioja 1980) or in subsequent years (Parker and Houston 1971, Haukioja 1990, Tuomi et al. 1990).

Physiological responses of forest trees to changes in their environments in turn can affect the ability of these trees to defend their foliage against insect herbivores. In most cases, insect responses have been found to correspond to associated changes in foliar toughness, water content, nutrient content, or levels of defensive compounds.

Because different insect species respond differently to particular defensive compounds, and may differ in nutritive requirements, it is often inaccurate to make statements in regards to plant defenses without examining specific responses of herbivores.

Shade stress has been associated with both increases (Bryant et al. 1987, Bultzman and Faeth 1988, Nichols-Orians 1991, Dale and Causton 1992, Lowman 1992, Shure and Wilson 1993, Johnson et al. 1997) and decreases (Coley 1983, Lincoln and Mooney 1984, Bultzman and Faeth 1988, Bassett 1991, Koricheva et al. 1998) in herbivory and/ or herbivore densities. Nutrient availability also has variable effects on herbivores. Fertilization has generally corresponded to increased performance of individual herbivores, but can have negligible or negative effects on herbivores at the population level (Kytö et al. 1996). Defoliation-induced changes in host foliage may increase or occasionally decrease the host's resistance to the defoliating herbivore (Tuomi et al. 1988, Karban and Baldwin 1997) or co-occurring species in the same feeding guild (Denno et al. 1985).

As indicated above, studies on single environmental variables, such as light, nutrient availability, and herbivory have given insight into the independent effect of these variants on plant physiology. However, it is rare that in natural settings plants will be facing only one of these stresses. With this in mind, recent research has focused on plant responses to multiple stresses (Chapin et al. 1987, Chapin 1991). The number of studies on interactions among multiple stresses remains relatively low, but is growing as evidence builds that there is an interactive aspect of plant response to environmental stress.

When examining stress effects on trees, it is important to keep in mind the life history strategy of the tree species of interest. Different plant species have evolved different strategies for dealing with environmental perturbations, and other selective forces in their habitats (e.g. competition and herbivory). Species adapted to low resource environments are often slower growing, more highly defended, and less responsive to environmental perturbation than species adapted to high resource environments (Coley 1983, Bryant et al. 1983, Bryant et al. 1985, Coley et al. 1985).

Although at present no one theory sufficiently explains or predicts plant responses to environmental stresses the carbon/ nutrient balance hypothesis (Bryant et al. 1983, Tuomi et al. 1984) and the growth/ differentiation balance hypothesis (Loomis 1932, Lorio 1986, Herms and Mattson 1992) are closely related theories that have been useful in conceptualizing potential mechanisms of plant response to their environments. Both theories address resource limitations that restrict and guide patterns of resource allocation among life processes, especially between growth and defense.

The objectives of this thesis were two fold. First, I sought to determine the simultaneous effects of light, nutrient availability, and natural defoliation on resource acquisition and allocation patterns of sugar maple (*Acer saccharum* Marsh.) and paper birch (*Betula papyrifera* Marsh.). These objectives are addressed in Chapter 2. Second, I sought to determine corresponding effects of treatments on the lepidopteran defoliators gypsy moth (*Lymantria dispar* (L.) Lymantriidae), forest tent caterpillar (*Malacosoma disstria* Hubner, Lasiocampidae), and whitemarked tussock moth (*Orgyia leucostigma* (J.

Smith), Lymantriidae) as mediated through effects on the quality of host foliage. These objectives are addressed in Chapter 3.

Light treatments were designed to mimic roughly successional canopy closure, with the first three years since planting spent in high light environments followed by two years of shade which simulated overstory closure. In the final year of the experiment, the shade was eliminated and once again all trees were exposed to full sun, mimicing gap formation.

In contrast to most studies which have used artificial defoliation treatments, forest tent caterpillar larvae were used to naturally defoliate randomly selected trees.

Defoliation effects on foliar traits were measured both the season of defoliation and the subsequent season so that any delayed effects could be detected. Carbon acquisition was measured as net photosynthetic rate, while nitrogen assimilation was indirectly reflected by foliage nitrogen content. Treatment effects on resource allocation were measured as changes in plant growth, specific leaf mass, and foliar concentrations of total phenolics and condensed tannins. Treatment effects on herbivore resistance were determined by quantifying larval survival, development time, relative growth rates and nutritional indices, as well as pupal mass.

Paper birch and sugar maple trees have contrasting life history strategies, with birch being a fast-growing, shade intolerant, early-successional species and sugar maple being a slow-growing, shade tolerant, climax species. To my knowledge, this is the first study to examine simultaneous responses of both a pioneer and a climax species to simultaneous shade, nutrient enrichment, and defoliation. An improved understanding of the interrelated nature of plant responses to stress, coupled with an improved

understanding of the mechanistic links between environmental factors and insect performance, is essential for developing accurate predictions of the effects of natural and human disturbance on forested ecosystems, and on the herbivores residing within these ecosystems. Understanding how tree stress in turn affects populations of the outbreak defoliators gypsy moth and forest tent caterpillar is especially important, as these species are the most important defoliators of deciduous forest trees in North America (Mattson et al. 1991). Defoliation effects on host quality can illuminate how outbreaks of one species may indirectly regulate the population dynamics of another through host-mediated interspecific competition. A mechanistic understanding of environmental effects on tree physiology and herbivore resistance is essential for understanding the dynamics of forest ecosystems, and for their effective stewardship.

CHAPTER 2:
EFFECTS OF LIGHT, NUTRIENT AVAILABILITY AND DEFOLIATION ON
RESOURCE ACQUISITION AND ALLOCATION IN PAPER BIRCH
(*Betula papyrifera*) AND SUGAR MAPLE (*Acer saccharum*)

INTRODUCTION

Forest ecosystems are mosaics of light, nutrient, and water resources. The distribution of tree species is determined in part by their differential responses to given resource conditions. The resources available to an individual tree can change dramatically over its lifetime as stochastic events alter the environment. Gap formation after tree-fall can increase the availability of both light and nutrients. Temporal and spatial distributions of resources also shift as community succession progresses. Canopy closure reduces the amount of light available to understory trees; neighbours compete for nutrients and water. Environmental factors can have large impacts on plant physiology, influencing both resource acquisition and resource allocation. Patterns of resource allocation among growth, reproduction, storage, maintenance, and defense in turn determine future ability both to compete for resources and to retain resources previously acquired.

Life History Differences in Resource Allocation.

Allocation of resources to any one process (growth, reproduction, storage, maintenance, defense) necessitates a reduction of resources allocated to others (Cody

1966, Mooney 1972, Bazzaz et al. 1987). In environments where resource limitations constrain an individual's ability to replace lost tissue (e.g. foliage loss resulting from herbivory), resource retention may be especially important (Coley et al. 1985). Perhaps for this reason, species found in low resource environments, especially those in low light areas (typically slow growing, shade tolerant species) often have higher levels of constitutive defenses than species typical of resource rich environments (Bryant et al. 1983, Bryant et al. 1985, Coley et al. 1985, Mole et al. 1988, Poorter 1989, Nichols-Orians 1991, Coley 1993, Shure and Wilson 1993, Dudt and Shure 1994, Klepzig et al. 1995, Coley and Barone 1996). The occurrence of fast-growing, poorly defended plants in high-resource areas and slow-growing, well-defended plants in low-resource areas has been observed in many systems both among (Coley 1983, Bryant et al. 1985, Bryant et al. 1992, Walters et al 1993, Ashton and Berlyn 1994) and within (Winn and Evans 1991, Niesenbaum 1992, Dale and Causton 1992) species. Slow growth in species adapted to low resource environments can be viewed as a consequence of selection for traits that enable success in unfavorable conditions (Chapin 1991, Lambers and Poorter 1992), intrinsic differences in abilities to acquire resources or hormonally stimulate growth (Chapin 1991), or as a result of environmental constraints on growth (see Carbon/Nutrient Balance Hypothesis, below).

The extent to which resource allocation patterns are genetically determined versus the extent to which they are determined by the environment is uncertain. Phenotypic plasticity in plant responses to environmental stress has been shown to be greater in species adapted to high-resource (especially high light) environments (Smith 1991, Riddoch et al. 1991, Thompson, Huang and Kiedemann 1992, Thompson, Kriedemann

and Craig 1992, Chazdon and Kaufmann 1993, Ashton and Berlyn 1994, Lusk et al. 1997).

Effects of Light, Nutrients, and Defoliation on Resource Acquisition and Resource Allocation.

Light.

Light is an essential resource for higher plants. Light levels to which forest trees are exposed vary from deep shade, with less than 2% of incident photosynthetically active radiation (PAR) penetrating discrete openings in dense forest canopies (Larcher 1995) to levels near to those in full sun at the center of large gaps. Woody forest species are often classified as shade tolerant or intolerant. Classically, this division was based upon perceived abundance in the forest understory, with abundant understory species identified as shade-tolerant (Baker 1949). More recent studies quantitatively base classifications on relative growth rates and/ or low light survivorship (Kitajima 1994, Kobe et al. 1995, Walters and Reich 1996). Shade intolerant species generally grow faster in high relative to low light and/or have reduced survival in low light. Additional physiological differences between shade tolerant and intolerant species are noted by Givnish (1988).

A reduction in available light limits photosynthetic assimilation, thereby limiting carbon available for growth, primary and secondary metabolism, and storage. Plants may acclimate to reduced light by reallocating dry matter, changing leaf morphology, chlorophyll and protein content, decreasing respiration, decreasing enzyme activity, decreasing electron transport activity, altering whole plant architecture (Holt 1995), and

increasing, decreasing, or maintaining photosynthetic rates (Turnbull 1991, Givnish 1986, Holt 1995, Mulkey et al. 1993).

Low light levels often result in reduced plant growth (Schaffer and Mason 1990, Denslow et al. 1990, Niesenbaum 1992, McKee 1994, Groninger et al. 1996, Johnson et al. 1997), especially in shade intolerant species. Decreased irradiance has been associated with decreased nitrogen content in foliage and a range of other plant tissues (Hollinger 1996, Schaffer and Mason 1990, Dale and Causton 1992), although in some studies no effect of irradiance on nitrogen content was observed (Cui and Caldwell 1997). Because light is used for carbon-fixation, shade may limit the carbon content of plant tissues.

Leaf morphology is often altered by changing light environments as plants attempt to maintain adequate photosynthetic rates. Shaded individuals often have thinner but larger leaves than conspecifics in the sun (Potter 1992). Leaf mass per unit area within species correspondingly tends to increase with increased irradiance (Niinemets and Kull 1994, Gottschalk 1994), and among species is higher in shade intolerant species (Niinemets and Kull 1994).

While plant species adapted to shade may have higher levels of constitutive defenses than species adapted to sun due to differing life history strategies (discussed above), the opposite pattern seems to hold within a species (Coley 1993). Shaded individuals are often less defended than conspecifics in higher light environments (Bryant et al. 1992, Coley 1993, Dudt and Shure 1994, Johnson et al. 1997). Shade-induced reduction of carbon-based foliar defenses may be especially pronounced in nutrient-rich

soils, as these soils promote growth and thereby enable increased use of carbon in primary rather than secondary metabolism (Bryant et al. 1983).

Nutrients.

Although nitrogen is one of the most abundant elements in Earth's atmosphere, it is one of the most biologically limiting nutrients because it cannot be used by plants in its most common form ($N_{2(g)}$). Among its many uses, nitrogen is richly employed in photosynthetic cells where it is an element of light-harvesting apparatus and photosynthetic enzymes (Chapin et al. 1987). Much research has documented a strong correlation between foliar nitrogen and plant photosynthetic rates (Gulmon and Chu 1981, Mooney et al 1981, DeJong 1982, Field et al. 1983, Field and Mooney 1986, Reich et al. 1991). Alleviation of nitrogen deficiencies in plants by fertilization can lead to increased foliar nitrogen (Floater 1997, Lawler et al. 1997). Therefore, individuals grown in nutrient rich areas may have increased photosynthetic capacities relative to individuals in nutrient-deficient regions.

Fertilization has been shown to increase plant growth (Linder and Rook 1984, McDonald et al. 1986, Bryant et al. 1987, Muzika 1993), perhaps in association with increased photosynthetic carbon acquisition. Fertilization can increase above ground plant growth proportionally more than root growth, resulting in decreased root/ shoot ratios.

Nutrient addition has been correlated with increased starch storage (Wargo et al. 1972, McDonald et al. 1986, Burke et al. 1991), and increased leaf size in hardwoods (reviewed in Linder and Rook 1984). Increased foliar nitrogen levels have generally been

associated with decreased polyphenolic levels (Bryant et al. 1992, Muzika 1993, Kinney et al. 1997, Lawler et al. 1997), perhaps as a result of decreased carbon/ nitrogen ratios in plant tissues (see Carbon/ Nutrient Balance Hypothesis, below).

Defoliation.

In addition to abiotic stresses such as low levels of light and nutrients, plants may be exposed to herbivory, a key biotic stress. Loss of leaf area resulting from herbivory may result in increased photosynthetic rates in remaining foliage (Ovaska et al. 1992, Lovett and Tobiessen 1993). Often this compensatory photosynthesis is not sufficient to maintain needed assimilation, and growth rates are reduced in the presence of herbivores (Schaffer and Mason 1990, Fox and Morrow 1992, May and Killingbeck 1995), although in some cases compensatory increases in growth have been observed (Houle and Simard 1996).

Modelling work has indicated that the location and severity of herbivory can influence patterns of nitrogen distribution in the plant canopy (Stockhoff 1994). Defoliation is often associated with reductions in levels of foliar nitrogen (Fox and Morrow 1992). Perhaps for this reason, fertilization has been shown to mitigate defoliation-induced resistance (Hunter and Schultz 1995). Foliar carbon content is less effected by herbivory than foliar nitrogen content, as carbon can be mobilized from storage in response to damage (Wargo et al. 1972).

Defoliation has been both positively and negatively correlated with levels of foliar phenols and other foliar chemical defenses (Haukioja et al. 1985, Faeth 1992, Bryant et al. 1992). Plant responses to defoliation may be a result of shifting carbon/ nitrogen

balances in individuals (Bryant et al. 1983, see below), and/ or may be elicited by specific herbivore cues (e.g. salivary chemicals) which trigger defenses, as natural defoliation sometimes elicits a stronger response from the plant than artificial foliage removal (Haukioja and Neuvonen 1985). Defoliation induced responses can occur in the season of damage (Haukioja 1980) or in following years (Parker and Houston 1971, Haukioja 1990, Tuomi et al. 1990).

Environmental Variation at the Scale of the Individual.

While much research has focused on differences among individuals subjected to different levels of environmental stresses, less attention has been given to the variation that an individual will experience over its lifetime. As discussed above, both light and nutrients vary over the course of succession and in response to disturbance. Defoliation, too, varies annually. Although there is generally some base level of defoliation experienced by an individual, the cyclic population dynamics of many outbreak defoliators result in high herbivory stress in some years and in relatively negligible stress in others years.

The response of plants to long-term successional changes in light availability (declining with canopy closure and increasing with random gap formation, which was found to contribute over 65% of the total seasonal photosynthetically active radiation received in the understory of one northern United States hardwood forest, Canham et al. 1990) may have important effects on successional trajectories. This would especially be true if species that differ in life history strategies also differ in the nature of their response to fluctuations in the light environment. Response time, duration, and strength of plant

responses may all be important variables to consider when examining the impact of long-term responses on plant fitness and on interactions among individuals of the forest community. For example, herbivore populations may be indirectly affected by successional changes as they consume plant parts morphologically or chemically responsive to changing environments.

Theory on Mechanisms Driving Resource Allocation Patterns.

Physiological processes underlying observed plant responses must be examined to understand and predict interactions among stress factors. At present no single theory sufficiently explains or predicts plant responses to environmental stresses. The carbon/ nutrient balance hypothesis (Bryant et al. 1983, Tuomi et al. 1984) and the growth/ differentiation balance hypothesis (Loomis 1932, Lorio 1986, Herms and Mattson 1992) are closely related theories that have been useful in conceptualizing potential mechanisms of plant response to their environments. Both theories address resource limitations that restrict and guide patterns of resource allocation among life processes including growth, storage, maintenance, and defense.

Carbon/ Nutrient Balance Hypothesis.

The carbon/ nutrient balance hypothesis (Bryant et al. 1983, Tuomi et al. 1984) predicts allocation to growth and secondary metabolism based upon the carbon/ nutrient (or nitrogen) (C/N) ratio of the plant. Specifically, carbon-based secondary metabolism is predicted to vary directly with the C/N ratio of the plant. Growth receives resource priority in favorable environments, but can be limited by either carbon or nitrogen

availability. Because moderate nutrient deficiency (infertile soils, loss of nutrients to herbivores) limits growth more than photosynthetic capacity, C/N ratios will be increased in nutrient deficient plants and excess carbon will be available for allocation to the production of secondary metabolites. In shaded environments, low levels of photosynthetically active radiation will limit carbon fixation, reducing C/N ratios. Limited carbon availability will decrease concentrations of carbon based secondary metabolites. However, nutrients that accumulate in excess of growth requirements (e.g. when other factors limit growth, such as low light) may be converted to nitrogen-based defensive compounds such as alkaloids.

It is relevant to note that the carbon/ nutrient balance hypothesis assumes that there is no “cost” of defense, as defense compounds are produced with resources acquired in excess of those needed for growth (Bryant et al. 1983, Tuomi et al. 1988). However, nutrient loss due to herbivory can induce higher levels of defensive compounds in regrowth foliage in the same or the following season (Neuvonen and Haukioja 1984, Neuvonen et al. 1987, Haukioja 1990, Tuomi et al. 1990) by shifting the C/N balance of the plant upward. This “delayed induced resistance” should be especially strong when defoliation occurs early in the season when leaves contain proportionally more nitrogen than carbon. Because they result from nutrient loss, defoliation-induced responses are predicted to be weak or non-existent in fertilized environments. Empirical studies show that induced resistance can also occur within damaged and undamaged foliage of the host tree within hours or days of damage (Haukioja 1980). Environmental effects on the strength of “rapid induced resistance” are not as easily predicted by the carbon/ nutrient balance hypothesis.

Growth/ Differentiation Balance Hypothesis.

The growth/ differentiation balance hypothesis (Loomis 1932, Lorio 1986, Herms and Mattson 1992) subsumes the carbon/ nutrient balance hypothesis, placing it within an expanded framework, which broadens the range of predictions. Unlike the carbon/ nutrient balance hypothesis, the growth/ differentiation balance hypothesis emphasizes physiological processes over environmental factors and therefore predicts shifts in resource allocation based on a wider range of environmental conditions including nutrient availability, temperature, and drought (Herms and Mattson 1992). The growth/ differentiation balance hypothesis also addresses the importance of plant developmental stage, which is not addressed by the carbon/ nutrient balance hypothesis.

The growth/ differentiation balance hypothesis distinguishes among resource allocation to “processes and structures further enhancing resource acquisition” (i.e. growth; Herms and Mattson 1992, p.297) versus “non-growth processes and structures required to retain and make efficient use of resources under adverse environmental conditions and/ or intense herbivory” (i.e. differentiation; Herms and Mattson 1992, p.297). While many growth and differentiation processes are co-dependent, some differentiation processes (e.g., manufacture of anti-herbivore secondary metabolites) may limit growth by diverting resources from growth processes (Herms and Mattson 1992). This implies a potential cost of defense not acknowledged by the carbon/ nutrient balance hypothesis.

The growth/ differentiation balance hypothesis holds that the effects of environmental variation on allocation patterns of constitutive carbon-based secondary

metabolism can be predicted by the impact of the variant on plant growth relative to photosynthesis (resource acquisition). Environmental factors that slow growth more than photosynthesis (moderate nutrient deficiency, drought, temperature stress) are predicted to increase carbon availability for secondary metabolism and storage reserves because carbon will accumulate in excess of growth requirements. Growing meristems are strong carbon sinks. Weak sink strength of slow growing meristems in resource-limited environments will reduce phloem transport, thus increasing carbon accumulation in strong source leaves which continue to maintain high rates of photosynthesis. This carbon is often diverted to biosynthesis of carbon-based secondary metabolites. Developmental stage of the plant can also affect this relationship (Herms and Mattson 1992), with young rapidly growing tissues being stronger sinks than older tissues. Active meristems utilize carbon in the processes necessary for growth, such as protein synthesis and respiration. Limited carbon constrains secondary metabolism in strong sinks. For factors such as shade, which limit source strength, both growth and secondary metabolism are predicted to be carbon limited. Effects of environmental stresses on defoliation induced resistance are not addressed by the growth/ differentiation balance hypothesis.

Objectives and Predictions.

The objectives of this study were to determine the simultaneous effects of light, nutrient availability, and natural defoliation on resource acquisition and allocation patterns of sugar maple (*Acer saccharum* Marsh.) and paper birch (*Betula papyrifera* Marsh.). Shade treatments were designed to mimic successional canopy closure, with all

trees grown in full sun for three years, after which half the trees were exposed to two years of shade, thereby simulating natural overstory closure. In the final year of the experiment, all trees were once again exposed to full sun, mimicking gap formation or overstory defoliation. Defoliation effects on foliar traits (see below) were measured both during the season of defoliation and during the following season so that any delayed effects could be detected.

Treatment effects on plant acclimation to shade were measured as morphological changes in foliage (specific leaf mass and leaf area) and light response curves. Treatment effects on acquisition of carbon were measured as net photosynthesis, while effects on acquisition of nitrogen were estimated by foliage nitrogen content. Resource allocation was determined by quantifying plant growth and levels of total phenolics and condensed tannins in foliage. Phenolics (assayed in both the total phenolic measure and the condensed tannin measure) are the predominant class of carbon-based secondary metabolites in birch and maple and have been implicated in constitutive and induced herbivore resistance in a wide range of systems (Feeny 1970, Haukioja et al. 1985; but see Bernays 1981, Ayres et al. 1997).

I focused on paper birch and sugar maple because these species have contrasting life history strategies, and therefore are expected to respond differently to environmental stresses. Additionally, both species are economically important, have overlapping ranges in Michigan, and are important hosts of the forest tent caterpillar, *Malacosoma disstria* (Hubner, Lepidoptera: Lasiocampidae) (Goodman et al. 1990, Safford et al. 1990), which was used to implement the natural defoliation treatment (see below). To my knowledge,

this is the first study to examine simultaneous effects of defoliation, light, and nutrient stress on species with contrasting life history strategies.

Paper birch is a fast-growing, early-successional, shade-intolerant species (Loehle 1988, Safford et al. 1990). This species develops best on cool moist sites and is sensitive to nutrient availability (Safford et al. 1990). It produces a diversity of foliar carbon-based secondary metabolites (Palo 1984).

At the opposite end of the life history continuum, sugar maple is a slow-growing, late-successional, shade-tolerant species (Loehle 1988, Goodman et al. 1990). This species is also sensitive to soil fertility and is common to cool, moist sites (Goodman et al. 1990). It produces both carbon- and nitrogen-based foliar defenses (Barbosa et al. 1990).

Based upon life history theory, I predicted that: (1) light response curves of birch would have higher light-saturated photosynthetic rates and higher light saturation points than those of maple; (2) in high resource environments (high light, nutrients), birch would have faster growth and higher ambient photosynthetic rates than maple; (3) birch responses (growth, photosynthetic activity, foliar chemistry) to environmental variability (light, nutrients, defoliation) would be more plastic than maple responses; (4) birch foliage would contain lower concentrations of defensive compounds than maple foliage (lower concentrations of condensed tannins and total phenolics than maple foliage).

Based upon the growth/ differentiation hypothesis, I predicted that (1) if shade decreases photosynthesis of birch and maple, then they would have lower levels of C-based secondary defense chemicals (condensed tannin and total phenolic concentrations)

than conspecifics grown in the sun; (2) if fertilization increases growth more than photosynthesis, then carbon-based defenses would be lower in fertilized treatments; and (3) if defoliation decreases foliar nitrogen, then levels of carbon-based defenses would be higher in defoliated trees in 1996 and 1997.

MATERIALS AND METHODS

Experimental Site and Design.

Studies were conducted in established research plots at the Dow Gardens in Midland, MI. Climatological information for Midland is provided by the U.S. Department of Commerce National Oceanic and Atmospheric Administration (1971). The research plots consisted of forty-eight 1 m² cells arranged in 6 rows of 8. Rows were spaced 2 m apart. Walls of cells were lined with 30 mil poly-vinyl chloride liners extending 1 m into the ground and 10 cm above ground level. Overlapping edges were heat-sealed, which effectively isolated the soil environment of each cell. Bottoms of cells were open. Neighbouring rows were grouped in sets of 2 to comprise blocks (3 blocks total).

One sugar maple and one paper birch tree were transplanted into each cell in April of 1992, at which point maple were entering their fifth growing season and birch their third. Trees within species are open-pollinated half siblings. The maternal source of maple was native to Minnesota, close to the Wisconsin border, while the maternal source of birch was native to eastern Wisconsin.

Light intensity, soil nutrients, and defoliation treatments were administered in complete factorial fashion. The experiment was designed as a randomized, complete

block, split plot with light as the whole plot and nutrients and defoliation as subplots.

Each of the 16 treatment combinations (species*light*nutrient *defoliation) were replicated twice in each of the three blocks for a total of 96 trees in the experiment.

Experimental Treatments.

Light Manipulation.

One row of each block was randomly selected to be shaded, while the other experienced ambient light conditions. Shading was accomplished by using a wooden frame to support an 80% black shade cloth (E. C. Geiger, Inc.) over the top of selected rows and hanging 30% shade cloth from the top of the frame supports to the base of the canopy. In both 1995 and 1996, plots were shaded from May (as leaves reached full size) until initiation of leaf senescence in October.

Values of quantum flux recorded during photosynthetic measurements (see below) verified that shade cloth reduced light in treated plots by 80%. Average photosynthetically active radiation (PAR) measured in full sun on measurement days was $1285 \pm 48 \mu\text{mol m}^{-2} \text{s}^{-1}$ and in shade treatments was $273 \pm 49 \mu\text{mol m}^{-2} \text{s}^{-1}$. Air circulation, precipitation, insect herbivores, invertebrate predators and avian species were not noticeably affected by the presence of shade structures.

Soil Nutrients.

Four cells per row were randomly selected to receive a 225 kg/ha/yr of actual nitrogen (N) fertilizer with phosphorous (P) and potassium (K) applied in the ratio of

18:5:9 NPK. The remaining cells were not fertilized. Nitrogen in the fertilizer was in the form of urea N (16.05%, with 6.75% sulfur coated for slow-release) and ammonia N (1.95%) with the balance as nitrate. Phosphorus was in the form of P_2O_5 and potassium in the form of K_2O . Half of the annual amounts of fertilizer were applied in May and the remainder in October in 1994, 1995, and 1996.

Natural Defoliation.

Forest tent caterpillar, one of the most important outbreak defoliators of deciduous trees in North America (Mattson et al. 1991), was used to implement the defoliation treatment. On 16 May 1996, half of the experimental trees were inoculated with forest tent caterpillar egg bands, which were held in mesh packets attached to limbs (10 to 15 egg bands per tree). Egg bands were field collected from quaking aspen (*Populus tremuloides*) stands in Cochrane, Ontario on 7 May 1996. Prior to deployment, egg bands were surface sterilized by rinsing with bleach for 3 minutes, followed by a 5 minute tap water rinse (Grisdale 1968). Egg hatch occurred on 16 May, corresponding with bud break as it does in natural populations. Trees first broke bud between 8 and 19 May, and all buds had opened by 14-30 May. Larval feeding on control trees was restricted by manual redistribution of larvae, restraining of overlapping treatment and control tree branches with twine, and applying sticky bands to the trunks of control trees. Estimates of defoliation (percent leaf area removed) were determined as the average of visual estimates by four independent observers. Defoliation levels were $1 \pm 3\%$ and $8 \pm 3\%$ (range 0-32.5%) in maple and $6 \pm 3\%$ and $52 \pm 3\%$ (range 0.5-98.5%) in birch.

Forest tent caterpillar avoided eating maple even when continually placed on this species. Although some herbivores, including gypsy moth, do not tend to prefer maple and other hosts which contain alkaloids (Barbosa et al. 1990), this behavior in forest tent caterpillar was unexpected as Dankert (1995) found forest tent caterpillar to prefer maple over birch in a study using trees from the same cohort as those used in the present study.

Plant Physiological Responses.

Acclimation to Shade.

Photosynthetic Capacity (Light Response Curves). Between 26 and 31 August, 1996, photosynthetic responses of trees to varying levels of photosynthetically active radiation were determined for all non-defoliated trees using a CI-301PS open photosynthesis system (CID, Inc., Vancouver, Washington State USA). Carbon assimilation was measured for 55-second intervals at 0, 50, 100, 200, 300, 450, 600, 800, and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface. To account for changing intensity of light with distance from the light source to the leaf, the above light levels were achieved by dividing these target values by 0.61 and then setting the light source intensity to adjusted values. One leaf per tree was measured. Only fully-expanded leaves in the exterior of the canopy with minimal or no herbivore or other damage were used. On birch, only short shoot foliage was sampled. A preliminary study conducted earlier had indicated a possible species*nutrient interactive effect on carbon assimilation at maximal light intensity (A_{max}). In order to increase power for detecting fertilization effects, a sampling protocol was developed in which fertilized and non-fertilized trees of the same species and light

treatment were sampled consecutively. Light response curves were fit to an equation of the general form:

$$A = ((b \cdot I) / (1 + a \cdot I)) - \text{Resp},$$

where A = carbon assimilation rate (measured), I = irradiance (measured), and “ a ”, “ b ”, and “Resp” are parameters fit with the Marquardt-Levenberg algorithm. Parameter “ a ” (“curve”) is inversely related to photosynthetic carbon assimilation at light saturation. “ b ” indicates the initial slope of the light response curve, and “Resp” is the dark respiration rate (carbon assimilation at $I=0$).

Specific Leaf Mass and Leaf Area. Twelve leaves from each tree were collected from throughout the canopy on 15 June 1995, 11 June 1996 and 16 June 1997 (base 42°F degree days at Saginaw, Michigan: 15 June 1995 = 1039, 11 June 1996 = 827, 1 August 1996 = 2268, and 16 June 1997 = 911; data from Jeff Andresen, Michigan State University Geography Department). Leaf area was measured using a digital image analysis system (AgVision Digital Image Analysis System, Decagon Devices, Inc. in 1995 and 1996, and CI400 Computer Image Analysis System, CID, Inc. in 1997), and average leaf area was calculated. Samples were oven-dried for 48 h at 45°C, then weighed. Specific leaf mass (SLM) was calculated with the equation [SLM = dry mass/leaf area (g/m²)].

Resource Acquisition.

Photosynthetic Assimilation at Ambient Conditions. Net photosynthetic carbon assimilation at ambient conditions was measured for all trees using a closed-system

LICOR 6200 infrared gas analyser (LICOR Inc., Lincoln, NE). Measurements were made on sunny days with clear skies between 1000 h and 1300 h EST. In Midland, morning skies are typically clear with patchy clouds approaching from the NW between 1230 h and 1300 h. One fully-expanded leaf in full sun with minimal or no damage was measured per tree. Birch trees were measured on 10 July and 16 July, and maple on 11 July and 22 July 1996. Stomatal conductance measurements were made only on the second measurement date per species. Sample sizes prohibited both species (96 trees) from being measured on the same day without introducing excessive diurnal variation.

Foliar Carbon and Nitrogen. Dried leaf matter collected for specific leaf mass measurements (see above) from each tree was ground to pass through #20 mesh in a Wiley mill, and analyzed for percent carbon and nitrogen content by ignition in a Carbo Erba model NA 1500 CHN Analyzer with methods described by Daun and DeClercq (1994).

Resource Allocation.

Height and Diameter Growth. Trunk diameters at 50 cm above ground level were measured at the beginning and end of 1994, 1995, and 1996 growing seasons (5 May 1994, 10 October 1994, 28 May 1996 and 26 October 1996). Diameters were measured with callipers consistently oriented east to west to minimize year-to-year variation in measurements due to trunk asymmetries. Trunk heights were measured with a telescoping ruler on 12 May and 3 October 1994, 23 September 1995, and 26 October 1996. Increase in diameter and height within years was calculated as the difference

between height (or diameter) measured at the beginning and at the end of each growing season.

Foliar Condensed Tannin and Total Phenolic Concentrations. Concentrations of foliar condensed tannins were determined for samples of 12 birch or maple leaves per tree collected from throughout the canopy on 11 June 1996 and 16 June 1997, and for 12 “mature” (short shoots) and 12 “immature” (axial growth) leaves from each birch tree on 1 August of 1996. Mature leaves had expanded earlier in the season and were noticeably a darker green color than immature leaves which expanded relatively later in the season. Total phenolic concentrations were determined for all leaves sampled in 1996 but not in 1997. Leaves were submerged in liquid nitrogen within two minutes of sampling to prevent enzymatic degradation and phenolic oxidation. Leaves were transported on dry ice to the laboratory, and stored frozen until they could be lyophilized. Leaves were ground to pass through #40 mesh in a Wiley mill, and 70-80 mg aliquots were weighed out for polyphenolic extractions. Except during grinding and weighing, leaves were kept refrigerated at all times after lyophilization.

Total phenolic activity was determined by the Folin-Denis assay (Swain and Hillis 1959) using an RFA-300 Rapid Flow Analyzer (Astoria-Pacific International, Clackamas, OR). Phenolics were extracted by 30 minute agitation in 50% methanol. Samples were run with tannins from Alaska paper birch (*Betula resinifera*, purified from Alaska populations by Tom Clausen, University of Alaska, Fairbanks) and sugar maple (purified from the study population by James Nitao, Michigan State University) as standards to obtain values for percent dry weight total phenolics on a species-specific basis.

Condensed tannins concentrations (CT) were determined by the n-butanol proanthocyanadin assay (Hagerman 1995). Phenolics were extracted from 70-80 mg foliage samples in 8 ml of 70% acetone at 5°C for 18 h on a shaker table. Tannins from 4 g of ground leaves from bulk samples of each species (collected the same day as foliage sampled for chemistry) were extracted in 70% acetone and purified for use as standards. Tannin purification was accomplished by removing acetone from bulk extracts by rotoevaporation, packing aqueous extract on to a Sephadex LH-20 column, eluting phenolic monomers by flushing 95% ethanol through the column until a negative Prussian Blue spot test (Hagerman 1995) was obtained, and collecting larger molecular weight phenolics by washing the column with 70% acetone until a negative Prussian Blue spot test indicated all phenols had eluted. Acetone was then again removed by rotoevaporation and samples were frozen and lyophilized.

Statistical Analyses.

Main and interactive effects of treatments on the initial slope (parameter “b”) and curve (parameter “a”) of light response curves, stomatal conductance, net photosynthesis rates, foliar carbon, nitrogen, condensed tannin and total phenolic concentrations and C/N ratios, specific leaf mass, and leaf area were analysed using univariate analysis of variance (PROC GLM; SAS Institute, Inc. 1990). Tree height, annual height growth, and annual diameter growth were analyzed using analysis of covariance (ANCOVA; SAS Institute, Inc. 1990), with May 1994 height and diameter measures as covariates, respectively. Assumptions of normality and equal variances required for ANOVA were

verified with Shapiro-Wilkes normality tests (SAS PROC UNIVARIATE) and visual examination of normality plots, respectively. Extreme outliers (greater than 3 standard deviations from the mean) were removed from analyses only if they previously had been noted as unusual, or if the datum was biologically infeasible. Block was treated as a random effect and species, light, nutrient, and defoliation were treated as fixed effects. Models were adjusted to account for the split-plot design by testing for shade effects (whole plot factor) with the block*light mean square error (MSE) in the denominator of the *F* statistic, as opposed to the complete model MSE. Data are reported as least square means $\pm$ standard errors, unless noted. Standard errors of shade effects were calculated using block*light as the error term.

Within a species, the relationship between percent defoliation and tree height and diameter growth, absolute tree height, foliar carbon and nitrogen content, C/N ratios, condensed tannin, total phenolic content, and specific leaf mass were also tested using Pearson correlation coefficients (SAS PROC GLM) and treating defoliation as a continuous variable.

RESULTS

Acclimation to Shade.

Photosynthetic Capacity (Light Response Curves).

Light response curves generated in 1996 for each light*fertilization treatment combination for birch and maple are reported in Table 2.1. Birch had higher photosynthetic rates than did maple for all intensities of photosynthetically active

Table 2.1. Average photosynthetic assimilation (A , $\mu\text{moles CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as a function of irradiance (I , $\mu\text{mol m}^{-2} \text{ s}^{-1}$) in non-defoliated trees. See text for measurement and calculation details. $N = 4\text{-}6$ per treatment combination.

Treatment	Equation
Maple	
Shaded	
Fertilized	$A = (0.0279 I / (1 + 0.0193 I)) - 0.6013$
Non-fertilized	$A = (0.0258 I / (1 + 0.0060 I)) - 0.4567$
Non-shaded	
Fertilized	$A = (0.0375 I / (1 + 0.0112 I)) - 0.6299$
Non-fertilized	$A = (0.0207 I / (1 + 0.0048 I)) - 0.6901$
Birch	
Shaded	
Fertilized	$A = (0.0327 I / (1 + 0.0044 I)) - 0.4633$
Non-fertilized	$A = (0.0340 I / (1 + 0.0038 I)) - 0.7217$
Non-shaded	
Fertilized	$A = (0.0295 I / (1 + 0.0034 I)) - 0.6817$
Non-fertilized	$A = (0.0312 I / (1 + 0.0032 I)) - 0.8150$

Table 2.2. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, species and nutrient effects and their interactions on the initial “curve” (parameter a) and initial slope (parameter b) of light response curves, and on assimilation at PAR = 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (RESP), 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A100), 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A200), 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A400) and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (A1000). *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	curve	slope	RESP	A100	A200	A400	A1000
Block	2	1.20	1.00	5.33 **	1.75	2.77 #	3.11 #	2.56 #
Light	1	1.51	0.01	6.18	0.38	0.09	0.03	0.49
Block*Light	2	0.51	0.91	0.49	2.82 #	1.78	0.76	1.49
Species	1	4.47 *	1.04	0.86	29.21 ****	46.16 ****	48.28 ****	36.23 ****
Species*Light	1	0.38	0.47	0.02	0.09	0.05	0.44	0.78
Nutrient	1	2.58	1.08	0.87	0.08	0.12	0.45	0.50
Species*Nutrient	1	2.19	2.06	2.09	0.00	0.05	0.06	0.05
Light*Nutrient	1	0.33	0.90	0.06	1.22	0.57	0.09	0.09
Species*Light*Nutrient	1	0.25	0.99	1.00	2.94 #	1.00	0.10	0.10
Error	<i>df</i>							
	35							

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, **** = $p \leq 0.0001$.

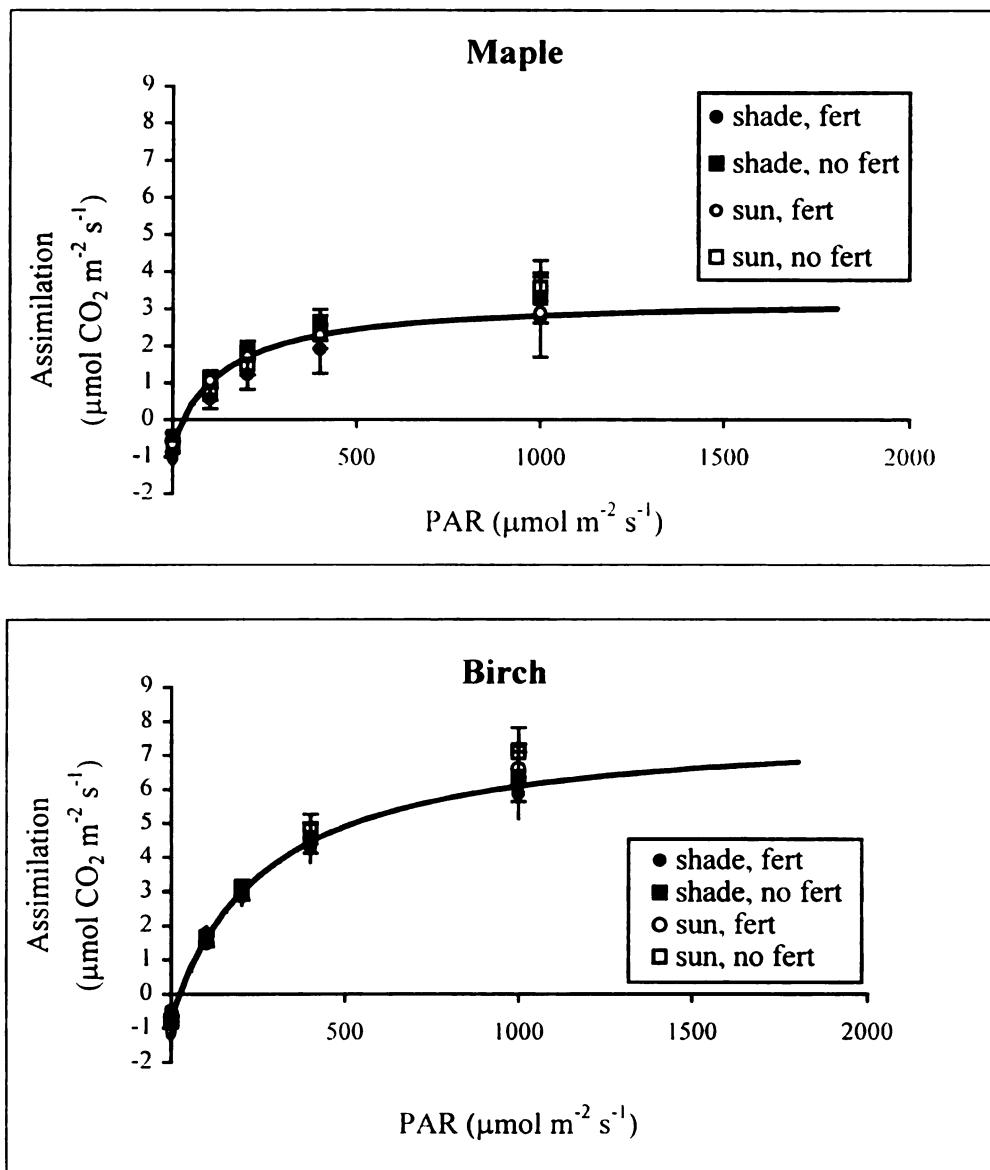


Figure 2.1. Photosynthetic light response curves for sugar maple (A) and paper birch (B) grown under factorial combinations of light and nutrient availability.

radiation (PAR) examined, but the species did not differ in respiration rates (Table 2.2, Figure 2.1). Although both species adjusted their leaf morphology in shaded environments (see Specific Leaf Mass, below), neither improved their capacity to assimilate carbon in low light or high nutrient environments (Table 2.2; Figure 2.1).

Specific Leaf Mass and Leaf Area.

Intrinsic Differences in Specific Leaf Mass and Leaf Area. Birch had greater specific leaf mass (SLM) than maple in 1995, 1996 and 1997 (Table 2.3). Within a species, 1997 values tended to be greater than 1996 values. In maple this may reflect the fact that shade decreased foliage mass per unit area in 1996 (see below), with the lack of shading in 1997 increasing the average mass per unit area of maple foliage. The SLM of birch increased over the 1996 season. Both mature and immature leaves harvested in August weighed more per unit area than leaves sampled in June. This was likely a result of tree-wide variation rather than a function of leaf age, as immature foliage sampled in August had greater SLM than mature foliage (Table 2.3).

Birch had smaller leaf areas than maple in 1995, 1996, and 1997 (Table 2.3). Leaf areas of both species were highest in 1995 before treatment implementation.

Light Effects on Specific Leaf Mass and Leaf Area. Shading had no effect on the specific leaf mass of birch or maple in June of 1995 (Table 2.4a). In June of 1996, the specific leaf mass of maple was 20% lower in shaded trees than in trees grown in full sun (Table 2.5a, Figure 2.2). Light effects on the SLM of birch were not significant in June; although, as in maple, specific leaf mass tended to be lower in shaded birch. In August,

Table 2.3. Foliar carbon and nitrogen concentration, carbon/nitrogen (C/N) ratio, condensed tannin and total phenolic content, specific leaf mass (SLM) and leaf area of paper birch and sugar maple foliage in 1995, 1996, and 1997 (least square means $\pm$ standard error).

<u>June 1995</u>		
	Paper Birch	Sugar Maple
Carbon (% dry leaf)	49.3 $\pm$ 0.3	49.4 $\pm$ 0.3
Nitrogen (% dry leaf)	3.03 $\pm$ 0.05	2.28 $\pm$ 0.05
C/N ratio	18.7 $\pm$ 0.4	19.6 $\pm$ 0.4
Specific leaf mass (g m ⁻²)	59.9 $\pm$ 1.2	49.6 $\pm$ 1.2
Leaf Area (cm ²)	28.7 $\pm$ 1.6	100.7 $\pm$ 1.6
<u>June 1996</u>		
	Paper Birch	Sugar Maple
Carbon (% dry leaf)	51.1 $\pm$ 0.3	49.4 $\pm$ 0.3
Nitrogen (% dry leaf)	4.06 $\pm$ 0.05	2.96 $\pm$ 0.05
C/N ratio	12.8 $\pm$ 0.4	16.9 $\pm$ 0.4
Condensed tannins (% dry leaf)	1.12 $\pm$ 0.08	1.32 $\pm$ 0.09
Total phenolics (% dry leaf)	12.4 $\pm$ 0.5	21.3 $\pm$ 0.5
Specific leaf mass (g m ⁻²)	36.4 $\pm$ 1.0	31.1 $\pm$ 1.0
Leaf Area (cm ²)	19.8 $\pm$ 0.7	81.4 $\pm$ 2.5
<u>August 1996</u>		
	Mature Birch	Immature Birch
Carbon (% dry leaf)	48.8 $\pm$ 0.3	48.3 $\pm$ 0.3
Nitrogen (% dry leaf)	2.60 $\pm$ 0.05	2.59 $\pm$ 0.05
C/N ratio	19.3 $\pm$ 0.4	18.9 $\pm$ 0.4
Condensed tannins (% dry leaf)	0.78 $\pm$ 0.08	0.88 $\pm$ 0.08
Total phenolics (% dry leaf)	11.8 $\pm$ 0.5	12.5 $\pm$ 0.5
Specific leaf mass (g m ⁻²)	55.3 $\pm$ 1.0	58.9 $\pm$ 1.0
Leaf Area (cm ²)	15.5 $\pm$ 0.9	25.0 $\pm$ 1.0
<u>June 1997</u>		
	Paper Birch	Sugar Maple
Carbon (% dry leaf)	47.1 $\pm$ 0.3	45.9 $\pm$ 0.3
Nitrogen (% dry leaf)	2.89 $\pm$ 0.05	2.19 $\pm$ 0.05
C/N ratio	16.6 $\pm$ 0.4	21.3 $\pm$ 0.4
Condensed tannins (% dry leaf)	1.70 $\pm$ 0.08	1.05 $\pm$ 0.09
Specific leaf mass (g m ⁻²)	43.4 $\pm$ 1.0	36.3 $\pm$ 1.0
Leaf Area (cm ²)	19.9 $\pm$ 0.8	78.4 $\pm$ 2.1

Table 2.4. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, and nutrient effects and their interactions on specific leaf mass (A) and leaf area (B) of birch and maple foliage sampled on 15 June 1995. There was no defoliation treatment in 1995. *F*-values for light were calculated with block*light as the error term.¹

Specific Leaf Mass June 1995			
A. Source of Variation	<i>df</i>	Birch	Maple
Block	2	2.48	2.61 #
Light	1	0.02	1.20
Block*Light	2	0.18	1.02
Nutrient	1	0.00	0.00
Light*Nutrient	1	0.84	0.99
Error <i>df</i>		40	37

Leaf Area June 1995			
B. Source of Variation	<i>df</i>	Birch	Maple
Block	2	0.12	2.34
Light	1	1.44	2.94
Block*Light	2	0.10	2.22
Nutrient	1	1.12	2.57
Light*Nutrient	1	0.01	1.19
Error <i>df</i>		40	37

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, **** = $p \leq 0.0001$.

Table 2.5. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on specific leaf mass (A) and leaf area (B) of birch and maple foliage sampled on 11 June 1996, birch foliage originating from buds formed during the previous season ("Mature birch") and from leaf primordia formed in the current season ("Immature birch") sampled on 1 August 1996, and from birch and maple foliage sampled on 16 June 1997. *F*-values for light were calculated with block*light as the error term.¹

a.	Source of Variation	<i>df</i>	June 1996				Specific Leaf Mass			
			Birch		Maple		August 1996		June 1997	
	Block	2	1.21		0.92		Mature birch	Immature birch	Birch	Maple
	Light	1	8.56 #		21.22 *		1.46	0.96	11.65 ****	2.08
	Block*Light	2	2.11		1.11		40.03 *	17.69 #	0.29	1.86
	Nutrient	1	2.43		0.03		0.71	3.87 *	1.82	1.19
	Light*Nutrient	1	4.38 *		0.21		0.02	0.27	0.04	0.07
	Defoliation	1	0.51		0.02		0.04	0.03	0.08	1.39
	Light*Defoliation	1	0.73		0.51		6.20 *	0.10	1.40	0.06
	Nutrient*Defoliation	1	0.88		0.02		3.62	0.88	0.55	0.01
	Light*Nutrient*Defoliation	1	2.92 #		0.12		0.26	0.34	0.17	0.97
	Error <i>df</i>		36		32		1.09	0.61	0.22	0.05
							36	36	36	33

b.	Source of Variation	<i>df</i>	June 1996				Leaf Area			
			Birch		Maple		August 1996		June 1997	
	Block	2	0.22		3.08 #		Mature birch	Immature birch	Birch	Maple
	Light	1	0.10		3.67		1.96	3.67 *	0.45	2.89 #
	Block*Light	2	2.46		0.67		7.38	17.71 #	5.26	0.34
	Nutrient	1	0.00		0.04		0.48	0.47	0.25	0.65
	Light*Nutrient	1	0.26		0.61		0.11	0.22	0.24	0.00
	Defoliation	1	0.12		0.50		0.06	0.22	0.25	1.88
	Light*Defoliation	1	1.50		1.77		1.31	0.57	0.49	0.00
	Nutrient*Defoliation	1	0.01		0.01		5.60	4.85 *	0.47	0.13
	Light*Nutrient*Defoliation	1	0.16		1.72		0.68	2.64	1.11	0.37
	Error <i>df</i>		33		33		0.11	0.17	0.01	0.30
							33	33	33	33

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, **** = $p \leq 0.0001$.

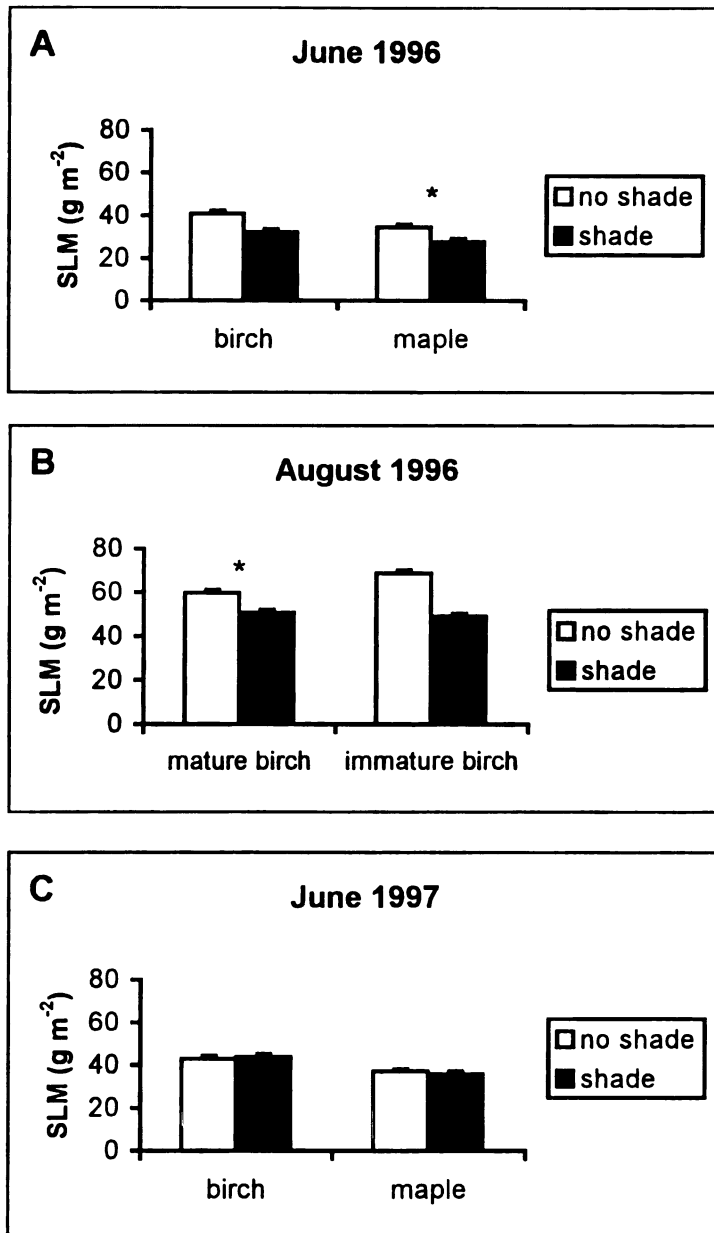


Figure 2.2. Effects of light on the specific leaf mass (SLM) of paper birch and sugar maple foliage in June (A) and August (B) of 1996 and June of 1997 (C). Asterisks indicate significant effects of light treatment within species or leaf age class ($p \leq 0.05$). See text for description of age classes.

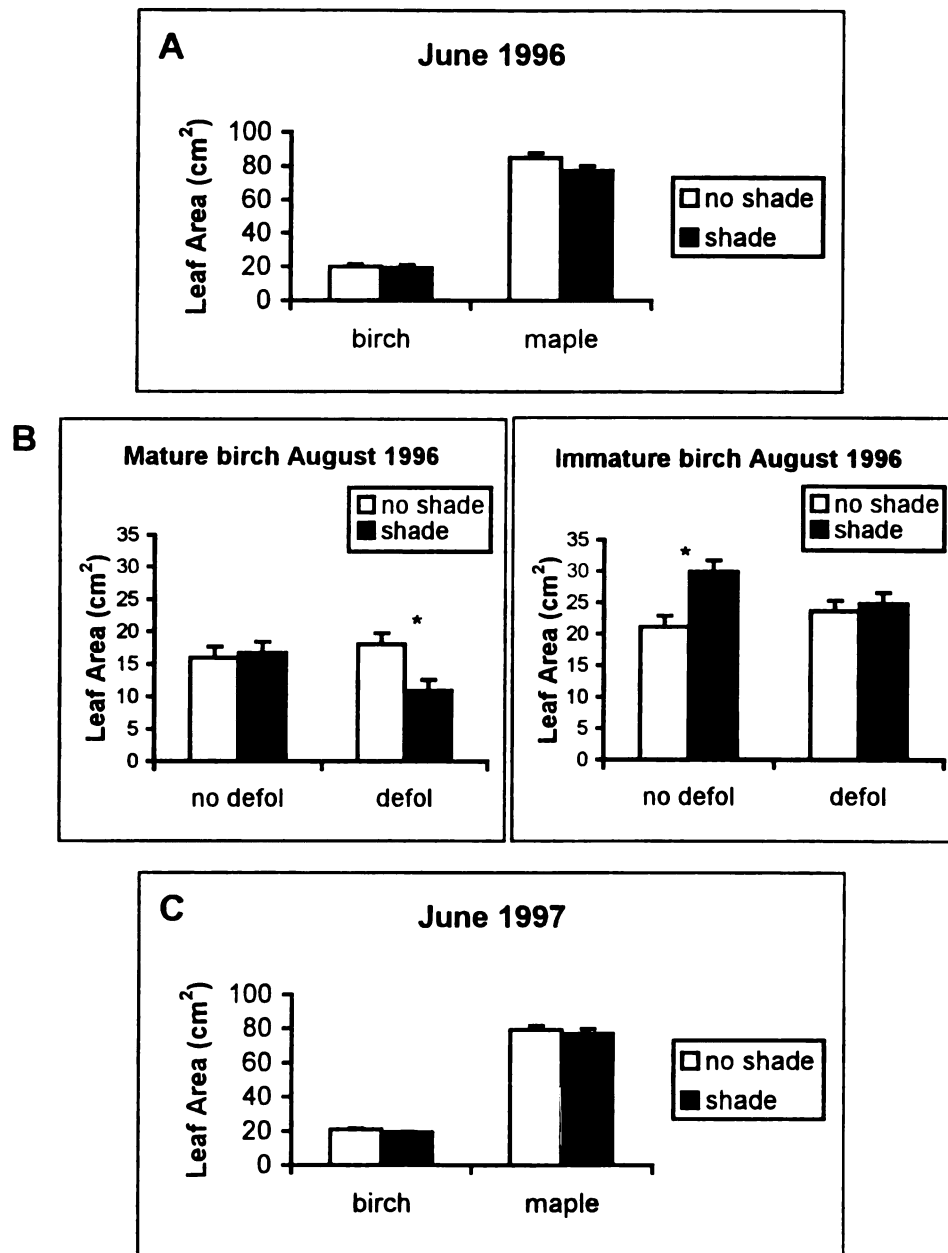


Figure 2.3. Effects of light on the leaf area of paper birch and sugar maple foliage in June (A) and August (B) of 1996 and June of 1997 (C). Asterisks indicate significant effects of the light treatment within species or leaf age classes ($p \leq 0.05$). See text for description of leaf age classes.

the specific leaf mass of both mature and immature foliage from birch trees was lower when grown in shade relative to full sun ($p \leq 0.05$ and $p \leq 0.10$, respectively) (Figure 2.2). In 1997 there was no evidence of prior season's shading on birch or maple SLM (Table 2.5a, Figure 2.2).

Shade did not affect the leaf area of birch or maple in June of 1995 or 1996, but did have an effect on birch leaf area measured in August of 1996 (Tables 2.4b and 2.5b). Shade increased the area of immature birch foliage, but only on trees that had not been defoliated (significant light*defoliation interaction, Table 2.5b, Figure 2.3). Shade also influenced the effect of defoliation on the leaf area of mature birch foliage (see below). Prior seasons' shading did not affect the leaf area of birch or maple in 1997 (Table 2.5b).

Nutrient Effects on Specific Leaf Mass and Leaf Area. The nutrient treatment did not affect the SLM of maple in June of 1995, 1996 or 1997, or the SLM of old or new birch foliage in August of 1996 (Tables 2.4a and 2.5a). In June of 1996, the SLM of birch foliage was greatest for non-shaded trees that had not been fertilized (significant light*nutrient interaction, Table 2.5a).

Fertilization did not affect the leaf area of birch or maple in 1995, 1996, or 1997 (Tables 2.4b and 2.5b).

Defoliation Effects on Specific Leaf Mass and Leaf Area. Both ANOVA (Table 2.5a) and correlation analyses indicated that defoliation had no effect on the SLM of maple in 1996 or 1997, perhaps due to the low levels of defoliation achieved.

In birch, defoliation did not affect the SLM of foliage sampled in June 1996. ANOVA indicated that the SLM of mature, but not immature foliage of defoliated birch collected in August was 8% higher ($57.15 \pm 1.19 \text{ g m}^{-2}$) than foliage from non-defoliated trees ($53.15 \pm 1.19 \text{ g m}^{-2}$, Table 2.5a). This was most likely an artefact of methodology and not indicative of true differences in SLM. Many of the defoliated birch were defoliated to the extent that the only foliage available for analyses was predominantly mid-rib with bits of leaf blade attached. Because the mid-vein is denser than the leaf blade, samples from such trees would therefore contain a higher proportion of mid-rib than samples of intact leaves.

There were no significant correlations between percent defoliation and the SLM of birch foliage sampled in June and August. There were also no delayed effects of defoliation on the SLM of birch in 1997, as indicated by either ANOVA or correlation analyses.

Defoliation in 1996 did not affect the leaf area of maple or birch foliage collected in June of that year or in June of 1997. However, the leaf area of mature but not immature birch foliage collected in August of 1996 was lower on defoliated trees in the shade (significant light*defoliation interaction, Table 2.5b, Figure 2.3). This result may have been observed because in many cases mature leaves remaining on defoliated trees had some leaf area removed by herbivores and therefore had lower leaf areas than leaves on non-defoliated trees. The restriction of this defoliation effect to shaded trees may reflect a preference for the defoliators to feed in shaded areas. Although there was no

statistically significant effect of shade on the defoliation treatment, there was a trend ($p = 0.08$) for defoliation to be greater in the shade.

Resource Acquisition.

Net Photosynthesis Under Ambient Conditions.

Statistical comparisons of relative photosynthetic rates of birch and maple could not be made as species effects were confounded by dates of measurement. However, in agreement with light response curves, the photosynthetic rate of birch ($7.49 \pm 0.27 \mu\text{mol m}^{-2} \text{s}^{-1}$) was higher than that of maple ($4.89 \pm 0.30 \mu\text{mol m}^{-2} \text{s}^{-1}$) as measured under ambient conditions in June 1996. Date of measurement did not significantly affect photosynthetic rates and did not interact with any treatment (data not shown).

In birch, shade reduced photosynthetic rates ca. 50% relative to trees in full sun on both measurement dates, although this effect was only statistically significant on the second date of measurement (for the first date, $p = 0.057$; Table 2.6, Figure 2.4). In maple, the light treatment did not have a significant effect on photosynthetic rates, although trends of decreased photosynthesis in the shade were apparent on both dates of measurement (Table 2.6, Figure 2.4). There was no effect of fertilization or defoliation on the photosynthetic rates of birch or maple (Table 2.6).

The stomatal conductance of maple ($0.09 \pm 0.01 \mu\text{mol CO}_2 \text{ cm}^{-1} \text{s}^{-1}$) tended to be lower than that of birch ($0.22 \pm 0.01 \mu\text{mol CO}_2 \text{ cm}^{-1} \text{s}^{-1}$), corresponding with lower photosynthetic rates. Although again no statistical comparisons can be made due to

Table 2.6. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on net photosynthesis and stomatal conductance of paper birch and sugar maple under ambient conditions. Photosynthesis was measured on two dates for each species in 1996. *F*-values for light were calculated with block*light as the error term.¹

BIRCH				
Source of Variation	<i>df</i>	Assimilation 10 July	Assimilation 16 July	Stomatal Conductivity 10 July
Block	2	0.22	0.41	1.96
Light	1	16.12 #	32.39 *	0.00
Block*Light	2	1.60	0.79	2.59 #
Nutrient	1	1.21	1.08	0.28
Light*Nutrient	1	2.59	0.13	3.71
Defoliation	1	0.14	0.53	0.00
Light*Defoliation	1	1.68	1.05	0.17
Nutrient*Defoliation	1	0.16	3.67 #	0.03
Light*Nutrient*Defoliation	1	0.42	0.52	0.28
Error <i>df</i>		35	35	36
MAPLE				
Source of Variation	<i>df</i>	Assimilation 11 July	Assimilation 22 July	Stomatal Conductivity 11 July
Block	2	0.40	3.31 #	0.82
Light	1	1.85	3.38	1.50
Block*Light	2	0.20	1.25	1.97
Nutrient	1	0.21	0.07	0.11
Light*Nutrient	1	0.36	0.11	0.48
Defoliation	1	0.18	1.56	0.13
Light*Defoliation	1	1.83	0.24	1.34
Nutrient*Defoliation	1	0.85	1.42	0.42
Light*Nutrient*Defoliation	1	0.74	0.27	2.94 #
Error <i>df</i>		33	33	33

¹ # = $p \leq 0.10$. * = $p \leq 0.05$.

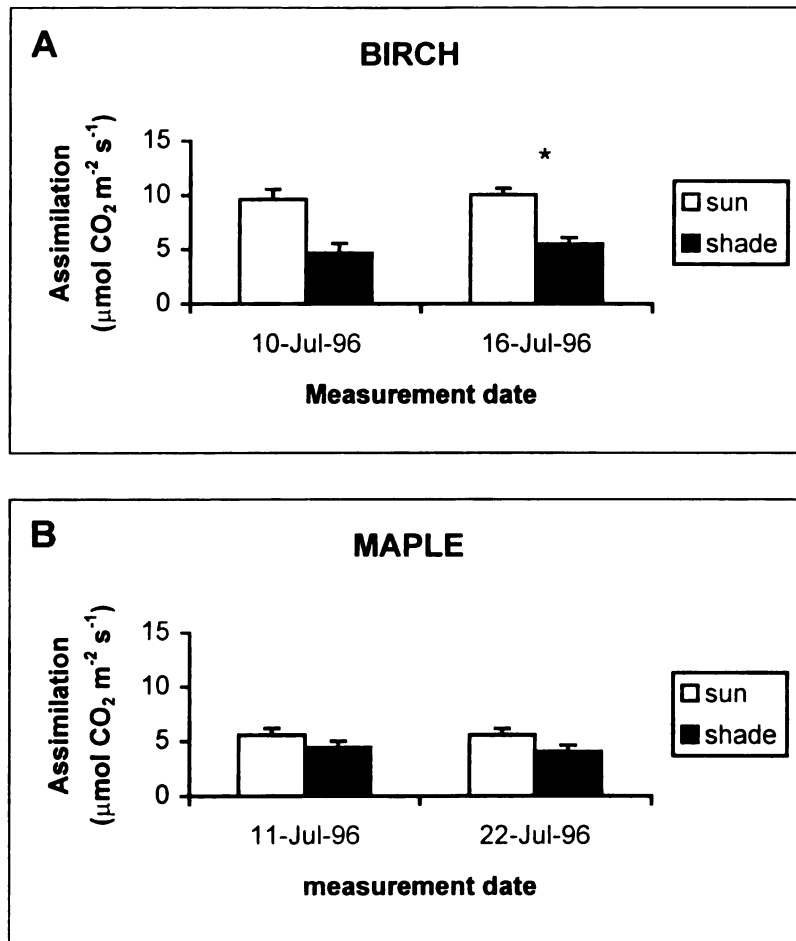


Figure 2.4. Light effects on net photosynthesis of paper birch and sugar maple. Asterisks indicate significant effects of light within species ($p \leq 0.05$).

Table 2.7. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, and nutrient effects and their interactions on carbon content (%C), nitrogen content (%N) and carbon/nitrogen ratios (C/N ratio) of paper birch and sugar maple foliage collected in June 1995. There was no defoliation treatment in 1995. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Birch		%N	C/N ratio	Maple		%N	C/N ratio
		%C	%N			%C	%N		
Block	2	0.46	0.05	1.02	0.18	0.25	0.81		
Light	1	0.40	5.36	0.09	0.74	22.76 *	0.20		
Block*Light	2	0.08	0.14	1.14	0.00	0.16	1.44		
Nutrients	1	0.41	0.10	0.16	2.38	4.41 *	0.46		
Light*Nutrients	1	0.67	0.15	2.51	2.33	0.04	0.07		
Error <i>df</i>		40	40	40	37	37	37		

¹ * = *p* < 0.05.

Table 2.8. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on carbon content (%C), nitrogen content (%N) and carbon/nitrogen ratios (C/N ratio) of paper birch and sugar maple foliage collected 11 June 1996. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Birch			Maple		
		%C	%N	C/N ratio	%C	%N	C/N ratio
Block	2	3.41 *	0.15	0.16	3.30 *	2.44	1.40
Light (block*light as error term)	1	13.05 #	15.39 #	12.77 #	0.09	2.11	1.89
Block*Light	2	0.16	1.69	2.29	2.81 #	2.52 #	2.59 #
Nutrient	1	2.44	3.58 #	3.42 #	0.81	3.29 #	3.55 #
Light*Nutrient	1	1.48	2.55	3.57 #	0.76	0.14	0.02
Defoliation	1	0.00	1.92	3.29 #	1.77	0.16	0.27
Light*Defoliation	1	0.28	0.27	1.54	0.05	0.14	0.05
Nutrient*Defoliation	1	0.11	1.17	2.78	0.95	0.75	1.09
Light*Nutrient*Defoliation	1	0.78	4.60 *	5.80 *	0.74	0.30	0.25
Error <i>df</i>		36	36	36	33	33	33

¹ # = $p < 0.10$, * = $p < 0.05$.

Table 2.9. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on carbon content (%C), nitrogen content (%N) and carbon/nitrogen ratios (C/N ratio) of paper birch foliage originating from buds formed the previous season ("Mature birch") and from the current season's leaf primordia ("Immature birch") collected 1 August 1996. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Mature birch			Immature birch		
		%C	%N	C/N ratio	%C	%N	C/N ratio
Block	2	0.56	1.30	0.47	0.02	0.12	0.34
Light	1	83.40 *	16.40 #	22.75 *	0.05	5.84	11.01 #
Block*Light	2	0.03	2.73 #	2.21	0.97	2.58 #	1.58
Nutrient	1	2.02	3.46 #	8.47 **	0.39	1.68	1.02
Light*Nutrient	1	3.94 #	6.21 *	4.59 *	0.49	0.38	1.01
Defoliation	1	0.04	6.86 *	5.24 *	3.85 #	0.95	0.08
Light*Defoliation	1	0.56	1.64	0.83	0.67	0.01	0.18
Nutrients*Defoliation	1	3.41 #	5.80 *	2.68	0.81	0.28	1.54
Light*Nutrient*Defoliation	1	1.26	0.72	0.95	0.88	7.30 *	11.15 **
Error <i>df</i>		36	36	36	36	36	36

¹ # = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$.

Table 2.10. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on carbon content (%C), nitrogen content (%N) and carbon/nitrogen ratios (C/N ratio) of paper birch and sugar maple foliage collected 16 June 1997. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Birch			Maple		
		%C	%N	C/N ratio	%C	%N	C/N ratio
Block	2	0.96	0.23	0.52	7.38 **	0.83	0.54
Light	1	2.32	1.88	0.16	0.28	2.77	0.77
Block*Light	2	3.95 *	1.45	3.60 *	6.90 **	0.47	1.33
Nutrient	1	0.85	2.65	3.77 #	2.98 #	10.25 **	6.43 *
Light*Nutrient	1	1.63	4.27 *	3.93 #	0.56	0.03	0.03
Defoliation	1	0.13	2.70	4.18 *	0.18	2.40	3.13 #
Light*Defoliation	1	0.43	0.21	0.37	0.01	0.09	0.01
Nutrient*Defoliation	1	0.71	0.02	0.81	1.53	1.48	1.15
Light*Nutrient*Defoliation	1	0.06	1.44	3.16 #	0.96	0.11	0.77
Error <i>df</i>		36	36	36	31	31	31

¹ # = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$.

confounding date effects. Stomatal conductivity in both species was not affected by light, nutrient, or defoliation treatments (Table 2.6).

Foliar Carbon and Nitrogen.

Intrinsic Differences in Carbon and Nitrogen Concentration and C/N Ratios.

Birch foliage had greater carbon (C) and nitrogen (N) concentrations, and lower C/N ratios than did maple foliage in 1995, 1996, and 1997 (Tables 2.3, 2.7, 2.8, 2.10), with the exception that in 1995 there was no difference in carbon concentration.

In August of 1996, immature and mature birch leaves did not differ from each other in carbon or nitrogen composition, or in the ratio of C to N (Tables 2.3 and 2.9). Both nitrogen and carbon content declined and C/N ratios increased in birch over the growing season, as indicated by comparison of birch foliage sampled in June and in August.

Light Effects on Foliar Carbon and Nitrogen Concentration and C/N Ratios. In the first year of the light treatment (1995), light had no effect on the foliar carbon content or C/N ratios of either species (Table 2.7). In maple, but not birch, shade was associated with an 8% increase in foliar nitrogen content (Table 2.7).

In June of 1996, the second season of shading, there was again no main effect of light on foliar carbon levels or C/N ratios, nor was there an effect of light on the nitrogen content of birch or maple foliage sampled in June (Table 2.8). Although effects were not statistically significant, shade tended to increase carbon content ($p = 0.0688$) and nitrogen content ($p = 0.0593$) and to decrease C/N ratios ($p = 0.0702$) of birch foliage sampled in

June. Light effects on birch foliage did become statistically significant as the season progressed and leaves aged. In August, shade decreased foliar carbon content by 2%, increased C/N ratios by 20%, and tended ($p = 0.0559$) to increase the nitrogen content of mature but not immature birch foliage. Seasonal effects of light treatments on maple were not monitored.

Light availability had no long-term effects on leaf chemistry. In 1997, all trees were exposed to ambient light conditions as may occur in previously shaded trees following gap formation. There was no evidence of differences in foliar carbon, nitrogen, or C/N ratios between birch of maple trees that had been shaded and those which had always existed in full sun (Table 2.10).

Nutrient Effects on Foliar Carbon and Nitrogen Content and C/N Ratios. In 1995, the first year of the nutrient treatment, fertilization of maple increased foliar nitrogen 9% (Table 2.7, Figure 2.5), but had no effect on foliar carbon or C/N ratio. In birch, however, fertilization had no effect on any of these foliar traits.

In June of 1996, the effect of fertilization on foliar nitrogen content of maple was no longer statistically significant. There also was no effect of fertilization on foliar carbon content or C/N ratio of birch and maple, or on foliar nitrogen content of birch. As the season progressed, however, mature foliage of birch grown in full sun responded to fertilization with a 16% increase in foliar nitrogen, and a corresponding 14% reduction in C/N ratio. In contrast, mature foliage of shaded birch did not respond to fertilization (light*nutrient interaction, Table 2.9). Fertilization had no effect on the carbon or nitrogen content or C/N ratio of immature birch foliage (Table 2.9).

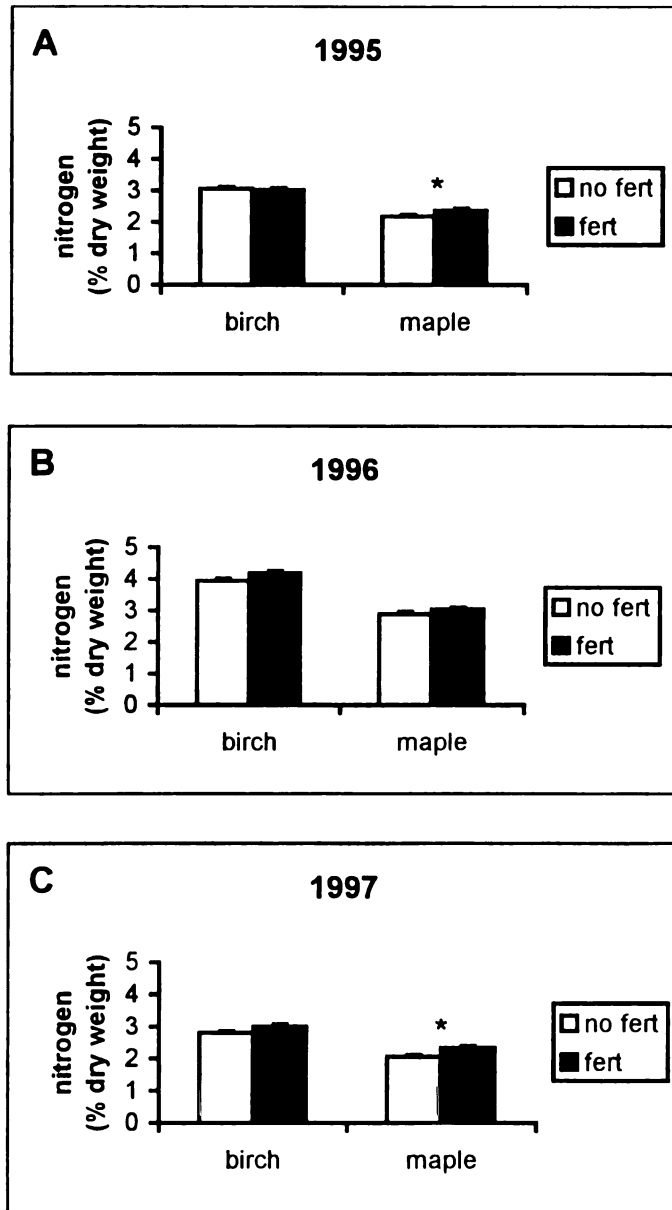


Figure 2.5. Effects of nutrient treatment on the foliar nitrogen content of paper birch and sugar maple in June of 1995 (A), 1996 (B), and 1997 (C). Asterisks indicate significant effects of fertilization within species ($p \leq 0.05$).

In 1997, fertilization increased the foliar nitrogen content of maple by 14% (Figure 2.5), and decreased C/N ratios by 10%, but had no effect on carbon content (Table 2.10). Although fertilization had no effect on the nitrogen content of birch in 1995 and 1996, in 1997 fertilization increased the nitrogen content of birch by 15%. Although trees were not shaded in 1997, fertilizer only affected birch trees that had not been shaded the previous two seasons (1995 and 1996) (light*nutrient interaction, Table 2.10). Nitrogen content of previously shaded birch was not affected by fertilization (Table 2.10). Fertilization did not affect C/N ratios or the carbon content of birch in 1997.

Defoliation Effects on Foliar Carbon and Nitrogen Concentration and C/N

Ratios. Defoliation did not affect foliar carbon content, but did decrease foliar nitrogen and C/N ratios. Fertilization often mitigated the effects of defoliation on leaf chemistry.

Defoliation during spring of 1996 had no effect on foliar carbon or nitrogen, or on C/N ratios of maple foliage sampled in June (Table 2.8). However, in maple there was a weak positive correlation between percent defoliation and levels of foliar nitrogen ($r = 0.33$, $p = 0.026$, $n = 45$; Figure 2.6), and a corresponding weak negative correlation between percent defoliation and C/N ratio ($r = -0.29$, $p = 0.047$, $n = 48$).

In 1996, birch foliage from defoliated trees generally had a lower nitrogen content and a greater C/N ratio than foliage from non-defoliated trees. This effect was present both in June and August, was influenced by light treatments, and was negated by fertilization. Defoliation had no effect on the foliar nitrogen content or C/N ratio of fertilized birch in June or August of 1996. However, in non-fertilized birch trees grown in full sun, defoliation decreased foliar nitrogen by 17% and increased the C/N ratio by

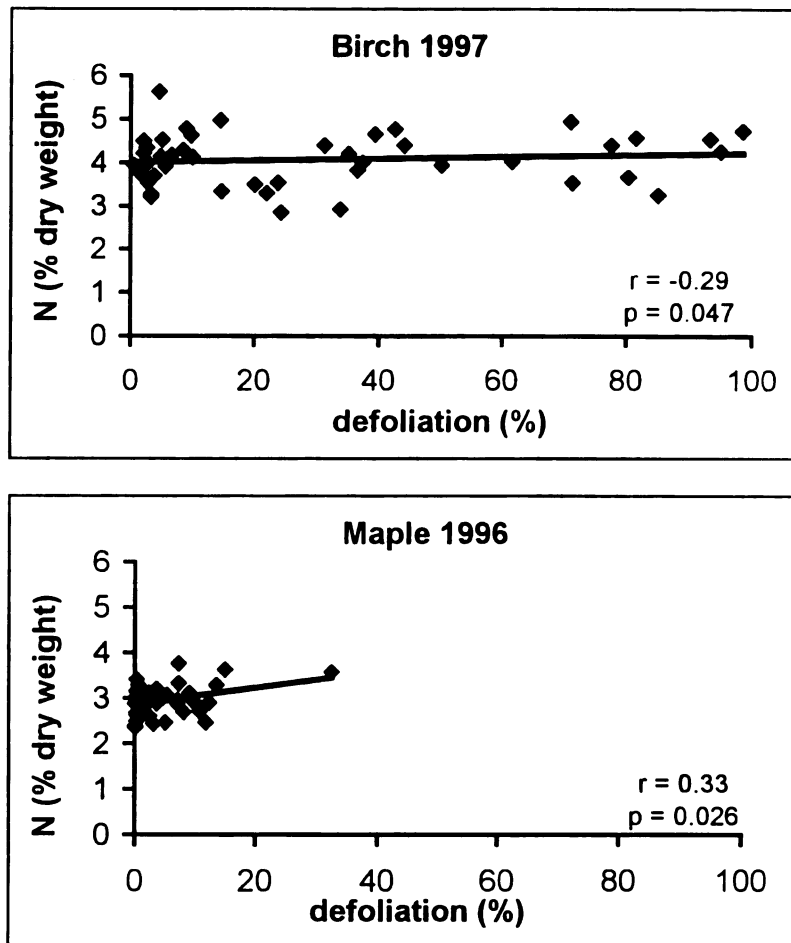


Figure. 2.6. Correlations between percent defoliation by forest tent caterpillar in 1996 and foliar nitrogen content in paper birch in 1997 (A) and sugar maple in 1996 (B).

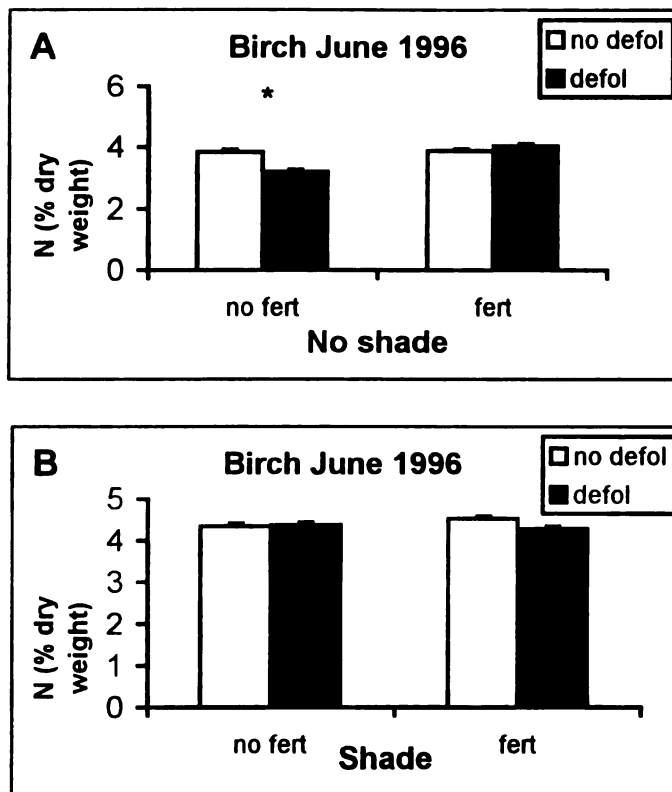


Figure 2.7. Effects of fertilization and defoliation on the foliar nitrogen content of paper birch in full sun (A) and in shade (B) in June of 1996. Asterisks indicate significant effects of defoliation within fertilization treatment ($p \leq 0.05$).

20% in the foliage sampled in June (light*nutrient*defoliation interaction, Table 2.8, Figure 2.7). There was no effect of defoliation on the nitrogen content of shaded birch.

Similar patterns were seen later in the season. As in June, the nitrogen content in mature birch foliage harvested in August 1996 was reduced 15% on defoliated hosts, but only on those trees that had not been fertilized (significant nutrient*defoliation interaction, Table 2.9). Defoliation increased the C/N ratio of mature foliage in both fertilized and non-fertilized birch by 8%. Defoliation also decreased the nitrogen content of immature leaves from non-fertilized birch trees by 13%, but only for those trees grown in full sun (light*nutrient*defoliation interaction, Table 2.9).

In 1997, both ANOVA (Table 2.10) and correlation analyses indicated that defoliation had no effect on carbon or nitrogen content, or C/N ratios of maple foliage. In birch, previous season's defoliation had no effect on C or N content, but did increase foliar C/N ratio by 7% (Table 2.10). Correlation analyses did not reveal any relationship between percent defoliation and carbon content, nitrogen content, or C/N ratios in birch in 1997.

Resource Allocation.

Height and Radial Growth.

Intrinsic Differences in Birch and Maple Growth. Birch growth prior to implementation of treatments (1992-1993) was greater than that of maple (data not shown). At the beginning of 1994, although birch were three years younger, they were an average of 32 cm (12%) taller than maple. During the 1994 growing season, birch

Table 2.11. *F*-values and degrees of freedom (*df*) from ANCOVA of blocking, light, species, nutrient, defoliation effects, and their interactions on the trunk height and diameter growth during the 1994, 1995, and 1996 seasons with height and diameter in May of 1994 as covariates, respectively; and over-all trunk height at the end of 1994, 1995, and 1996, with May 1994 height as a covariate. Models for each year correspond to treatments in effect during that year. *F*-values for light were calculated with block*light as the error term.¹

1994				
Source of Variation	<i>df</i>	Diameter growth	Height growth	Over-all height
Block	2	0.13	0.41	0.41
Species	1	9.46 **	17.68 ****	17.82 ****
Nutrient	1	0.03	0.01	0.01
Species*Nutrient	1	0.07	0.29	0.28
May 1994 diameter or height (covariate)	1	0.33	0.89	66.56 ****
Error <i>df</i>		86	86	86

1995				
Source of Variation	<i>df</i>	Diameter growth	Height growth	Over-all height
Block	2	3.13 *	8.01 ***	4.82 *
Light	1	32.35 *	0.04	0.74
Block*Light	2	0.29	1.59	1.32
Species	1	1.44	1.92	14.36 ***
Species*Light	1	0.16	0.60	0.57
Nutrient	1	1.15	4.66 *	3.65 #
Species*Nutrient	1	0.10	1.10	0.34
Light*Nutrient	1	0.69	0.12	0.01
Species*Light*Nutrient	1	0.78	0.27	0.82
May 1994 diameter or height (covariate)	1	0.07	0.23	28.78 ****
Error <i>df</i>		80	77	77

1996				
Source of Variation	<i>df</i>	Diameter growth	Height growth	Over-all height
Block	2	6.93 **	0.85	5.39 **
Light	1	11.74 #	79.70 *	1.98
Block*Light	2	2.30	0.13	1.54
Species	1	0.01	1.15	14.91 ***
Species*Light	1	1.04	0.24	0.00
Nutrient	1	0.00	0.30	2.56
Species*Nutrient	1	0.61	0.25	0.11
Light*Nutrient	1	1.21	2.44	1.12
Species*Light*Nutrient	1	4.69 *	0.02	1.55
Defoliation	1	1.69	6.45 *	3.10 #
Species*Defoliation	1	0.08	3.24	1.77
Light*Defoliation	1	0.03	0.48	5.84
Species*Light*Defoliation	1	1.92	0.01	0.37
Nutrient*Defoliation	1	0.74	4.82 *	1.72
Species*Nutrient*Defoliation	1	0.42	0.01	0.33
Light*Nutrient*Defoliation	1	5.22 *	2.63	0.70
Species*Light*Nutrient*Defoliation	1	1.70	1.09	0.93
May 1994 diameter or height (covariate)	1	2.25	0.38	38.70 ****
Error <i>df</i>		72	69	72

¹ # = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

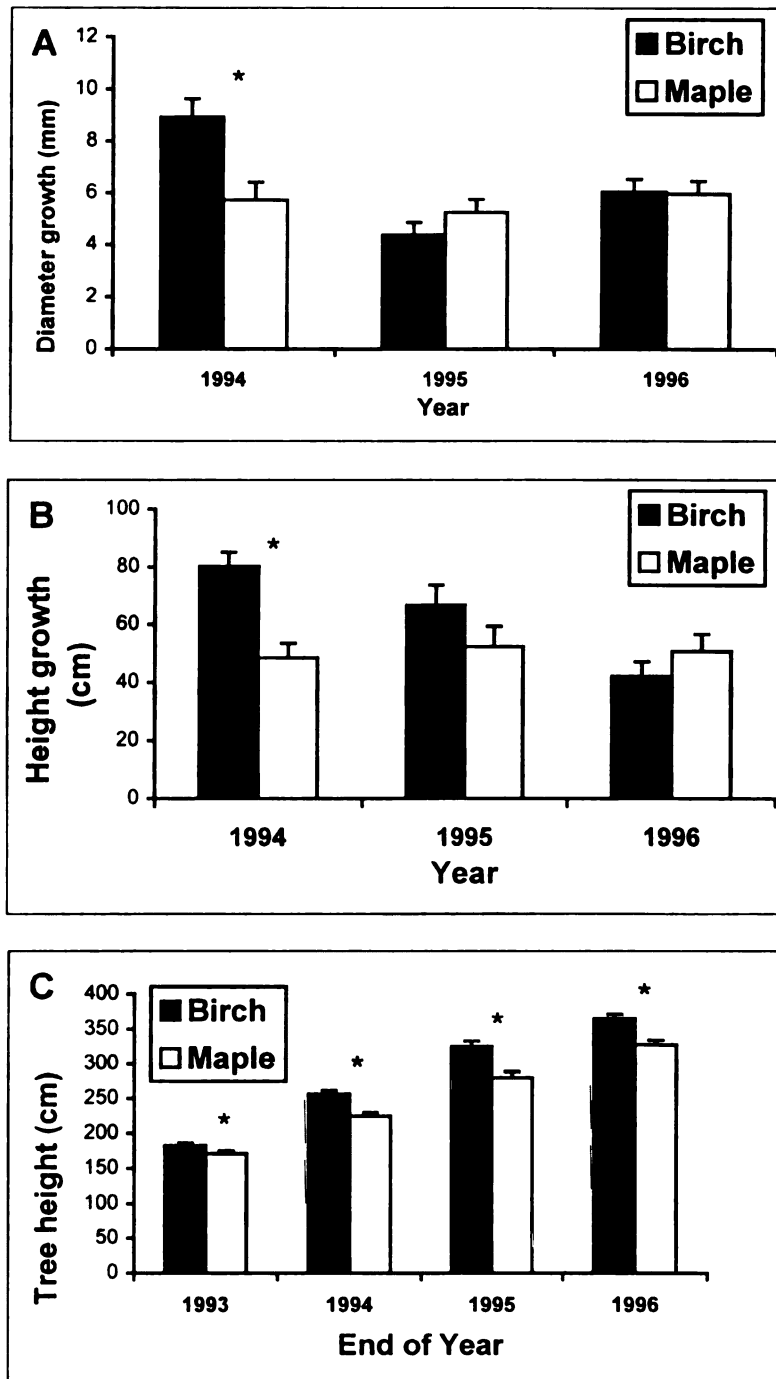


Figure 2.8. Diameter growth (A), height growth (B), and absolute height (C) of paper birch and sugar maple in 1994, 1995, and 1996. Asterisks indicate significant differences in species growth or height within years ($p \leq 0.05$).

growth in both height and diameter was greater than that of maple, as predicted based upon comparative life history strategies (Table 2.11, Figure 2.8).

From 1995-1996, height growth of both species was equal, but because of faster growth prior to 1994, birch remained significantly taller than maple. Both diameter and height growth of birch and maple were equivalent in 1995 and 1996 (Table 2.11, Figure 2.8). On average, birch was 46 cm (14%) taller than maple at the end of the 1995 growing season, and 38 cm (10%) taller than maple following 1996.

Light Effects on Birch and Maple Growth. Shade reduced both height and diameter growth of both species, but effects differed by year and were affected by interactions with other treatments. Effects of shade were not strong enough to affect the overall height of either birch or maple in 1995 or 1996. From 1992-1994, all trees grew in full sun. Shade treatments were first implemented in 1995, mimicking canopy closure with succession. That year, shade decreased diameter growth of birch by 44% and diameter growth of maple by 30% (Table 2.11, Figure 2.9). Height growth of both birch and maple was unaffected by shade in 1995.

In contrast, in the second year of the light treatment (1996), shade led to strong reductions in height growth of both species (41% reductions in birch and 45% in maple; Table 2.11, Figure 2.9). Shade reduced diameter growth in fertilized birch and in non-fertilized maple (significant species*light*nutrient interaction, Table 2.11, Figure 2.9). Shade interacted with nutrient and defoliation treatments (significant light*nutrient*defoliation interaction, Table 2.11). Birch and maple in full sun generally showed the greatest diameter growth in the absence of fertilization and defoliation. In

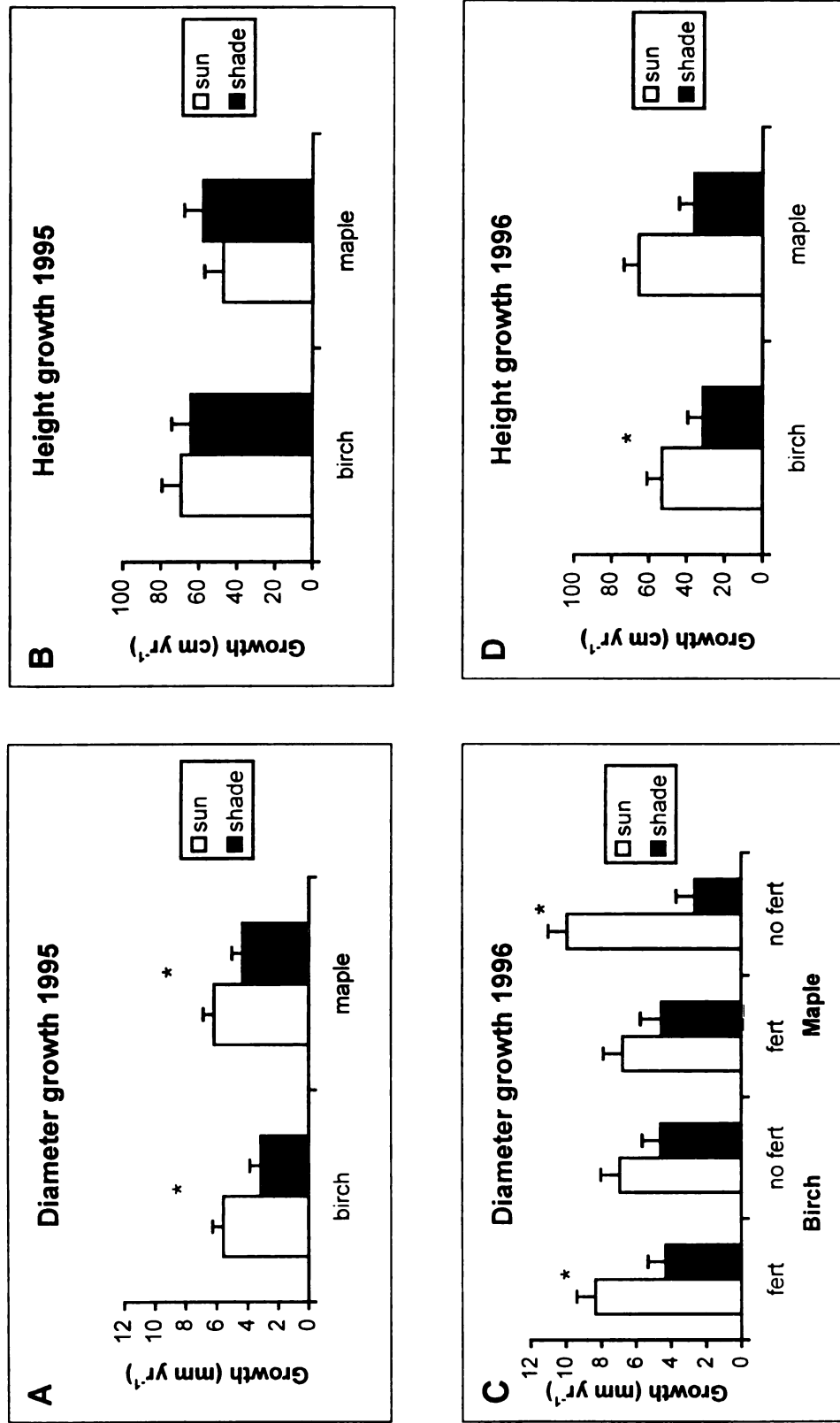


Figure 2.9. Effects of light availability on diameter growth (A) and height growth (B) in 1995 and on diameter growth (C) and height growth (D) of paper birch and sugar maple in 1996. Asterisks indicate significant effects of light within species ($p \leq 0.05$).

contrast, fertilization and defoliation had no effect on diameter growth of shaded trees (Figure 2.9).

Nutrient Effects on Birch and Maple Growth. Contrary to expectations, fertilization did not generally increase tree growth, and in some cases actually was associated with reductions in growth. During the first year of the treatment (1994), fertilizer had no effect on height or diameter growth, or on the over-all height of birch or maple (Table 2.11).

In 1995, fertilization did not affect diameter growth of either species, but unexpectedly reduced height growth by an average of 31%, (39% reduction in birch height growth and 19% reductions in maple height growth) (Table 2.11, Figure 2.10). The effect of fertilization on height growth was not substantial enough to result in treatment effects on over-all height.

In 1996, the third season of treatment, fertilization had no effect on height or diameter growth, or on over-all height of birch or maple (Table 2.11). However, fertilization did influence the effect of the light (see above) and defoliation (see below) on plant growth.

Defoliation Effects on Birch and Maple Growth. Forest tent caterpillar defoliation in 1996 reduced the height growth of birch and maple by 57% and 10%, respectively (Table 2.11, Figure 2.11). The effect of defoliation on height growth was strongest in fertilized trees, where defoliation reduced height growth 55% (nutrient*defoliation interaction, Table 2.11, Figure 2.11). Defoliation influenced the effect of shade on

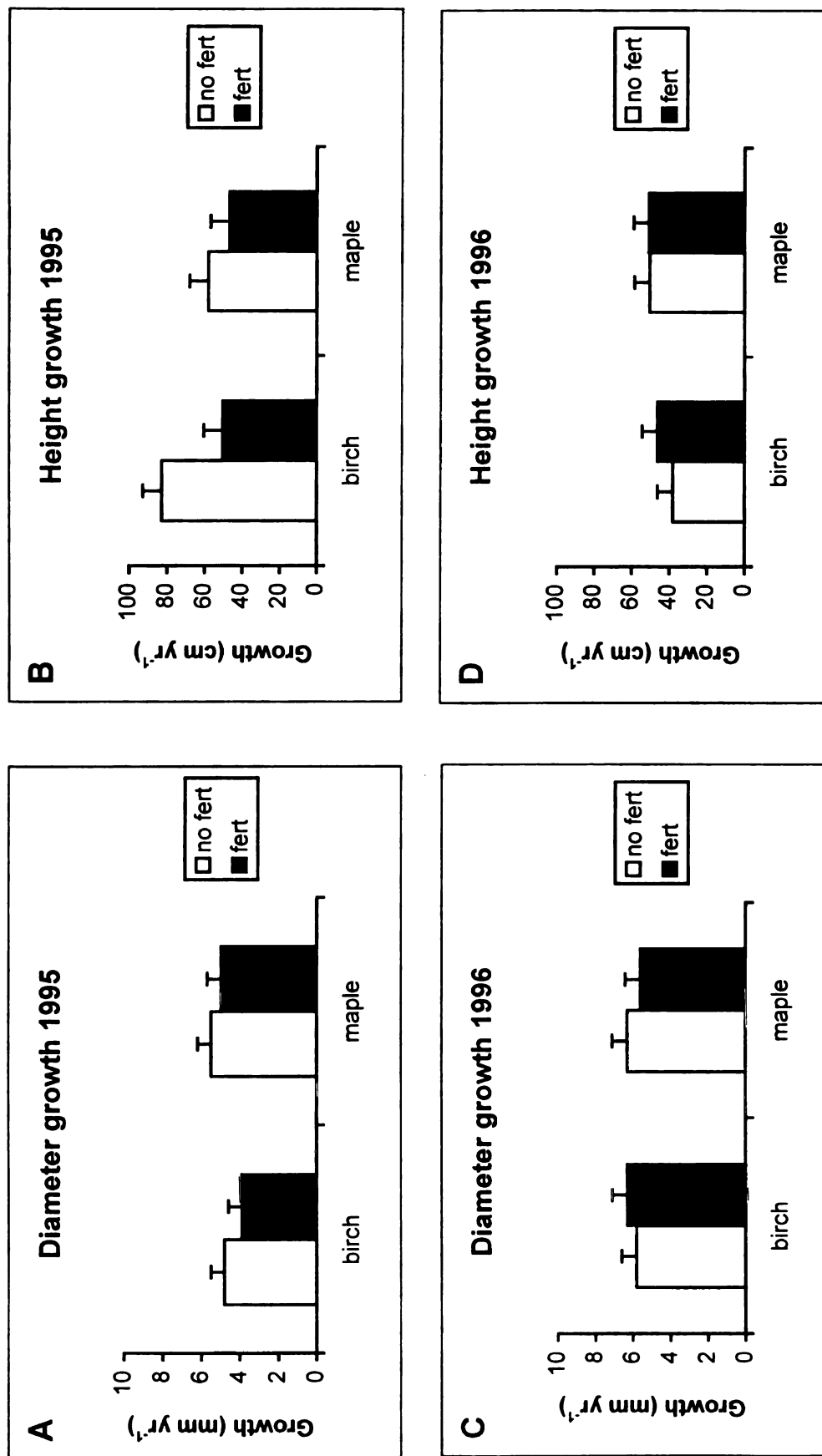


Figure 2.10. Effects of nutrient availability on diameter growth (A) and height growth (B) in 1995 and on diameter growth (C) and height growth (D) of paper birch and sugar maple in 1996. Asterisks indicate significant effects of fertilization within species ($p \leq 0.05$).

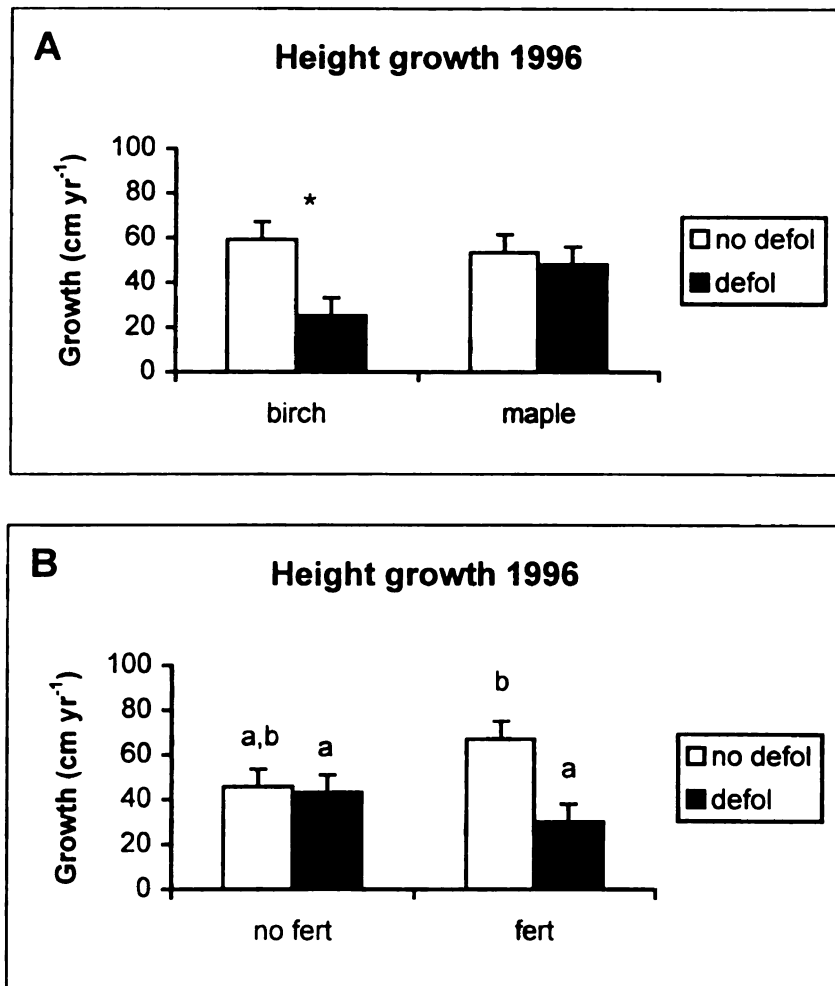


Figure 2.11. Effects of defoliation on height growth of paper birch and sugar maple in 1996 (A) and the influence of fertilization on the magnitude of the defoliation effect (B). In (A) asterisks indicate significant effects of defoliation within species ($p \leq 0.05$). In (B) bars with the same letter above them are not significantly different at $\alpha = 0.05$.

diameter growth of birch and maple in 1996, but did not influence over-all height of either species.

Foliar Condensed Tannin and Total Phenolic Concentrations.

Intrinsic Differences in Condensed Tannin and Total Phenolic Content. In 1996, birch and maple foliage sampled in June did not differ from each other in condensed tannin content, but in June of 1997 birch foliage had higher condensed tannin concentrations than did maple foliage (Table 2.3). Mature and immature birch foliage sampled in August of 1996 did not differ from each other in condensed tannin content, but had lower condensed tannin levels than birch foliage sampled in June of that year, reflecting a seasonal decline in condensed tannins in birch. Birch condensed tannin levels were lower in 1996 than in 1997 while maple condensed tannin levels were higher in 1996.

Total phenolic concentrations also varied between birch and maple. Birch appeared to have lower total phenolic concentrations than maple in 1996 (Table 2.3). As for condensed tannin content, mature and immature birch foliage sampled in August did not differ from each other in total phenolic content. There was no evidence of seasonal variation in birch total phenolic levels, as foliage sampled in June and foliage sampled in August had equivalent total phenolic contents. Total phenolic content was not measured in 1997, so no comparisons can be made across years.

Light Effects on Condensed Tannin and Total Phenolic Content. In June of 1996, shade was associated with a 37% reduction in birch condensed tannin levels, but did not

Table 2.12. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on total phenolic and condensed tannin content (% foliar dry weight) of paper birch and sugar maple foliage collected 11 June 1996. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Birch		Maple	
		total phenolics	condensed tannins	total phenolics	condensed tannins
Block	2	0.83	0.08	0.06	1.01
Light	1	8.06	36.23 *	19.18 *	2.29
Block*Light	2	0.80	0.22	0.68	3.11 #
Nutrient	1	1.74	1.76	0.98	0.60
Light*Nutrient	1	0.90	1.28	1.13	2.14
Defoliation	1	1.29	0.30	0.08	0.77
Light*Defoliation	1	0.54	0.17	0.42	0.18
Nutrient*Defoliation	1	0.20	0.06	0.01	0.37
Light*Nutrient*Defoliation	1	2.96 #	0.46	0.01	0.53
Error <i>df</i>		36	36	30	33

¹ # = $p < 0.10$, * = $p < 0.05$.

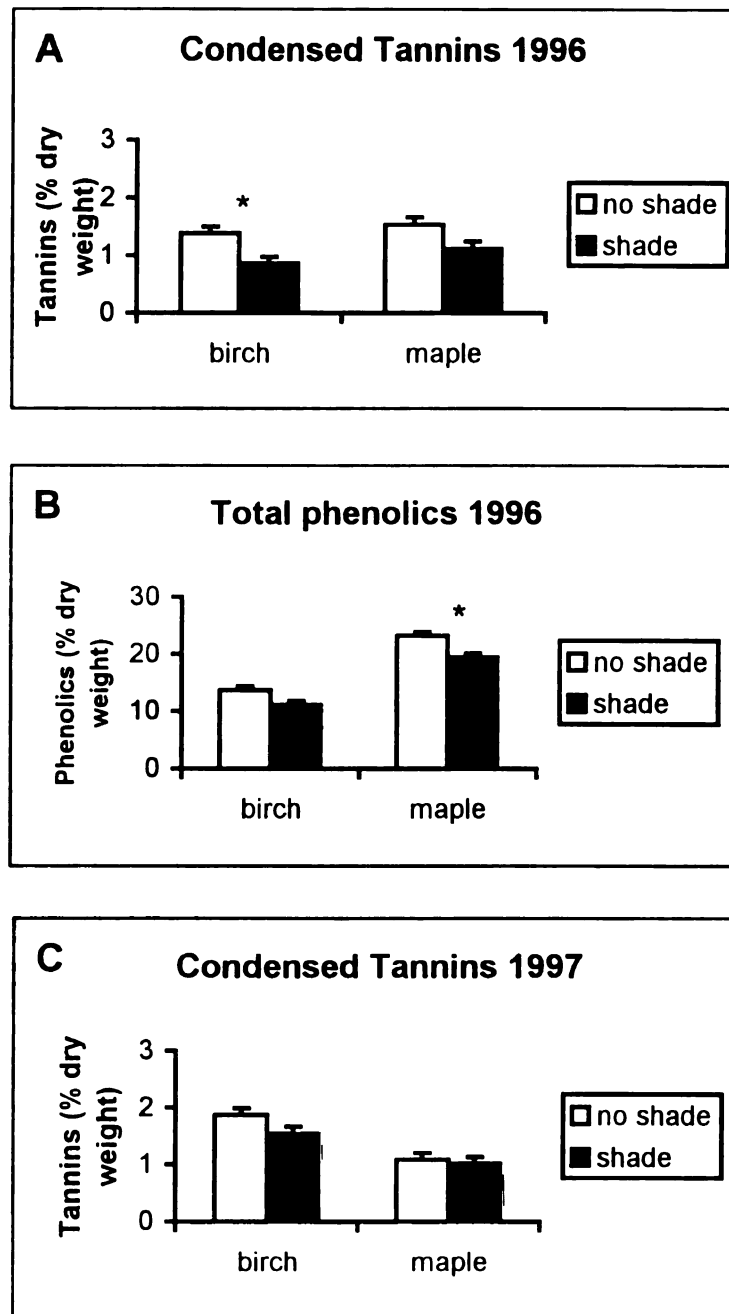


Figure 2.12. Effects of shade on the concentrations of carbon-based secondary metabolites of paper birch and sugar maple: condensed tannin levels in June of 1996 (A), total phenolic levels in June of 1996 (B), and condensed tannin levels in June of 1997 (C). Asterisks indicate significant effects of light treatment within species ($p \leq 0.05$).

Table 2.13. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on total phenolic and condensed tannin content (% foliar dry weight) of paper birch foliage originating from buds formed the previous season ("Mature birch") and from leaf primordia formed in the current season ("Immature birch") collected 1 August 1996. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Mature birch		Immature birch	
		total phenolics	condensed tannins	total phenolics	condensed tannins
Block	2	1.38		0.71	1.42
Light	1	3.66	637.04 **	6.24	12.81 #
Block*Shade	2	1.42	0.02	1.09	1.49
Nutrient	1	1.11	0.77	4.48 *	0.95
Light*Nutrient	1	0.82	0.02	0.42	0.03
Defoliation	1	1.65	0.02	0.22	0.00
Light*Defoliation	1	0.60	0.07	0.00	0.62
Nutrient*Defoliation	1	0.78	0.16	1.61	0.02
Light*Nutrient*Defoliation	1	0.90	0.06	0.30	0.19
Error <i>df</i>		36	36	36	36

¹ * = $p < 0.05$. ** = $p < 0.01$.

Table 2.14. *F*-values and degrees of freedom (*df*) from ANOVA of blocking, light, nutrient, and defoliation effects and their interactions on condensed tannin content (% foliar dry weight) of paper birch and sugar maple foliage collected 16 June 1997. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	1997 condensed tannins (% dry weight)	
		Birch	Maple
Block	2	1.04	1.50
Light	1	1.61	0.71
Block*Light	2	1.36	0.32
Nutrient	1	1.53	0.06
Light* Nutrient	1	0.13	3.27 #
Defoliation	1	0.01	1.14
Light*Defoliation	1	0.07	0.16
Nutrient*Defoliation	1	1.63	0.07
Light*Nutrient*Defoliation	1	0.11	0.01
Error <i>df</i>		36	32

¹ # = $p < 0.10$

influence total phenolic concentrations (Table 2.12, Figure 2.12). Conversely, in maple, shade reduced total phenolic concentrations by 17%, but did not influence condensed tannin concentrations (Table 2.12, Figure 2.12).

In late season birch, shade reduced the condensed tannin concentration of foliage from the mature age class by 35% and tended to reduce the condensed tannin concentration of immature birch foliage by 41% ($p = 0.0700$) (Table 2.13). As in June, shade did not affect total phenolic levels of August birch foliage.

In 1997, there was no evidence of an effect of prior season's shading on condensed tannin concentrations of birch or maple foliage (Table 2.14). Total phenolics were not measured in 1997.

Nutrient Effects on Condensed Tannin and Total Phenolic Content. Fertilization did not affect condensed tannin or total phenolic concentrations of birch or maple foliage in June of 1996 (Table 2.12) or condensed tannin concentrations of either species in June of 1997 (Table 2.14). However, in August of 1996, total phenolic concentrations of immature birch foliage were decreased 17% in fertilized treatments (fertilized: $11.37 \pm 0.78\%$, non-fertilized: $13.69 \pm 0.78\%$, Table 2.13). Condensed tannin levels of immature birch were not effected by fertilization. Neither condensed tannin nor total phenolic content of mature birch foliage was affected by fertilization (Table 2.13).

Defoliation Effects on Condensed Tannin and Total Phenolic Content. ANOVA indicated no significant rapid or delayed effects of defoliation on condensed tannin or

total phenolic content of foliage from either species in either 1996 or 1997 (Tables 2.12, 2.13, and 2.14). Correlation analyses, however, revealed weak negative relationships between defoliation and levels of condensed tannins in June sampled birch ($r = -0.29$, $p = 0.047$, $n = 48$) and immature (but not mature) leaves of birch sampled in August ($r = -0.30$, $p = 0.038$, $n = 48$).

DISCUSSION

Birch and maple differed in many physiological characteristics. Patterns of resource acquisition and allocation in both species were influenced by light, nutrient, and defoliation treatments, acting alone or in combination. While some generalizations can be made regarding the influences of these treatments in the present study, often each tree species responded differently to environmental stressors.

Intrinsic Differences in Paper Birch and Sugar Maple Physiology.

As predicted based on life history, shade-intolerant paper birch had higher photosynthetic capacities (i.e. higher light-saturated photosynthetic rates, indicated by light response curves), and correspondingly higher rates of carbon acquisition in both sun and shade than did maple. Although prior work has suggested that shade-tolerant species are more successful in low-light areas because of relatively higher photosynthetic rates at low irradiances but lower maximum assimilation rates in full sun than shade-intolerant species (Bazzaz 1979), this study affirms more recent work indicating that this is not the determinant of shade tolerance in all cases (Kobe et al. 1995). Shade tolerance in sugar maple cannot be explained by a lower respiration rate than birch (which would result in

more efficient utilization of acquired carbon), as birch and maple had equivalent respiration rates. However, this study only measured respiration over a short time frame (days) in mid July. There is likely to be diurnal and seasonal variation in respiration, as well as possible differences in whole plant (including root, trunk, and branch) respiration. First year paper birch have been shown to have higher respiration rates than first year sugar maple (Walters et al. 1993) and first year red maple (*Acer rubrum*, Kubiske and Pregitzer 1996). Phenological variation in allocation and metabolic processes may be very important in differential shade tolerance and successional mechanisms.

Birch had a greater specific leaf mass than maple in 1995, 1996 and 1997. This is likely related to the presence of higher nitrogen and photosynthetic apparatus in the leaves. Specific leaf masses appeared to be higher within species in 1997 than in 1996. This most likely reflects release of birch and maple from shade, which depressed specific leaf mass during the second year of shading (1996).

Corresponding to its higher photosynthetic rates, birch contained higher levels of foliar nitrogen than maple in all years examined. This may indicate higher nitrogen acquisition rates in addition to higher carbon acquisition rates, or may reflect intrinsic differences in N-allocation patterns between these species.

Also in agreement with life history predictions, paper birch exhibited greater growth (both in height and diameter) than sugar maple in the first year of the study. However, in subsequent years there were no differences between the species in height or diameter growth. This convergence in growth appeared to be due to decreased growth of birch over time, while growth of maple held constant (Figure 2.8). Although birch remained taller than maple throughout the experiment, if these trends were to continue

over time, maple would soon surpass birch in height. This would be expected to occur naturally, as the persistent maple replaces the pioneering birch. Even in cases where the species were able to coexist, maple would be expected to surpass birch in height, as maple trees reach 27-37 m (90-120 ft) in height while birch will reach only 21 m (70 ft) on average (Goodman et al. 1990, Safford et al. 1990). Similarly, birch can be expected to reach 25-30cm (10-12" dbh) and live 140 – 200 years (Safford et al. 1990), while maple can reach 79-91 cm (30-36") dbh and live 300 – 400 years (Goodman et al. 1990).

Reduction in height growth in birch over time is expected as individuals age. However, the slowed growth observed early in the trees' lives in this study should not necessarily be expected considering the highly competitive environment in which this species commonly occurs, and considering that birch do not reach reproductive maturity until 15 years of age (Safford et al. 1990). Birch responded more strongly to treatments than did maple, and reductions in birch height growth likely reflect these treatment effects. In 1995, birch height growth was unexpectedly reduced 31% by fertilization. In 1996, birch height growth was reduced 41% by shade and 57% by defoliation. These results correspond to the progressively reduced average height growth in birch over the course of the study.

Birch had lower concentrations of total foliar phenolics than maple, in agreement with other studies showing that pioneer species often allocate fewer resources to defense than late successional species (Bryant et al. 1985, Coley et al. 1985, Dudt and Shure 1994). In contrast to total phenolic trends, maple had higher levels of condensed tannins than birch. Condensed tannins are components of the total phenolics measure, and total

phenolics are only one of the array of defenses available to birch and maple. Birch also contains papyricific acid (in stems), a deterrent to browsing mammals and other herbivores (Bryant et al. 1992). Maple contains alkaloids, which are associated with deterrence of gypsy moth (Barbosa et al. 1990) and other forest Lepidopterans. Because plants contain a diversity of secondary metabolites which have defensive attributes, and because herbivores are not equally deterred by all defenses, it is not possible to make accurate statements of the relative strength of plant defense without reference to a specific herbivore or class of herbivores. Defensive quality may not be directly correlated with the quantity of resources allocated to particular secondary metabolites (see Chapter 3).

Seasonal and Age Related Changes in Birch Foliar Chemistry.

There were no strong differences in nutritive, chemical, and morphological traits of mature (short shoot) and immature (axial growth) birch foliage collected in August of 1996 although the age classes did differ in their sensitivity to treatments.

Carbon and nitrogen levels increased over the season as did SLM, while condensed tannin content decreased. Baldwin et al. (1987) found that hydrolyzable tannin content in yellow birch (*Betula allegheniensis*) decreased as the season progressed, while condensed tannin content increased toward midseason and then declined again as the season progressed.

It is possible that there were not stronger differences between old and new foliage because although the foliage differed in age, all foliage was fully expanded when sampled. Many differences in young and old foliage may be attributed to their differential source/ sink status, which in turn can affect the susceptibility of individual leaves to environmental stresses (Coleman 1986). The source/ sink transition normally occurs when leaves are two-thirds expanded, therefore the leaves sampled were probably acting as photosynthetic sources. Even so, differences do exist (apparently in traits not measured here) between birch mature and immature foliage. Swallowtail butterfly larvae (*Papilio* sp.) showed a preference for older foliage both in the lab (Harms unpublished data) and the field (personal observation). In other systems, younger foliage has been preferred by herbivores (Stamp and Bowers 1990, Faeth 1992).

Effects of Light on Birch and Maple Physiology.

The light treatment under which leaves developed did not affect light-saturated photosynthetic rates (A_{max}) in either birch or maple, indicating that there was no effective photosynthetic acclimation to shade, even though leaf morphology did respond to the light treatment (discussed below). In contrast, Riddoch et al. (1991) found higher initial slopes of light response curves and higher light-saturated photosynthetic rates in one pioneer and one climax tropical tree species under high light. Likewise, Thompson, Huang, and Kriedemann (1992) found a strong positive correlation between light-saturated photosynthesis and environmental light availability in both shade-tolerant and

shade-intolerant rainforest trees. Similar results have been found by other researchers (Turnbull 1991, discussed in Givnish 1988). Tropical species may be more responsive to light conditions when developing, as leaves in these species are generally longer-lived than those of deciduous temperate trees. Alternatively, low-light levels in the present study (20% ambient), while extreme enough to influence other aspects of plant physiology (discussed below), may not have been low enough to influence the efficiency of photosynthetic apparatus. Finally, shade supports in the present study were implemented shortly after budbreak, such that initial foliage expansion of all plants occurred in full sun as is the case in nature as expanding leaves provide little shade. It is possible that light-availability affects development of photosynthetic apparatus during differentiation of foliar tissues, and that acclimation potential is decreased after foliar expansion.

Although there was no effect of the light treatment on photosynthetic capacity, specific leaf mass and leaf area did respond to shading. Trees acclimated to temporal shifts in light availability in 1996 by decreasing leaf mass per unit area ratios (specific leaf mass). In maple, this response was evident early in the season (with June-sampled foliage) and was not associated with an increase in leaf area. In birch the response was not evident until August, and in immature foliage, corresponded to an increase in leaf area in non-defoliated but not defoliated trees. Decreased specific leaf mass may result from increased leaf size, from compositional differences in sun versus shade-grown foliage, or from a combination of these and other factors. By spreading out photosynthetic apparatus over a larger area by producing larger but thinner leaves, plants

may more efficiently harvest the limited incident radiation in shaded environments. Shade led to increased foliar nitrogen per unit mass in maple (discussed below), suggesting that decreases in other foliar components may have occurred. In 1997 the shade cloth was not erected, thus previously shaded trees experienced ambient light conditions as they might in nature after gap formation. Foliage on trees that were previously shaded had specific leaf masses equivalent to foliage on trees which had always grown in full-sun. This indicates that birch and maple trees may quickly adjust to gap formation.

Shade reduced photosynthetic rates of birch, but did not have a significant effect on maple photosynthesis, reflecting the greater tolerance of maple to shade and greater plasticity of pioneer species to environmental perturbation (Riddoch et al. 1991, Ashton and Berlyn 1994).

As predicted, shade reduced plant growth in both species. Height growth was equal in sun and shade during the first year of shading (1995), while diameter growth suffered. Equivalent height growth in sun and shade at a cost to diameter growth may be expected as plants attempt to compete for light. In 1996 height growth was not maintained but was decreased in shade, while there was no influence of light on diameter growth. Additionally, primordia for height growth are formed in the previous season, which may explain the lagging effect of shade observed in this study. Alternatively, trees may need to maintain optimal height/diameter ratios to ensure structural integrity. If carbon reserves from the previous year are used to drive height growth in the following

year, then shade in 1995 may have resulted in an insufficient carbon supply to maintain height growth in 1996.

Shade also increased foliar nitrogen content of maple during the first year of the treatment (1995) but did not influence the nitrogen content of birch foliage. Similarly, Kubiske and Pregitzer (1996) found that 84% shade increased foliar nitrogen in *Acer rubrum* (80% shade the present study) but had no effect on foliar nitrogen content of paper birch.

Although shade did not have a strong effect on foliar carbon or C/N ratios, it was associated with reductions in carbon-based defenses in both birch and maple in 1996, as predicted by the carbon/ nutrient balance hypothesis. However, birch and maple did not respond the same way. Total foliar phenolics of birch were not affected by shade, but the composition of phenolics changed, as condensed tannin content was reduced. Shade also induced compositional changes in maple phenolics, but in this case shade decreased total phenols and had no effect on condensed tannin levels. Ellsworth and Reich (1993) found that shade increased foliar phenolics in sugar maple. It is very likely that the compositional structure of plant defenses is more important than total levels of groups of compounds, as specific herbivores will respond to only some subset of the chemicals present (Kinney et al. 1997). Therefore, while it is clear that shade altered the composition of foliar defenses in birch and maple in this study, it is not clear how these alterations will affect their resistance to herbivores (see Chapter 3).

The effects of light on foliar defensive compounds disappeared in 1997 when trees were again exposed to full sun. This reinforces the conclusion that birch and maple respond quickly to long-term shifts in light availability such as may be experienced over the course of succession.

Effects of Nutrients on Birch and Maple Physiology.

Fertilization did not affect photosynthetic rates of birch or maple. However, fertilization did increase the foliar nitrogen content of sun-grown birch in August but not June of 1996. Increased foliar nitrogen levels are often associated with increased photosynthetic rates (Reich et al. 1991), therefore the lack of response of photosynthesis to fertilization in the present case is a bit surprising. This lack of response in birch could reflect the fact that photosynthesis was measured in July of 1996, which may have been before fertilization had its effect of increasing levels of nitrogen in birch foliage that year. Also, although foliar nitrogen content of birch was increased in August, this was measured on a per mass basis. Ellsworth and Reich (1992) found a significant relationship between light-saturated photosynthetic rates and nitrogen on a per leaf area but not per leaf mass basis. The distribution of nitrogen per unit area but not per unit mass may reflect optimal distribution of photosynthetic apparatus in foliage.

Surprisingly, fertilization reduced height growth of birch and maple in 1995. In contrast, increased nutrient levels have been shown to increase the growth rate of small birch (*Betula pendula*, Linder and Rook 1984, McDonald et al. 1986) and juvenile paper birch (*B. papyrifera*, Bryant et al. 1987). Observed reductions in height growth in 1995

may be the result of an interaction of fertilization with shade that was undetected that year. Although there was no significant effect of shade on height growth in 1995, a reduction of height growth in shaded treatments in 1996 was observed only in fertilized trees. Both shade and fertilization are expected to decrease plant C/N ratios, which might explain the interdependence of these two stressors. Height growth may be a function of C/N ratios in the plant. However, this speculation is not supported by the observation that although shade decreased height growth in 1996, there was no effect of shade on C/N in any year of this study.

In addition to fertilization being associated with reduced height growth, diameter growth was greatest in non-fertilized trees (with fertilization effects influenced by light and defoliation). Even so, for the most part fertilization had no effect on birch or maple growth rates. This indicates that nitrogen was not limiting in the non-fertilized treatments. In fact, nitrogen levels in fertilized treatments may have been sufficiently high such that some other micronutrient became growth-limiting. Subsequent effects of fertilization may be due to shifts in concentrations of one or more micronutrients, not in concentrations of nitrogen. This is supported by field observations of chlorotic leaves on both species, which may be indicative of manganese deficiency (data not shown).

Although nitrogen was not growth-limiting, increases in available nitrogen and micronutrients with fertilization did decrease defensive allocation in late season birch. Levels of total phenolics were decreased in young, fully expanded birch foliage of fertilized trees in August. This response was in agreement with carbon/ nutrient balance predictions although it was not accompanied by significant increases in C/N ratios in foliage of fertilized birch. In contrast to the August response, fertilization did not

influence C-based defenses of foliage sampled in June in either species in either year. These results suggest that subtle differences in mineral and/ or nutrient availability can influence defensive allocation and therefore herbivore resistance (Chapter 3), even when nutrient availability is not at levels low enough to depress growth. These effects may vary over the season, and are likely to depend on the plant species. Dudt and Shure (1994) found no influence of fertilization on phenolic levels or herbivory of dogwood or poplar trees.

Effects of Defoliation on Birch and Maple Physiology.

Defoliation was not associated with compensatory increases in photosynthesis in this study, although others have found increased photosynthesis in the remaining foliage of defoliated trees (Ovaska et al. 1992, Lovett and Tobiessen 1993). However, this study is not a strong test of defoliation effects on average photosynthetic rates as there is much canopy-wide variation in photosynthetic rates (Ellsworth and Reich 1993) and in this study photosynthesis was measured on only two leaves per tree.

Defoliation effects often mimicked those of nutrient deficiency in that fertilization often mitigated the effects of defoliation and *vice versa*. The depression of birch photosynthetic rates associated with fertilization was not seen in defoliated trees (see above). Defoliation decreased foliar nitrogen levels in non-fertilized birch. Correspondingly, defoliation was associated with increased C/N ratios, but this was true for both fertilized and non-fertilized trees. Hunter and Schultz (1995) also found

fertilization to mitigate defoliation effects in oak. Defoliation may cause nutrient deficiency because foliage contains the majority of the plant's nitrogen supply (Chapin et al. 1987), while other resources such as carbon are available from stored reserves in other plant parts.

Defoliation did reduce height growth in both species and there was no evidence that fertilization mitigated this effect. Because defoliation levels differed between birch and maple, no comparisons of relative species response can be made. However, Schaffler and Mason (1990) found herbivory to have a greater effect on growth in sun-grown rather than in shade-grown tropical trees.

Defoliation had no strong effects on levels of carbon-based defenses in maple foliage. This was most likely due to the low levels of defoliation experienced by this species. In birch, defoliation levels were more severe and were weakly negatively correlated with condensed tannin levels of June-sampled birch foliage in 1996, but no delayed effects of defoliation were observed in 1997. For insects sensitive to total phenols, this effect could lead to increased resistance of birch in the season of defoliation (see Chapter 3 for examination of foliage feeding Lepidopteran responses). Related to these observations, Dudt and Shure (1994) found herbivory and total phenolics to be inversely related on shade tolerant dogwood trees, but they found no relationship between herbivory and total phenolics in the shade intolerant tulip poplar. Faeth (1992) found increased hydrolyzable tannins, but lower condensed tannins in reflush foliage of defoliated oak relative to levels found in mature primary leaves.

Conclusions.

Paper birch and sugar maple trees differed in intrinsic physiological traits as predicted based upon life history considerations. Shade-intolerant paper birch had greater photosynthetic rates than shade tolerant maple. The higher level of shade tolerance in maple could not be explained by superior low light photosynthesis or respiration rates, as birch had greater photosynthetic rates at all light levels and respiration rates did not differ between species. Rather shade tolerance may be more dependent upon low light survival (Kobe et al. 1995) which may be influenced by patterns of carbohydrate storage (Kobe 1997). Paper birch grew faster than sugar maple, but was more likely to suffer reduced growth in response to environmental stress, especially shade. Birch also exhibited more plastic responses to shading and fertilization treatments. Although leaf morphology differed in sun and shade treatments, two years of shading did not affect photosynthetic light response curves in birch or maple, suggesting that neither species effectively acclimated to shade.

Resource acquisition in both species was influenced by shade, fertilization, defoliation, and the interaction of these stressors. Shade limited carbon acquisition of both birch and maple, and decreased foliar C/N ratios as predicted by the carbon/ nutrient balance hypothesis. However, this decrease in the C/N ratio was driven by increases in foliar nitrogen content, not by decreased carbon content which was not affected. Fertilization did not affect carbon acquisition, and there was only limited evidence of fertilization influencing nitrogen acquisition. It is not possible from these data to say if defoliation directly influenced nitrogen acquisition; however, defoliation was associated

with decreases in foliar nitrogen levels. This may indicate that nitrogen uptake was reduced in defoliated trees, perhaps as a result of decreased leaf area available for transpiration to drive root uptake. Alternatively, shifts in the allocation of available nitrogen may have occurred as nitrogen in the foliage was removed by herbivores.

Resource allocation patterns in both birch and maple were also influenced by shade, fertilization, and defoliation. Shade reduced growth of both tree species and altered the composition of foliar defenses. The carbon-based defense compounds of shaded trees differed compositionally from those of conspecifics grown in full sun. In birch, total phenolic levels remained constant although compositional changes occurred, while in maple total phenolic levels decreased. Shade decreased specific leaf mass, presumably resulting from the plant's attempts to maximize photosynthetic capacities under limiting light conditions. Fertilization also influenced resource allocation, but did not have effects as strong as those of shade. Fertilization decreased height growth in 1995 in both birch and maple, but overall did not have a strong effect on tree growth, suggesting that non-fertilized treatments were not strongly nitrogen limiting. In fact, fertilized treatments may have had a surplus of nitrogen resulting in other nutrient limitations. Fertilization did have subtle effects on defensive allocation in birch but did not influence the defensive allocation of maple. The effects of defoliation were often dependent upon the fertilization treatment of the plant even though fertilization was not generally growth-limiting. Defoliation resulted in reductions in allocation to height growth, but did not influence defensive allocation.

Shade, fertilization, and defoliation had influential effects on both resource acquisition and allocation in paper birch and sugar maple. Tree responses to these stresses were influenced by intrinsic characteristics, and therefore often differed among the two species. Moreover, the effects of one stress on tree health was often dependent upon simultaneous exposure to other stresses. The current understanding of plant responses to their environments will benefit greatly from more mechanistic studies aimed at determining the physiological steps involved in these responses. Only with a mechanistic understanding of plant responses will we be able to accurately predict the impact of interacting environmental stresses on individuals and populations.

CHAPTER 3:
EFFECTS OF LIGHT, NUTRIENT AVAILABILITY, AND DEFOLIATION ON
THE RESISTANCE OF PAPER BIRCH (*Betula papyrifera*) AND SUGAR MAPLE
(*Acer Saccharum*) TO CHEWING FOLIVORES

INTRODUCTION

Light and nutrient levels vary in forests as a result of natural events, such as gap formation when trees fall, and anthropogenic perturbations, such as logging, road construction, and nutrient loading from atmospheric nitrogen deposition (Vitousek et al. 1997). Herbivorous forest insects can be affected both directly and indirectly by abiotic factors such as light and nutrient availability, and by biotic factors such as defoliation of their host plants.

Shade stress has been associated with both increases (Bryant et al. 1987, Bultzman and Faeth 1988, Nichols-Orians 1991, Dale and Causton 1992, Lowman 1992, Shure and Wilson 1993, Johnson et al. 1997) and decreases (Coley 1983, Lincoln and Mooney 1984, Bultzman and Faeth 1988, Bassett 1991, Koricheva et al. 1998) in herbivory or herbivore densities. Changes in herbivore abundance and in observed amounts of herbivory (reflected in lost leaf area) can result from behavioural responses of herbivores to light. These can include increased abundance in areas of high light for purposes of thermoregulation or higher abundances in shaded areas where insects may be less visible to predators. Differences in total herbivory observed between sunny and

shaded areas may reflect differences in herbivore abundance, or indirect effects of light on herbivores mediated through changes in the quality of host foliage. Increased feeding, observed as increased amounts of herbivory, may reflect increased preference for a host or, alternatively, may reflect increased feeding to compensate for low nutritive quality of the host plant. To determine how host quality affects herbivore performance, physiological and fitness parameters such as growth rate, nutritional indices, survival, and fecundity must be examined. However, surprisingly few studies linking light environment to host quality have directly measured insect performance (but see Lawler et al. 1997).

Nutrient availability, like light availability, has been shown to have variable effects on herbivores. Fertilization most frequently has been implicated in increased performance of individual herbivores (reviewed in Kytö et al. 1996). Population-level effects are more variable, but for populations of leaf-chewing insects fertilization more frequently had negative effects than positive effects (Kytö et al. 1996). On some occasions, host nitrogen availability had no effect on herbivores (Floater 1997, Dudt and Shure 1994). It is unlikely that nutrient levels directly affect herbivore populations. Rather, effects are linked to the nutrient content of host foliage, nutrient-induced changes in concentrations of defensive chemicals, and to the effects of fertilization on community structure (e.g. increased ground cover supporting increased predator and parasitoid populations) (Scriber 1984, Kytö et al. 1996).

Defoliation-induced changes in host foliage often increase, and occasionally decrease, the host's resistance to the defoliating herbivore (Tuomi et al. 1988, Karban and Baldwin 1997) or other species with which it shares the host plant (reviewed in Denno et

al. 1995). Effects of defoliation on insect performance (growth, survival, fecundity) and herbivory may be observed during the same season (Haukioja 1980, Roland and Myers 1987, Hanhimaki 1989, Hunter 1987, Dankert 1995, Wold and Marquis 1997), or in following years (Roland and Myers 1987, Haukioja 1982, Haukioja 1990, Tuomi et al. 1990), and can be environmentally dependent (Dankert et al. 1997). Effects of severe defoliation on herbivores feeding concurrent to the defoliation event may reflect direct density-dependent behavioral or physiological changes in individuals in response to competition, in addition to changes in host quality. For example, individuals in high-density populations may disperse to escape competition, regardless of defoliation-induced changes in host quality.

Indirect effects of abiotic and biotic environmental stresses, including light, nutrients, and defoliation, on herbivores are mediated through changes in the quality of host foliage. This “quality” is often associated with levels of foliar nitrogen (Tuomi et al. 1984, Nichols-Orians 1991) and water content, which can be major limiting resources for insects (Scriber 1977, Mattson 1980, Scriber and Slansky 1981). Host quality is also a function of morphological traits of foliage such as the presence of trichomes and leaf thickness, and can be dependent on temporal synchronization of insect and plant phenology (Kolb and Teulon 1991). Host quality is often inversely related to levels of secondary metabolites (such as carbon-based polyphenols), which may play a defensive role (Feeny 1970, Haukioja et al. 1985, Johnson et al. 1997; but see Bernays 1981, Ayres et al. 1997). Stressed hosts may also protect themselves by attracting predators or parasitoids of their herbivores (Turlings et al. 1995). Nutrients, defensive chemicals, and morphological changes in foliage can slow the development time of insects and thereby

increase their time of exposure to predators and pathogens (Coley and Barone 1996). In Chapter 2, I discuss theoretical predictions regarding the effects of light, nutrient, and defoliation stress on these foliar traits and report observations on the effects of these stresses on paper birch and sugar maple. In the current chapter, I examine the performance of insects feeding on foliage of these trees to determine how treatment effects on host traits translate into effects on herbivore resistance.

The objectives of this study were to determine the indirect effects of defoliation, light, and nutrient availability on the lepidopteran defoliators gypsy moth (*Lymantria dispar*, (L.) Lymantriidae), forest tent caterpillar (*Malacosoma disstria*, Hubner, Lasiocampidae), and whitemarked tussock moth (*Orgyia leucostigma*, (J. Smith), Lymantriidae), as mediated through effects on the quality of sugar maple and paper birch.

Gypsy moth is an exotic species which was introduced into eastern North America from Europe in the 1860s (Michigan Department of Agriculture et al. 1984) and has since spread rapidly westward across the eastern United States. Because of its recent establishment, gypsy moth and forest tree populations in North America have not had an opportunity to co-evolve. A polyphagous feeder, gypsy moth eats a wide range of hosts including paper birch and sugar maple, which are abundant in the forests of the Great Lakes region. Gypsy moth is an outbreak species which undergoes cyclic changes in population sizes. It is univoltine in the United States and feeds early in the season as hatch occurs concurrent with bud break.

Whitemarked tussock moth is a native polyphagous folivore, and therefore has an evolutionary history with its North American hosts, including paper birch and sugar

maple. Whitemarked tussock moth is bivoltine in central Michigan, with the first generation feeding early in the season and the second generation feeding toward the middle to the end of the growing season. Second generation larvae hatch approximately six weeks after gypsy moth.

Forest tent caterpillar, like whitemarked tussock moth, is native to North America, ranging from northern Canada to northern Mexico (Fitzgerald 1995). Like gypsy moth, forest tent caterpillar is a univoltine, highly polyphagous outbreak species with a host range that includes paper birch and sugar maple. As early instars, forest tent caterpillar larvae are gregarious, but become solitary and wander upon reaching the fourth instar.

As indicators of insect performance, I measured survival, larval development time, pupal mass, relative growth rate, and nutritional indices of the insect species in 1996 and 1997. Insect bioassays were conducted concurrent with studies reported in Chapter 2, in which I reported effects of light, nutrient, and defoliation stress on a suite of foliar traits of paper birch and sugar maple (1996 data are summarized in Table 3.1 and 1997 data are summarized in Table 3.2). To elucidate potential mechanistic links between insect responses and foliar characteristics, I examined correlations between insect responses and foliar carbon, nitrogen, specific leaf mass, and polyphenols including total phenolic and condensed tannin concentrations. Insects were predicted to perform better (i.e. grow faster, develop more quickly, or have higher survival or pupal mass) on hosts with relatively high levels of foliar nitrogen and relatively low levels of total phenolics and condensed tannins. Predictions of how the responses of these variables to environmental treatments will affect insect performance are presented in Tables 3.1 and 3.2.

Table 3.1. Predicted effects of treatments on the performance (growth, pupal mass, and/ or duration of larval period) of chewing herbivores based on their effects on foliar traits in 1996 (percent change from control levels). Increases in foliar nitrogen and decreases in condensed tannin and/ or total phenolic content are predicted to be beneficial for chewing herbivores. Direction of light, nutrient, and defoliation treatment effects on foliar traits of paper birch and sugar maple relative to control levels are from Chapter 2.^{1,2}

Treatment	Paper birch			Sugar Maple		
		June	August		June	
SHADE	N	---	---	N	---	
	C	---	-2%	C	---	
	C/N	---	-20%	C/N	---	
	CT	-37%	-35%	CT	---	
	FD	---	---	FD	-17%	
	SLM	---	-15%	SLM	-20%	
Predicted insect performance:	improved		improved	improved		
FERTILIZATION	N	---	---	N	---	
	C	---	---	C	---	
	C/N	---	-9%	C/N	---	
	CT	---	---	CT	---	
	FD	---	---	FD	---	
	SLM	---	---	SLM	---	
Predicted insect performance:	no effect		no effect	no effect		
DEFOLIATION	N	---	-7%	N	---	
	C	---	---	C	---	
	C/N	---	+8%	C/N	---	
	CT	---	---	CT	---	
	FD	---	---	FD	---	
	SLM	---	+8%	SLM	---	
Predicted insect performance:	no effect		reduced	no effect		

¹Birch foliage was sampled in both June and August; maple was sampled only in June. Birch foliage sampled in August has flushed at budbreak ("mature foliage" *sensu* Chapter 2). Tussock moth fed on foliage closer in phenology to that sampled in August while gypsy moth fed on foliage closer in phenology to that sampled in June.

²Abbreviations: C= % carbon, N= % nitrogen, C/N= carbon/nitrogen ratio, CT= % condensed tannins, FD= % Folin- Denis reactive total phenolics, SLM= specific leaf mass (g/m²). CT, FD, C, and N were all measured in units of % foliar dry weight.

Table 3.1. Predicted effects of treatments on the performance (growth, pupal mass, and/or duration of larval period) of chewing herbivores based on their effects on foliar traits in 1997 (percent change from control levels). Increases in foliar nitrogen and decreases in condensed tannin and / or total phenolic content are predicted to be beneficial for chewing herbivores. Direction of light, nutrient, and defoliation treatment effects on foliar traits of paper birch and sugar maple relative to control levels are from Chapter 2.¹

Treatment	Paper birch		Sugar Maple	
PRIOR YEARS' SHADE	N	---	N	---
	C	---	C	---
	C/N	---	C/N	---
	CT	---	CT	---
	SLM	---	SLM	---
	Predicted insect performance: no effect		no effect	
FERTILIZATION	N	---	N	+14%
	C	---	C	---
	C/N	---	C/N	-10%
	CT	---	CT	---
	SLM	---	SLM	---
	Predicted insect performance: no effect		improved	
PAST YEAR'S DEFOLIATION	N	---	N	---
	C	---	C	---
	C/N	+7%	C/N	---
	CT	---	CT	---
	SLM	---	SLM	---
	Predicted insect performance: no effect		no effect	

¹ Abbreviations: C= % carbon, N= % nitrogen, C/N= carbon/nitrogen ratio, CT= % condensed tannins, SLM= specific leaf mass (g/m²), --- = no change. CT, FD, C, and N were all measured in units of % foliar dry weight.

An improved understanding of the mechanistic links between environmental factors and insect performance is essential for accurately predicting the effects of natural and human disturbance on herbivory in forested ecosystems. Understanding stress effects on gypsy moth and forest tent caterpillar is especially important as these species are the most important defoliators of deciduous forests in North America (Mattson et al. 1991). Furthermore, these environmental factors may mediate indirect interactions among herbivores. If defoliation alters host quality, then outbreaks of one species may naturally regulate the population dynamics of another through host-mediated interspecific competition. Data on stand susceptibility, strengthened by a mechanistic understanding of stress effects on tree resistance to herbivores, is essential for the effective stewardship of forested ecosystems.

MATERIALS AND METHODS

Experimental Site and Design and Treatments.

Studies were conducted with the same trees and research plots described in Chapter 2. Details of implementation of light, fertilization, and defoliation treatments are also provided in Chapter 2. To review, I examined two levels of light availability: 20% and 100% of ambient. Shading was implemented in 1995 and 1996 with the use of shade cloth. Fertilization levels were 0 and 225 kg ha⁻¹ yr⁻¹ of 18:5:9 NPK, with half administered at the beginning and the remainder at the end of the 1994, 1995, and 1996 growing seasons. In 1996, half of the trees were naturally defoliated by forest tent

caterpillar. Defoliation reached levels of (mean $\pm$ SE) $1 \pm 3\%$ and $8 \pm 3\%$ (range 0-32.5%) in sugar maple, and $6 \pm 3\%$ and $52 \pm 3\%$ (range 0.5-98.5%) in paper birch.

Insect Bioassays.

Bioassays with gypsy moth, whitemarked tussock moth, and forest tent caterpillar were conducted in laboratories at the Dow Gardens in Midland, MI in 1996 and at the Ohio Agricultural Research and Development Center, The Ohio State University, in Wooster, OH in 1997.

Gypsy moth.

In 1996, gypsy moth egg masses were obtained from the Canadian Forest Service, Insect Rearing Facility, Sault Ste. Marie, ON. Upon hatch (22 May), groups of 12 neonates were confined in arenas with detached foliage from one of each of the experimental trees. Egg hatch of lab-reared gypsy moth coincided with that of naturally occurring populations. Insects were reared in a growth chamber at 25°C with an 18L:6D photoperiod. Rearing containers were 150 x 25 mm diameter petri dishes with moistened charcoal-amended plaster bases that provided ample humidity for leaves to remain turgid between feedings. Foliage was changed three times per week, ensuring a constant and fresh food supply. Frass and uneaten leaves were removed from dishes as needed, at least once weekly and more often for later instars. To minimize growth chamber effects on insect growth, the position of individual dishes in the incubator were shifted on each feeding day.

Table 3.3. Abbreviations and calculation of nutritional indices. ¹⁻⁷

Nutritional index	Abbreviation	Units	Calculation
Relative growth rate	RGR	(mg mg ⁻¹ d ⁻¹)	$RGR = (\ln(\text{initial mass}) - \ln(\text{final mass})) / T$
Relative consumption rate	RCR	(mg mg ⁻¹ d ⁻¹)	$RCR = \text{consumption} / (W_e * T)$
Approximate digestibility	AD	(%)	$AD = (\text{assimilation} / \text{consumption}) * 100$
Efficiency of conversion of digested food to biomass	ECD	(%)	$ECD = (\text{growth} / \text{assimilation}) * 100$

¹ T = duration of study (days)
² consumption = initial food mass – final food mass
³ W_e (mean exponential mass) = (final mass – initial mass) / [ln (final mass/ initial mass)]
⁴ assimilation = consumption – mass of frass
⁵ growth = final insect weight – initial insect weight
⁶ Masses used for fourth and fifth instar indices are dry masses. RGRs of second instars were calculated as wet masses.
⁷ from Waldbauer 1968 and Gordon 1968

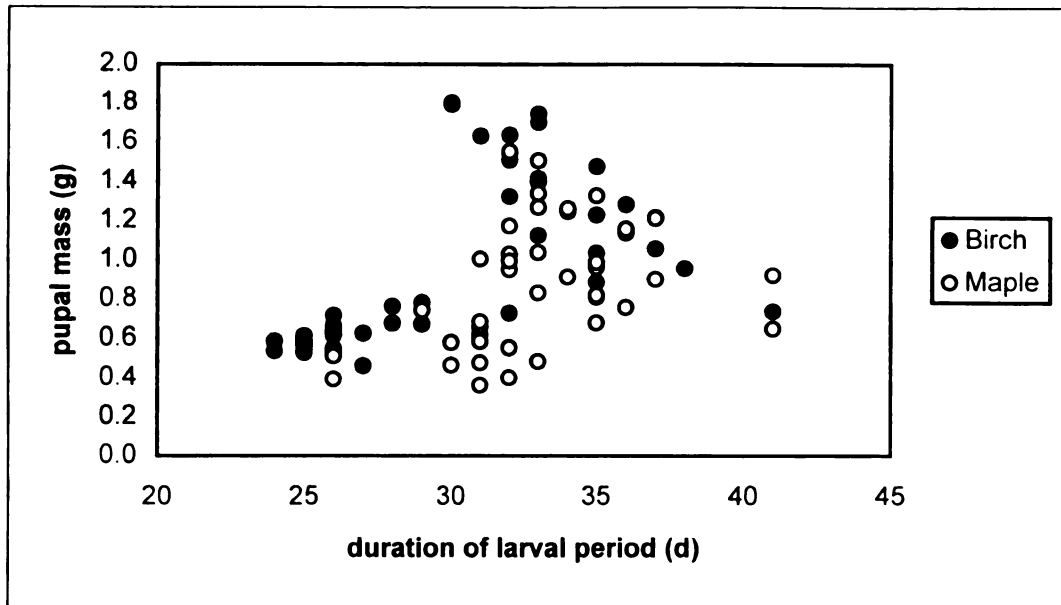


Figure 3.1. Gypsy moth pupal masses and duration of larval period in 1996. Data within host species form distinct clusters of high and low pupal mass/development time combinations. Insects with pupal masses < 0.80 g that complete larval development in ≤ 34 days were considered male.

Survival was measured after 12 d, at which point one larva per group, which by visual estimation appeared representative of the average size, was retained for continued rearing for later nutritional studies. Remaining surviving larvae in each group were disposed of.

Nutritional indices, including relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food to biomass (ECD) were calculated gravimetrically for one larvae per experimental tree using the equations listed in Table 3.3 (Waldbauer 1968, Gordon 1968). Growth, consumption, and frass production were quantified during 48 h feeding bioassays conducted within one day following molt to the fourth stadium (4-5 June). Larvae were weighed prior to being confined in plaster-filled petri dishes (as described above) with 90-120 cm² of fresh foliage (3-4 leaves) from the tree upon which they had been reared, and were allowed to feed undisturbed for 48 h in the growth chamber. After 48 h, larvae were again weighed, and area of unconsumed foliage was determined. Frass and the remaining foliage were then dried at 60°C for 2 d, and weighed.

Following the 48 h bioassay, larvae were reared until pupation. Larval development time (days from hatch to pupation) and pupal mass (without final instar exuviae) were recorded 2-3 d after pupation. Relative growth rate over the total larval period was calculated using 0.0006 g as the average initial mass of a neonate gypsy moth and pupal mass as the final mass of individuals.

Gypsy moth males generally have five instars while females have six. Hence, on average males develop faster and are smaller than females; therefore, insects were sexed

by examining the distribution of development times and pupal masses. Pupal mass was plotted against larval development time separately for both birch and maple-reared larvae (Figure 3.1). Data formed two distinct clusters for each tree species: one with quicker development times and smaller pupal masses and the other with relatively longer larval development times and larger pupal masses. Insects with larval development times under 34 d and pupal masses under 0.8 g were defined as male.

In 1997, fourth instar gypsy moth were field collected from hybrid poplars (*Populus x euroamericana* c.v. “Eugeneii”) in Kalamazoo County, MI on 16 June. These insects were part of a separate defoliation experiment, and were eclosed from eggs collected in Huron Township, Wayne County MI in late March 1997. Eggs were surface sterilized (10% formalin for 1 h) and then placed in the field prior to budbreak. When they reached the fourth instar, approximately 100 larvae were transported to Wooster, OH in petri dishes without food. Nutritional indices were determined with 48 h bioassays conducted on larvae 1-2 d into the fourth stadium. These bioassays were conducted as in 1996 (described above). Foliage from experimental trees (growing in Midland, MI) was collected on 16 June, stored in air-tight plastic bags, and kept on ice during transport to Ohio where it was refrigerated until bioassays were initiated on 17 June.

In 1997, second instar bioassays were conducted at the same time as the fourth instar bioassays with a separate cohort of insects. Larvae used in this bioassay were obtained as eggs from the Canadian Forest Service, Insect Rearing Facility, Sault Ste. Marie, ON. They were reared at 25°C with an 18L:6D photoperiod in groups of 50 – 150 on a constant supply of river birch and paper birch foliage (from Wooster, Ohio

populations). Beginning on 18 June, two second-instar larvae were reared for 48 h on detached foliage from each of the experimental trees. Relative growth rate was calculated gravimetrically as $RGR = [(\ln(\text{final group mass}) - \ln(\text{initial group mass})) / 2]$.

Whitemarked Tussock Moth.

In 1996, whitemarked tussock moth egg masses were obtained from the Canadian Forest Service, Insect Rearing Facility, Sault Ste. Marie, ON. Hatch occurred on 19 July. Groups of eight neonates were confined in a petri dish with detached foliage from one of the experimental trees, (one group per tree). Larvae were reared in growth chambers at 25°C with an 18L:6D photoperiod. Leaves were kept fresh and in ample supply, dishes were cleaned as needed, and positions of dishes in the chamber were changed regularly as described above.

Survival was measured after 14 d, at which point one larva per tree, which was deemed representative of the average group size, was retained for continued rearing for later nutritional studies. Additional larvae were discarded. Fourth instar nutritional indices, larval development time, pupal mass, and relative growth rate from egg to pupa were determined for one larva per experimental tree using methods identical to those described for gypsy moth in 1996 (above and Table 3.3). Relative growth rate over the entire larval period was calculated using 0.0004 g as the initial mass (estimated average mass of neonates) and pupal mass as the final mass. Adults were sexed following emergence.

In 1997, whitemarked tussock moth eggs were obtained from the Canadian Forest Service, Insect Rearing Facility, Sault Ste. Marie, ON. Insects were reared from egg hatch in growth chambers at 25 °C with an 18L:6D photoperiod. Hatch of a second cohort was delayed by refrigeration of eggs, and rearing temperature was adjusted as needed such that fourth instars were available for bioassays on 17 June and second instars on 18 June. Larvae were reared in groups of 50 – 150 on a constant supply of fresh paper birch and river birch foliage (Wooster, Ohio populations). Nutritional indices were calculated for fourth instars from gravimetric measures of larvae, foliage, and frass as described above for gypsy moth. Relative growth rate of groups of second instars was measured over 2 d. Second instar growth was determined using the equation $RGR = [(\ln(\text{final group mass}) - \ln(\text{initial group mass})) / 2]$. Group sizes ranged from two to three.

Forest tent caterpillar.

In 1997, fifth instar forest tent caterpillar were field collected from hybrid poplars (*Populus x euroamericana* c.v. “Eugeneii”) in Kalamazoo County, MI on 16 June (the same trees from which gypsy moth were collected), and transported to Wooster, Ohio in empty petri dishes. Nutritional indices were determined over 48 h (starting 17 June) for larvae feeding on detached foliage from experimental trees with methods identical to those used for gypsy moth in 1997.

Insect Performance in Relation to Foliar Traits.

Relationships between insect performance and foliar traits were examined during 1996 and 1997. In 1996, whitemarked tussock moth and gypsy moth relative growth rate, relative consumption rates, approximate digestibility, efficiency of conversion of digested food to biomass, pupal mass, and duration of the larval period were examined for correlations with foliar carbon, nitrogen, carbon/nitrogen ratios, specific leaf mass, condensed tannin, and total phenolic levels of birch and maple foliage collected 11 June 1996. Additionally, whitemarked tussock moth traits were tested for correlations with these same foliar traits of birch foliage sampled 1 August of 1996. Gypsy moth fourth instar 48 h feeding trials were run 4-7 June, one week prior to June sampling of foliage for chemical analyses. Whitemarked tussock moth fourth instar 48 h feeding trials were run 19-21 July, 5½ weeks after June sampling, and 2 weeks prior to August sampling of birch.

In 1997, relative growth rate, relative consumption rates, approximate digestibility, and efficiency of conversion of digested food to biomass in fourth instar gypsy moth, fourth instar whitemarked tussock moth, and fifth instar forest tent caterpillar were examined for correlations with foliar carbon, nitrogen, carbon/nitrogen ratios, specific leaf mass, and condensed tannin levels of birch and maple foliage collected 16 June (the same date as foliage was collected for bioassays). Details of foliage processing, chemical analyses, and effects of light, nutrient, and defoliation stress on plant physiology in both years are provided in Chapter 2.

Statistical Analyses.

Treatment effects on relative growth rate, nutritional indices (ECD, RCR, AD), pupal mass, and larval development time were analysed by analysis of variance (ANOVA) using SAS PROC GLM (SAS Institute, Inc.1990; version 6.11). Shapiro-Wilkes tests (SAS PROC UNIVARIATE) indicated that data met assumptions of normality required for ANOVA analyses, while visual examination of box plots (SAS PROC UNIVARIATE) indicated equal variances. Extreme outliers (greater than 3 standard deviations from mean) were removed from analyses only if they were associated with larvae that had molted during the 48 h study or had otherwise been noted to be unusual, or if the datum was obviously biologically unrealistic (ECD or AD < 0 or > 100; RCR or RGR excessively negative).

Blocks were treated as random effects, while species, light, nutrient, and defoliation were treated as fixed effects. Models were adjusted to account for the split-plot design with the addition of a separate test for shade effects (the whole plot factor). Effects of the light treatment were tested for with the block*light interaction mean square error (MSE) as the *F*-test denominator. All bar graphs report least square means $\pm$ standard error; standard errors of shade least square means were calculated by SAS with block*light as the error term. Relationships between percent defoliation and relative growth rate, ECD, AD, and RCR were also examined using correlation analysis (Pearson correlation coefficients, SAS PROC GLM) with defoliation treated as a continuous variable.

Table 3.4. Chi-square values, degrees of freedom (*df*), and *p*-values from nonparametric analyses (SAS CATMOD) of light, species, nutrient, defoliation effects and their interactions on gypsy moth 12 d survival and whitemarked tussock moth 14 d survival in 1996. $60 \leq N \leq 73$ insects on six trees per treatment combination.¹

Source of Variation	<i>df</i>	Survival	
		gypsy moth	whitemarked tussock moth
Intercept	1	62.63 ****	10104.34 ****
Species	1	3.67 #	5.27*
Light	1	3.19#	1.26
Species*Light	1	0.30	3.21#
Nutrient	1	3.88*	4.17*
Species*Nutrient	1	0.70	8.16**
Light*Nutrient	1	0.07	0.12
Species*Light*Nutrient	1	0.91	4.05*
Defoliation	1	2.86#	2.18
Species*Defoliation	1	0.39	3.36#
Light*Defoliation	1	1.75	3.10#
Species*Light*Defoliation	1	0.17	0.43
Nutrient*Defoliation	1	0.00	0.00
Species*Nutrient*Defoliation	1	0.22	0.31
Light*Nutrient*Defoliation	1	0.26	0.04
Species*Light*Nutrient*Defoliation	1	0.13	0.92

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$, **** = $p \leq 0.0001$.

Table 3.5. *F*-values and degrees of freedom (*df*) from ANOVAs of light, species, nutrient, defoliation effects and their interactions on gypsy moth relative growth rates measured from egg hatch to pupation (RGR total), and during the fourth stadium (RGR L4), and relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) measured during the fourth stadium. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	RGR total	RGR L4	RCR	AD	ECD
Block	2	2.08	1.96	1.04	2.10	3.55 *
Light	1	11.92 #	4.53	0.22	0.62	6.05
Block * Light	2	0.32	0.55	0.42	0.53	0.24
Species	1	5.15 *	0.02	56.08 ****	8.14 **	8.72 **
Species*Light	1	0.91	1.57	3.01 #	2.83 #	4.83 *
Nutrient	1	2.53	2.63	0.14	1.32	2.23
Species*Nutrient	1	0.26	1.61	0.84	1.32	3.00 #
Light*Nutrients	1	4.38 *	0.46	0.54	1.18	0.89
Species*Light*Nutrient	1	0.37	0.60	0.01	2.32	1.58
Defoliation	1	0.04	0.22	2.77	0.00	0.31
Species*Defoliation	1	1.81	1.38	0.00	0.59	0.07
Light*Defoliation	1	0.01	1.57	4.08 *	1.68	6.04 *
Species*Light*Defoliation	1	0.09	0.22	0.01	0.30	2.24
Nutrient*Defoliation	1	0.45	2.33	3.07	0.62	1.08
Species*Nutrient*Defoliation	1	0.29	0.37	0.61	0.96	1.15
Light*Nutrient*Defoliation	1	0.50	0.18	0.87	0.37	0.00
Species*Light*Nutrient*Defoliation	1	3.75 #	0.08	0.32	0.69	0.38
Error <i>df</i>		70	71	70	72	72

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$, **** = $p \leq 0.0001$.

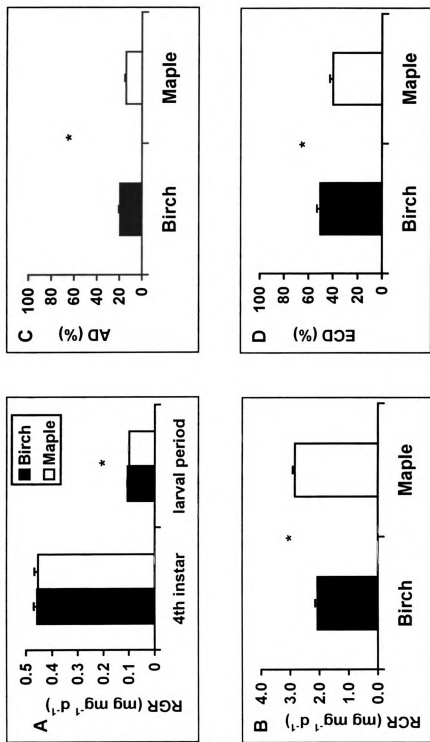


Figure 3.2. Relative growth rates (RGR) of gypsy moth measured over 48 h during the fourth instar and from egg hatch to pupation ("larval period") on paper birch and sugar maple in 1996 (A). Relative consumption rates (RCR) (B), approximate digestibility (AD) (C), and efficiency of conversion of digested food to biomass (ECD) (D), of fourth instar gypsy moth measured over 48 h. Asterisks indicate significant species effects on insect growth or nutritional indices ($p \leq 0.05$)

Table 3.6. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on gypsy moth pupal mass and larval duration in males and females. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Pupal Mass		Development time	
		male	female	male	female
Block	2	1.96	0.02	0.42	0.30
Light	1	4.18	34.88 *	8.64 #	7.38
Block*Light	2	0.33	0.23	0.20	0.22
Species	1	2.01	12.89 **	28.17 ****	0.03
Species*Light	1	0.01	0.23	0.00	1.06
Nutrient	1	3.93 #	0.63	1.01	0.02
Species*Nutrient	1	0.46	0.22	0.05	0.08
Light*Nutrient	1	0.02	1.57	0.02	2.07
Species*Light*Nutrient	1	0.14	0.14	0.09	0.79
Defoliation	1	1.16	1.59	0.62	7.17 *
Species*Defoliation	1	0.40	2.69	0.41	2.30
Light*Defoliation	1	0.01	0.38	0.00	0.68
Species*Light*Defoliation	1	0.12	0.61	0.17	1.98
Nutrient*Defoliation	1	0.47	0.05	0.07	0.01
Species*Nutrient*Defoliation	1	0.12	1.00	0.38	1.98
Light*Nutrient*Defoliation	1	0.00	0.30	0.09	0.14
Species*Light*Nutrient*Defoliation	1	.	0.10	.	0.45
Error <i>df</i>		22	29	22	31

¹ # = $p \leq 0.10$. * = $p \leq 0.05$. ** = $p \leq 0.01$. **** = $p \leq 0.0001$.

Table 3.7. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on gypsy moth relative growth rates over 48 h during the second (RGR L2) and fourth (RGR L4) stadii, and relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) measured during the fourth stadium. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	RGR L2	RGR L4	RCR	AD	ECD
Block	2	0.79	1.35	0.71	0.83	2.22
Light	1	0.50	0.74	1.98	3.25	0.52
Block*Light	2	2.25	1.16	0.77	0.39	0.63
Species	1	80.44 ****	16.72 ****	2.53	0.28	8.17 **
Species*Light	1	0.70	2.84 #	2.71	3.48 #	2.88 #
Nutrient	1	1.92	3.61 #	1.88	0.59	7.61 **
Species*Nutrient	1	7.04 **	1.21	0.75	0.46	0.11
Light*Nutrient	1	4.00 *	0.09	0.05	0.08	0.05
Species*Light*Nutrient	1	2.42	0.44	0.22	0.38	0.46
Defoliation	1	0.14	0.08	0.12	0.68	0.04
Species*Defoliation	1	3.91 #	0.37	0.16	0.00	0.43
Light*Defoliation	1	2.54	0.65	1.30	0.24	2.26
Species*Light*Defoliation	1	2.88 #	0.02	0.05	0.22	1.64
Nutrient*Defoliation	1	1.84	0.00	1.08	0.48	0.13
Species*Nutrient*Defoliation	1	7.78 **	2.84 #	1.83	1.17	0.23
Light*Nutrient*Defoliation	1	3.85 #	0.01	0.13	0.12	0.40
Species*Light*Nutrient*Defoliation	1	0.52	0.01	1.67	0.22	0.53
Error <i>df</i>		73	71	69	68	65

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$, **** = $p \leq 0.0001$.

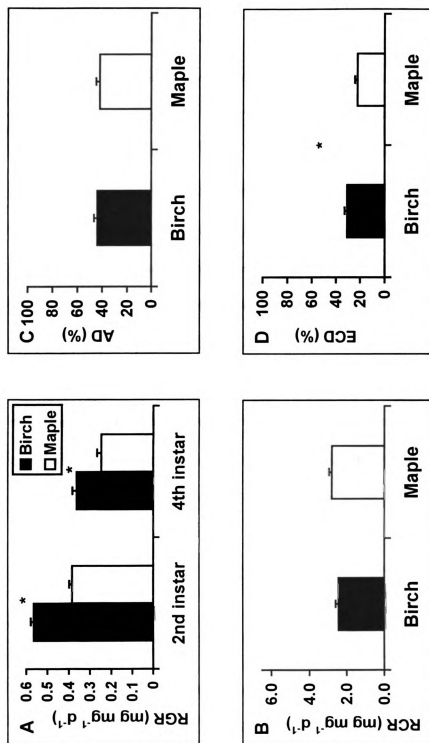


Figure 3.3. Relative growth rate (RGR) of gypsy moth measured during 48 h during the second and fourth instar on paper birch and sugar maple in 1997 (A). Relative consumption rates (RCR) (B), approximate digestibility (AD) (C), and efficiency of conversion of digested food to biomass (ECD) (D), of fourth instar gypsy moth measured over 48 h. Asterisks indicate significant species effects on insect growth or nutritional indices ($p \leq 0.05$).

Relative growth rates and relative consumption rates are ratios. Although some controversy exists over the use of ANOVA to analyze ratios (Packard and Boardman 1988, Raubenheimer 1995, Beaupre and Dunham 1995), I could not use the recommended analysis of covariance to analyze absolute growth and consumption because assumptions of equal response slopes across treatments were violated (Raubenheimer and Simpson 1992). Violation of assumptions was indicated by significant interaction terms between covariates and main treatment effects in ANOVA analyses (data not shown), indicating that the relationship between the covariate and the variable of interest was not independent of the experimental treatments.

Survival was analyzed by SAS PROC CATMOD (SAS Institute, Inc. 1990) for nonparametric analyses of categorical data. Data on foliar traits (foliar carbon, nitrogen, condensed tannin, and total phenolic content, carbon/nitrogen ratios, and specific leaf mass; details in Chapter 2) were correlated to insect performance by analysis of Pearson correlation coefficients (SAS PROC GLM). All results reported are statistically significant at $\alpha = 0.05$ unless noted.

RESULTS

Insect Bioassays.

Gypsy moth.

Birch was a better host for gypsy moth than was maple at all stages of insect development. In 1996, 12 d survival on maple ($93.4 \pm 1.0\%$) was 3% lower than that on birch ($96.6 \pm 1.0\%$) ($p = 0.056$, Table 3.4). Approximate digestibility of maple to fourth

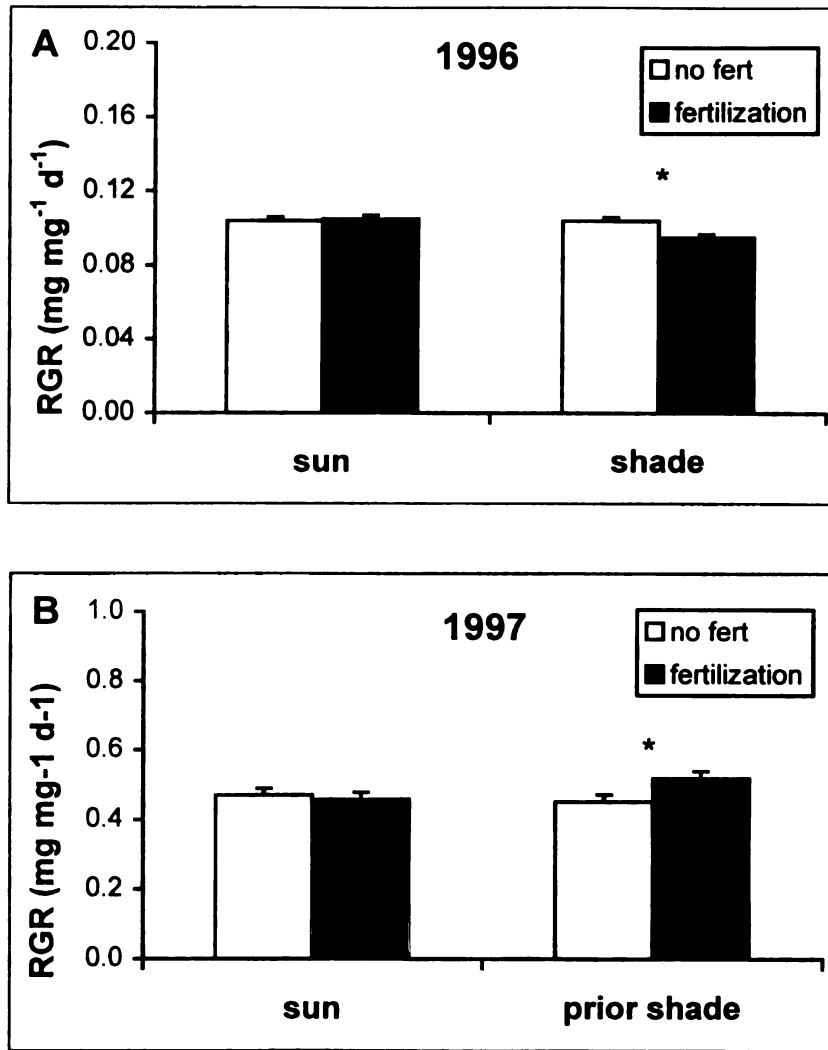


Figure 3.4. Effects of fertilization, influenced by shade, on gypsy moth relative growth rates as measured over the total larval period in 1996 (A) and growth of second instar gypsy moth measured over 48 h in 1997 (B). Asterisks indicate significant effects of fertilization on insect growth ($p \leq 0.05$).

instars was reduced 28% relative to that of birch, and digestive efficiencies (ECD) of fourth instars were decreased on maple by 21% (Table 3.5, Figure 3.2). However, larvae were able to compensate for decreased ECD on maple by increasing consumption rates 37% relative to those on birch, resulting in maintenance of growth rates. Growth rates over the duration of the total larval period were 5% lower on maple than birch (Table 3.5, Figure 3.2). Female pupal mass was 21% lower on maple (1.06 ± 0.05 g) than on birch (1.35 ± 0.06 g) (Table 3.6), while pupal masses of larvae defined as males (see Materials and Methods) averaged 0.59 ± 0.05 g for both host species. Male larval period was four days longer on maple (30 d) than on birch (26 d, Table 3.6).

In 1997, birch again was a better host for gypsy moth than was maple. Second instars grew 32% slower on maple than on birch (Table 3.7, Figure 3.3). Although approximate digestibility was equivalent on birch and maple (unlike in 1996), digestive efficiencies (ECD) of fourth instars were again 28% lower on maple (Table 3.7, Figure 3.3). Unlike in 1996, larvae were not able to compensate for poor host quality through increased consumption, and consequently growth was reduced 32% on maple (Table 3.7, Figure 3.3).

The light treatment did not have a strong effect on gypsy moth performance, but when effects were significant, shaded trees were lower quality hosts. Shade did not influence survival in 1996 (Table 3.4); however, digestive efficiencies (ECD) of fourth instar larvae fed from shaded birch (but not maple) were reduced 21% (Table 3.5). ECDs of gypsy moth fed birch grown in full sun were $56 \pm 4\%$, while ECDs of larvae fed foliage from shaded birch were $44 \pm 4\%$. Even so, shade did not decrease fourth instar

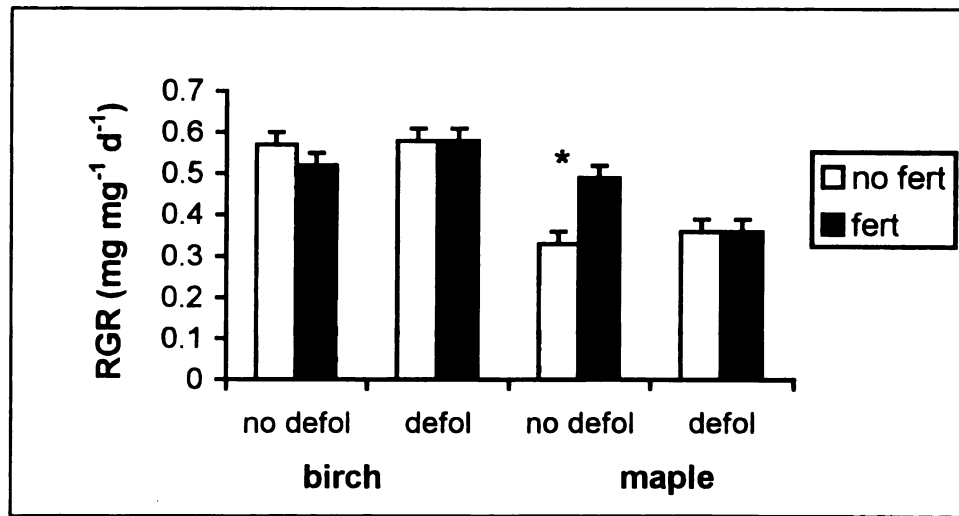


Figure 3.5. Interactive effects of fertilization and defoliation on the relative growth rate (RGR) of second instar gypsy moth on paper birch and sugar maple in 1997. Asterisks indicate significant effects of fertilization within defoliation treatments of birch or maple ($p \leq 0.05$).

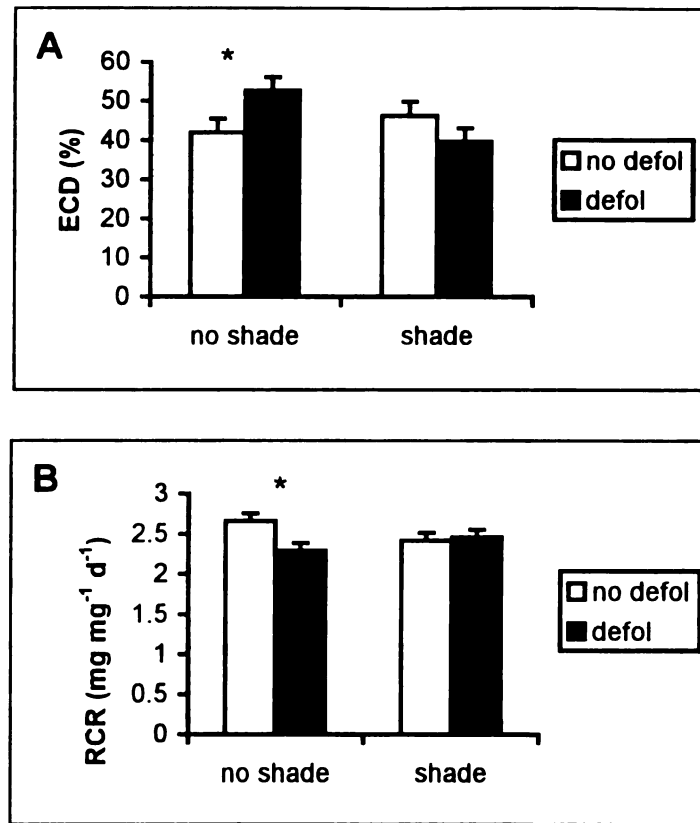


Figure 3.6. Interactive effects of defoliation and light treatment on digestive efficiency (ECD) (A) and consumption rate (RCR) (B) of fourth instar gypsy moth in 1996. Asterisks indicate significant effects of defoliation within light treatments ($p \leq 0.05$).

Table 3.8. Pearson coefficients for correlations between indices of gypsy moth (A), whitemarked tussock moth (B), and forest tent caterpillar (C) larval performance and host defoliation and variables of quality of foliage sampled in June from paper birch (upper value) and sugar maple (lower value) for 1996 and 1997 laboratory experiments ($34 \leq N \leq 48$).^{1,2}

A.	Foliage			Variable		
Gypsy moth performance variable	% defoliation	nitrogen	carbon/nitrogen ratio	condensed tannins	total phenolics	specific leaf mass
1996						
RGR L4	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
RGRtotal	-0.41 **	NS	NS	NS	NS	NS
	0.34 *	NS	NS	NS	NS	NS
RCR	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
ECD	NS	NS	0.30 *	NS	0.37 *	NS
	NS	NS	NS	NS	NS	NS
Pupal mass	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	0.31 *
Larval development time	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
1997						
RGR L2	NS	NS	NS	NS	NA	NS
	NS	NS	NS	-0.30 *	NA	NS
RGR L4	NS	0.44 **	-0.45 **	NS	NA	NS
	NS	0.32 *	NS	NS	NA	NS
RCR	NS	NS	NS	NS	NA	NS
	NS	NS	NS	NS	NA	NS
ECD	NS	NS	NS	NS	NA	-0.32 *
	NS	NS	NS	NS	NA	NS

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$, **** = $p \leq 0.0001$.

² Abbreviations and units: nitrogen (% dry weight), condensed tannins (% dry weight – birch or maple tannin equivalents), total phenolics (% dry weight – birch or maple tannin equivalents) measured only in 1996, specific leaf mass (g/m^2), RGR L4 = relative growth rate during 48 h of the fourth stadium ($\text{mg mg}^{-1} \text{d}^{-1}$), RGRtotal = relative growth rate during entire larval stage ($\text{mg mg}^{-1} \text{d}^{-1}$), RCR = relative consumption rate ($\text{mg mg}^{-1} \text{d}^{-1}$), ECD = efficiency of conversion of digested food to biomass (%), pupal mass (g), larval development time (d), RGR L2 = relative growth rate during 48 h of the second stadium ($\text{mg mg}^{-1} \text{d}^{-1}$), RGR L5 = relative growth rate during 48 h of the fifth stadium ($\text{mg mg}^{-1} \text{d}^{-1}$).

Table 3.8 (continued).

B.		Foliage		Variable		
Whitemarked tussock moth performance variable	% defoliation	nitrogen	carbon/nitrogen ratio	condensed tannins	total phenolics	specific leaf mass
1996						
RGR L4	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
RGRtotal	-0.61 ****	NS	NS	NS	NS	NS
	NS	NS	NS	0.39 *	-0.36 *	0.32 *
RCR	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
ECD	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
Pupal mass	NS	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS	NS
Larval development time	0.54 ****	NS	NS	NS	NS	NS
	NS	NS	NS	-0.40 **	0.32 *	NS
1997						
RGR L2	NS	NS	NS	NS	NA	NS
	NS	NS	NS	NS		NS
RGR L4	NS	0.37 *	-0.33 *	NS	NA	NS
	NS	NS	NS	NS		NS
RCR	NS	NS	NS	NS	NA	NS
	NS	NS	NS	NS		NS
ECD	NS	0.33 *	-0.35 *	NS	NA	NS
	NS	NS	NS	NS		NS

C.		Foliage		Variable	
Forest tent caterpillar Performance variable	% defoliation	nitrogen	carbon/nitrogen ratio	Condensed tannins	specific leaf mass
1997					
RGR L5	NS	NS	NS	NS	NS
	NS	NS	NS	NS	NS
RCR	NS	NS	0.32 *	NS	NS
	NS	NS	NS	NS	NS
ECD	0.33 *	NS	NS	NS	NS
	NS	0.35 *	NS	NS	NS

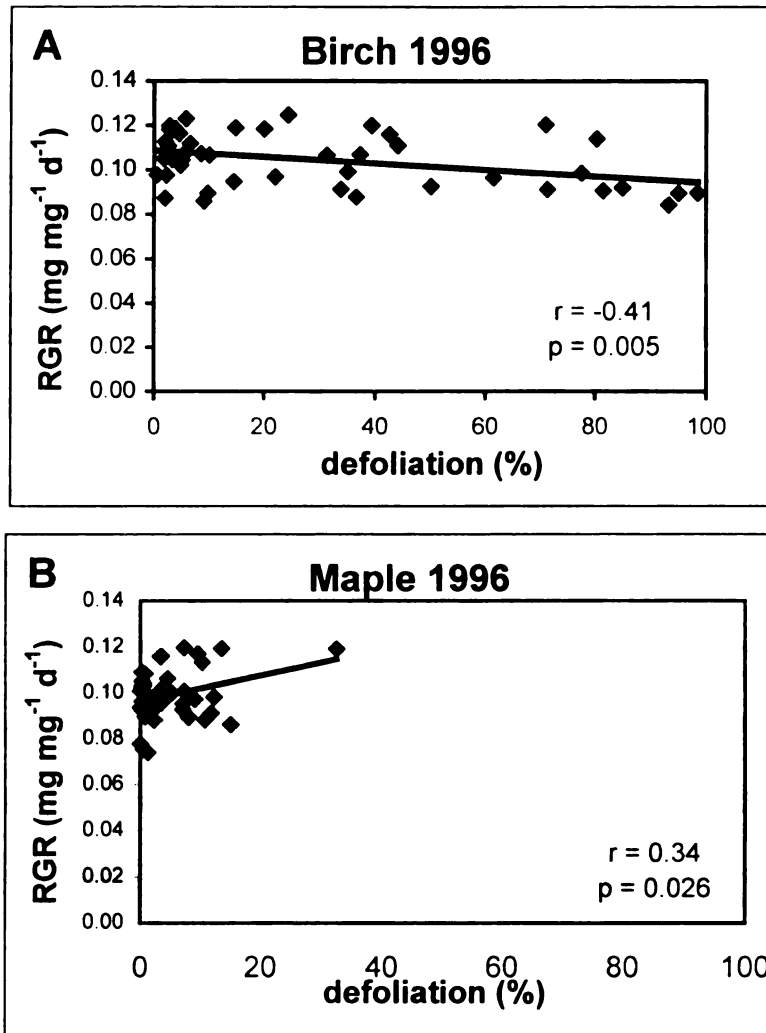


Figure 3.7. Relationship of defoliation with gypsy moth relative growth rate (RGR) as measured over the total larval period on paper birch (A) and sugar maple (B) in 1996.

growth rate (Tables 3.5). Furthermore, the light treatment did not influence the duration of the larval period of males or females, but did reduce the pupal mass of female (but not male) gypsy moth by 17% (Table 3.6). Pupal mass of females averaged 1.09 ± 0.03 g on shaded and 1.32 ± 0.03 g on non-shaded hosts. There were no effects of prior season's shading on second or fourth instar growth or nutritional indices in 1997 (Table 3.7).

The effects of nutrient treatments on gypsy moth performance were dependent upon host species, light, and defoliation. In 1996, fertilization was associated with a 2% increase in the survival of early instars (Table 3.4). Fourth instar growth and nutritional indices were not influenced by fertilization, but fertilization of both birch and maple grown in shade (not sun) reduced the growth rates of larvae as measured over the entire larval stage by 9% (significant light*nutrient interaction, Table 3.5, Figure 3.4). However, fertilization did not influence larval development time or pupal mass (Table 3.7).

In 1997, fertilization of maple (but not birch) increased relative growth rates of second instars by 23% (Table 3.7, Figure 3.5). Growth rates of second instars on fertilized maple were greatest if trees had not been previously defoliated (significant species*nutrient* defoliation interaction, Table 3.7, Figure 3.5). Fertilization also increased growth of second instars by 15% on previously shaded hosts, but not on hosts which had always grown in full sun (significant light*nutrient interaction, Table 3.7, Figure 3.4). Unlike in 1996, fertilization increased efficiency of conversion of digested birch and maple foliage to biomass by 37% in 1997 ($31 \pm 2\%$ and $22 \pm 2\%$ for fertilized

Table 3.9. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on whitemarked tussock moth relative growth rates measured from egg hatch to pupation (RGR total) and during the fourth stadium (RGR L4), and relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food to biomass (ECD) measured during the fourth stadium. *F*-values for light were calculated with block*light as the error term.

Source of Variation	<i>df</i>	RGR total	RGR L4	RCR	AD	ECD
Block	2	0.09	5.36 **	3.12 #	1.15	3.10 #
Light	1	4.90	0.34	0.83	0.84	0.07
Block*Light	2	2.76 #	3.71 *	1.65	0.97	0.50
Species	1	12.28 ***	8.34 **	3.60 #	2.17	25.16 ****
Species*Light	1	2.85 #	0.81	0.00	0.21	0.21
Nutrient	1	0.44	3.33	0.89	2.20	0.41
Species*Nutrient	1	0.65	0.54	0.10	0.30	1.35
Light*Nutrient	1	7.60 **	0.73	2.12	0.08	0.00
Species*Light*Nutrient	1	0.53	3.35 #	1.83	0.48	3.91 #
Defoliation	1	9.86 **	0.02	0.76	0.30	0.41
Species*Defoliation	1	5.18 *	2.35	0.26	0.14	0.81
Light*Defoliation	1	0.61	5.06 *	0.97	0.11	5.52 *
Species*Light*Defoliation	1	0.17	0.35	1.23	4.04 *	6.14 *
Nutrient*Defoliation	1	0.32	0.12	0.90	0.35	2.52
Species*Nutrient*Defoliation	1	1.55	0.26	0.00	2.84 #	1.17
Light*Nutrient*Defoliation	1	0.04	0.65	0.40	0.95	0.00
Species*Light*Nutrient*Defoliation	1	0.27	0.36	2.69	1.54	0.19
Error <i>df</i>		64	67	67	63	65

* # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

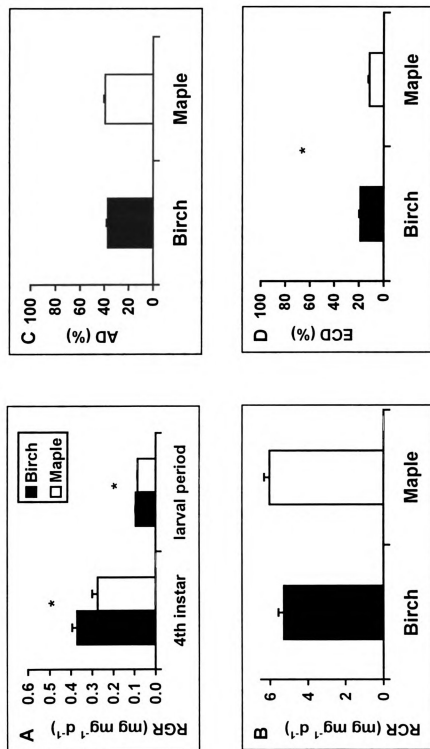


Figure 3.8. Relative growth rates (RGR) of whitemarked tussock moth measured over 48 h during the fourth instar and from egg hatch to pupation ("larval period") on paper birch and sugar maple in 1996 (A). Relative consumption rate (RCR) (B), approximate digestibility (AD) (C), and efficiency of conversion of digested food to biomass (ECD) (D), of fourth instar whitemarked tussock moth measured over 48 h. Asterisks indicate significant species effects on insect growth or nutritional indices ($p \leq 0.05$).

Table 3.10. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on whitemarked tussock moth pupal mass and larval durations in males and females. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	Pupal Mass		Larval Development Time	
		male	female	male	female
Block	2	0.15	0.09	0.16	0.52
Light	1	15.10 #	1.68	33.36 *	0.03
Block*Light	2	0.07	2.16	0.21	0.15
Species	1	0.12	0.42	0.70	0.25
Species*Light	1	0.15	0.30	3.09	0.13
Nutrient	1	0.44	0.20	0.00	0.93
Species*Nutrient	1	0.11	0.69	0.00	0.63
Light*Nutrient	1	0.51	0.29	0.78	0.24
Species*Light*Nutrient	1	0.20	.	3.09	.
Defoliation	1	0.03	0.00	0.52	3.77 #
Species*Defoliation	1	0.16	0.09	2.27	4.19 #
Light*Defoliation	1	0.52	0.00	0.10	0.94
Species*Light*Defoliation	1	.	0.32	.	0.23
Nutrient*Defoliation	1	0.23	0.12	0.02	0.03
Species*Nutrient*Defoliation	1	.	.	.	.
Light*Nutrient*Defoliation	1	0.25	.	4.14	.
Species*Light*Nutrient*Defoliation	1	.	.	.	.
Error <i>df</i>		4	16	4	16

¹ # = $p \leq 0.10$. * = $p \leq 0.05$.

Table 3.11. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on whitemarked tussock moth relative growth rates over 48 h during the second (RGR L2) and fourth (RGR L4) stadii, and relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) measured during the fourth stadium. *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	RGR L2	RGR L4	RCR	AD	ECD
Block	2	1.96	0.03	2.18	1.17	0.44
Light	1	12.49 #	2.25	6.50	0.33	9.28 #
Block*Light	2	0.21	0.43	0.02	0.75	0.04
Species	1	0.25	9.99 **	6.28 *	0.01	5.38 *
Species*Light	1	0.00	0.11	0.00	0.68	0.96
Nutrient	1	1.42	0.47	0.44	0.21	0.14
Species*Nutrient	1	0.13	0.33	0.13	0.08	0.00
Light*Nutrient	1	0.01	0.08	3.60 #	0.03	0.06
Species*Light*Nutrient	1	3.67 #	0.01	0.02	2.15	0.04
Defoliation	1	0.09	0.05	0.17	1.85	0.22
Species*Defoliation	1	0.25	0.05	1.65	0.06	0.42
Light*Defoliation	1	0.12	0.58	0.00	1.64	0.21
Species*Light*Defoliation	1	0.31	0.17	0.03	0.06	0.25
Nutrient*Defoliation	1	3.55 #	0.02	1.99	0.28	0.29
Species*Nutrient*Defoliation	1	1.05	0.19	1.53	1.59	0.07
Light*Nutrient*Defoliation	1	0.33	5.26 *	2.77	0.54	2.37
Species*Light*Nutrient*Defoliation	1	0.08	1.89	1.51	0.20	2.89 #
Error <i>df</i>		73	72	71	69	66

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, ** = $p \leq 0.01$.

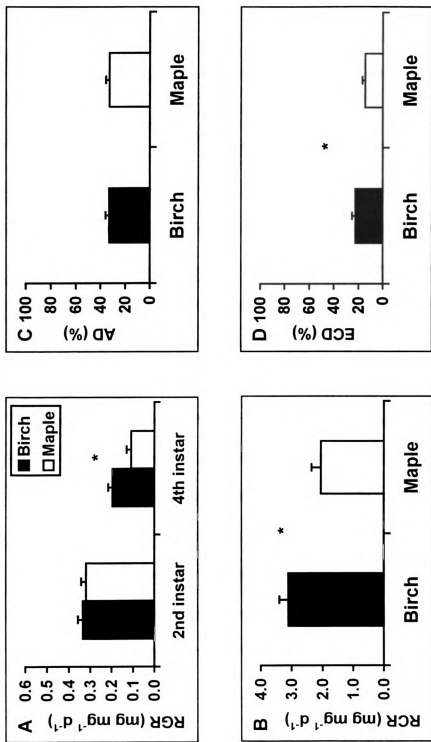


Figure 3.9. Relative growth rate (RGR) of whitemarked tussock moth measured over 48 h during the second and fourth instar on paper birch and sugar maple in 1997 (A). Relative consumption rate (RCR) (B), approximate digestibility (AD) (C), and efficiency of conversion of digested food to biomass (ECD) (D), of fourth instar whitemarked tussock moth measured over 48 h. Asterisks indicate significant species effects on insect growth or nutritional indices ($p \leq 0.05$).

and non-fertilized, respectively; Table 3.7). There was no effect of fertilization on fourth instar growth rates.

Defoliation generally appeared to improve host quality for gypsy moth, although some responses indicated poorer host quality of defoliated trees. In 1996, defoliation did not affect early instar survival on either host species (Table 3.4). In sun (but not shade), defoliation increased quality of both species, as indicated by 25% improvement in efficiency of conversion of digested food to biomass (ECD) and 14% lower relative consumption rates (RCR) of insects on defoliated hosts (significant light*defoliation interaction, Table 3.5, Figure 3.6). Results of ANOVA showed no significant effect of defoliation or species*defoliation interactions on gypsy moth growth as measured over the entire larval period. However, for insects feeding on maple, correlation analyses indicated a positive correlation between defoliation and growth during the total larval duration and a negative correlation between these same variables for insects feeding on birch (Table 3.8 A, Figure 3.7). Female gypsy moth took 2 d longer to pupate when feeding on defoliated hosts, but pupal mass was not affected by defoliation (Table 3.6).

There was no evidence of delayed induced resistance of birch or maple to gypsy moth. In 1997, prior season's defoliation was not correlated with growth rates of second instar gypsy moth, or with growth, relative consumption rate, or efficiency of conversion of digested food to biomass in fourth instar gypsy moth (Table 3.8 A).

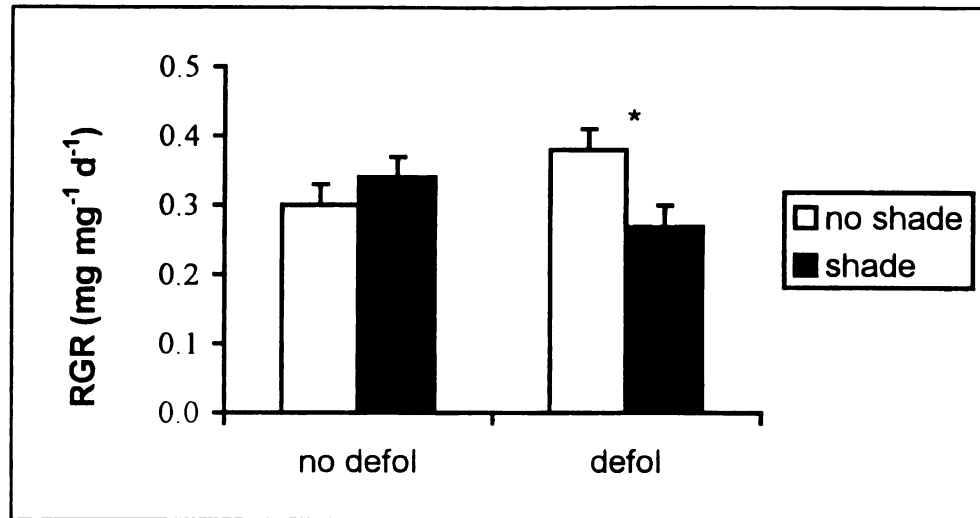


Figure 3.10. Interactive effects of light and defoliation on relative growth rate (RGR) of fourth instar whitemarked tussock moth in 1996. Asterisks indicate significant effects of shade on growth within defoliation treatments ($p \leq 0.05$).

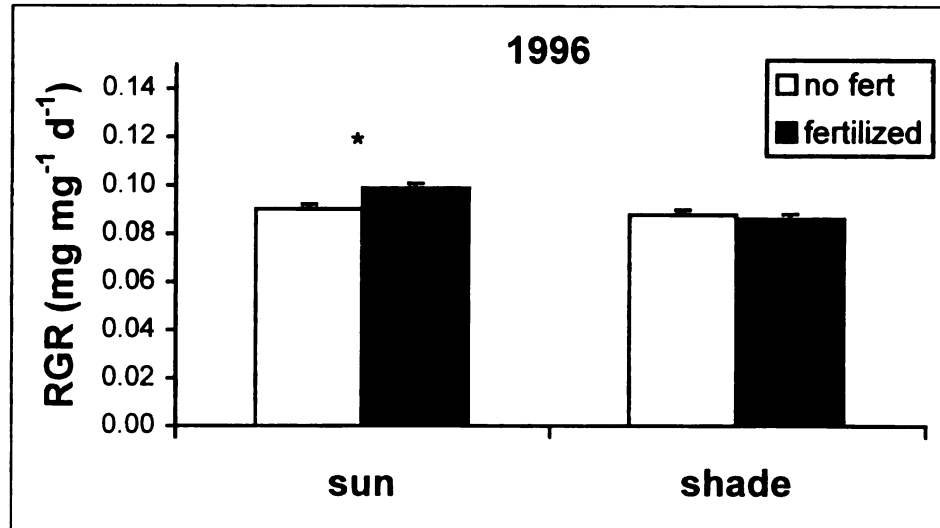


Figure 3.11. Interactive effects of fertilization and shade on whitemarked tussock moth relative growth rates measured over the total larval period in 1996. Asterisks indicate significant effects of fertilization within light treatments ($p \leq 0.05$).

Whitemarked tussock moth.

As with gypsy moth, birch was a better host than maple for whitemarked tussock moth. In 1996, survival of early instars was reduced 6% on maple ($87.5 \pm 2\%$) relative to birch ($93.2 \pm 2\%$) (Table 3.4). Fourth instars grew 26% more slowly on maple (Table 3.9, Figure 3.8), resulting from a 39% reduction in efficiency of conversion of maple foliage to biomass relative to birch (Table 3.9, Figure 3.8). Unlike gypsy moth, whitemarked tussock moth were unable to sufficiently increase consumption to compensate for reduced host quality, and growth rates measured over the total larval period were reduced 9% on maple relative to birch in 1996 (Table 3.9, Figure 3.8). Host species did not influence pupal mass (mean $\pm$ SD: female 0.31 ± 0.08 g; male 0.15 ± 0.04 g) or larval development time (mean $\pm$ SD: female 34 ± 7 d; male 28 ± 4 d, Table 3.10).

In 1997, host species did not affect second instar growth, but fourth instars grew 45% more slowly on maple (Table 3.11, Figure 3.9). As in 1996, reduced growth resulted from a 36% reduction in efficiency of conversion of maple foliage to biomass relative to birch (Table 3.11, Figure 3.9). Larval consumption rates on maple were reduced 34% relative to birch (Table 3.11, Figure 3.9).

Like gypsy moth, whitemarked tussock moth did not respond strongly to the light treatment. When effects were significant, shade decreased host quality, but these effects were generally dependent upon the defoliation treatment. In 1996, early instar survival was equivalent on shaded and sun-grown trees (Table 3.4). However, fourth instars grew slower on shaded hosts relative to sun-grown hosts, but only when trees were defoliated. Growth of fourth instars was reduced 30% on foliage from shaded hosts that had been

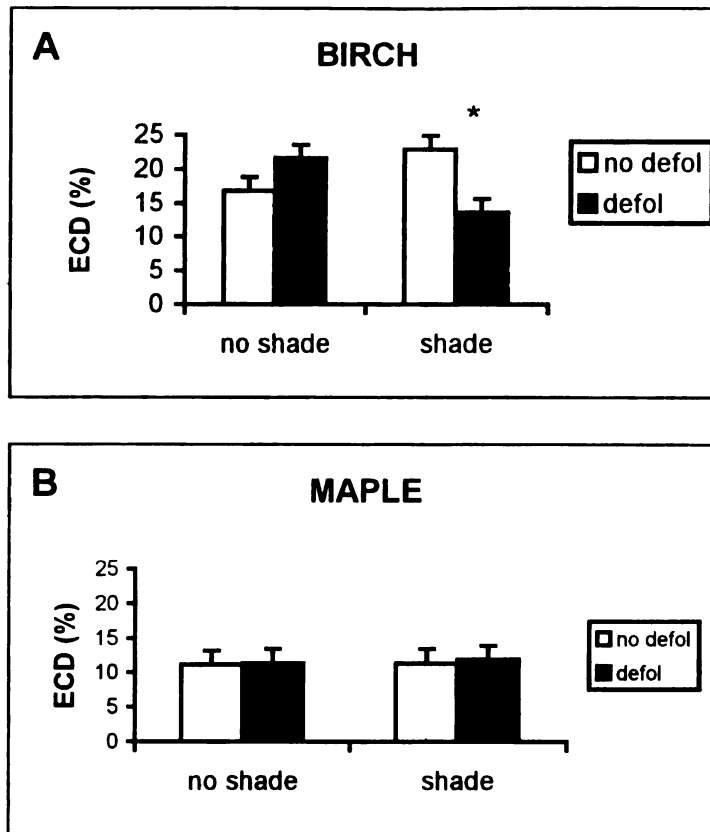


Figure 3.12. Interactive effects of light and defoliation on digestive efficiency (ECD) of fourth instar whitemarked tussock moth feeding on paper birch (A) and sugar maple (B). Asterisks indicate significant effects of defoliation on digestive efficiencies within light treatments ($p \leq 0.05$).

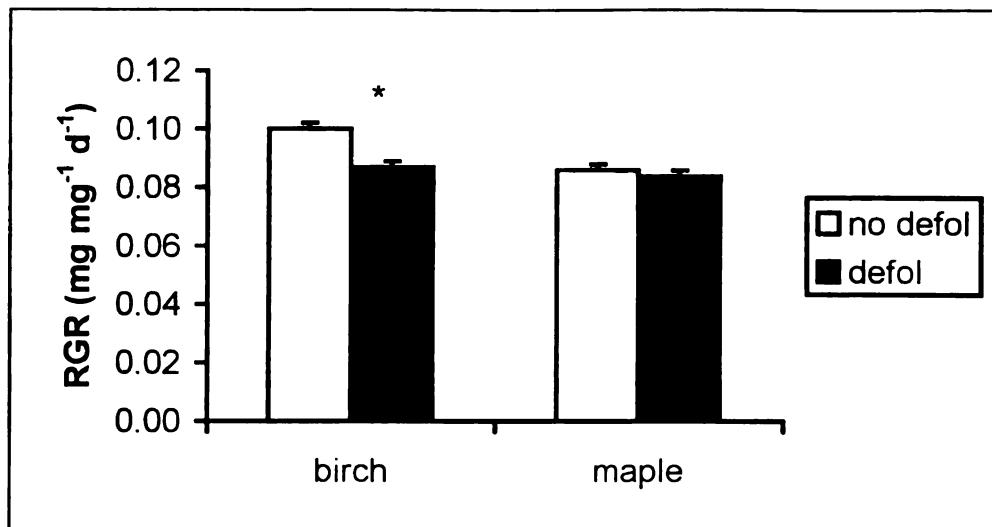


Figure 3.13. Effects of defoliation on relative growth rate (RGR) of whitemarked tussock moth measured over the entire larval period on paper birch and sugar maple. Asterisks indicate significant effects of defoliation within host tree ($p \leq 0.05$).

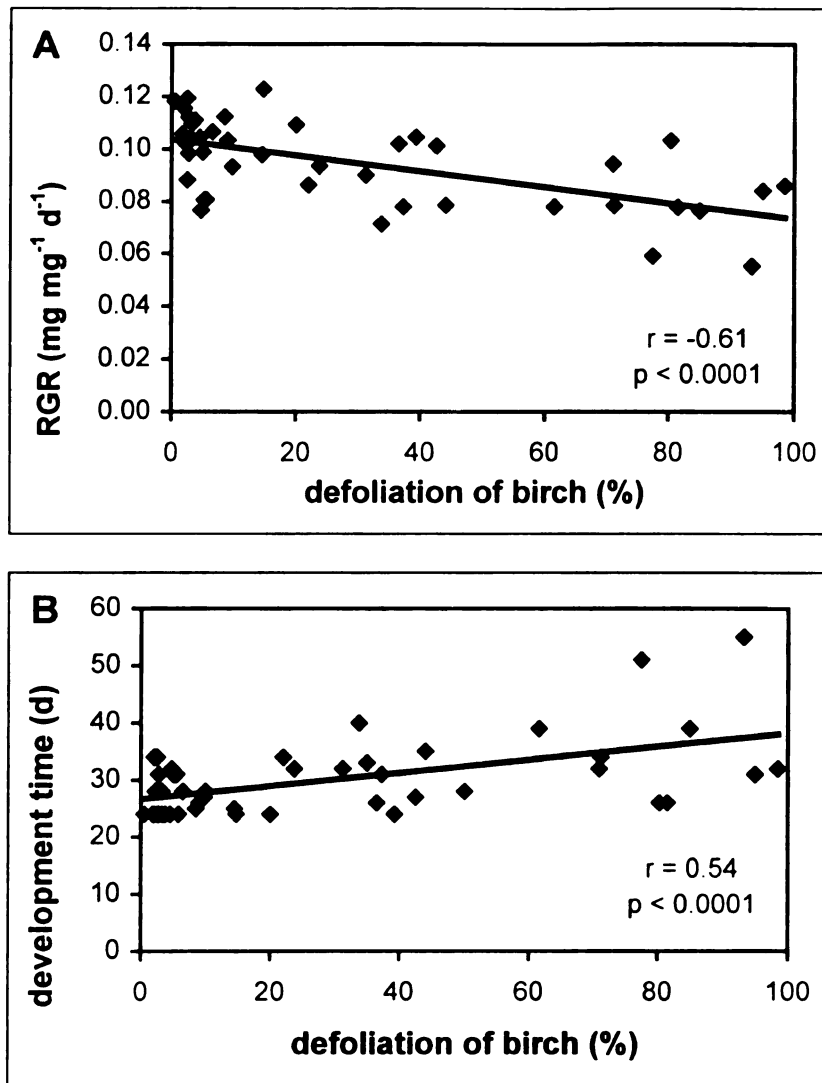


Figure 3.14. Relationship between percent defoliation and whitemarked tussock moth relative growth rate as measured over the total larval period (RGR) (A), and larval development time on birch (B) in 1996.

Table 3.12. *F*-values and degrees of freedom (*df*) from ANOVA of light, species, nutrient, defoliation effects and their interactions on fifth instar forest tent caterpillar relative growth rate (RGR L5), relative consumption rate (RCR), approximate digestibility (AD), and efficiency of conversion of digested food to biomass (ECD). *F*-values for light were calculated with block*light as the error term.¹

Source of Variation	<i>df</i>	RGR L5	RCR	AD	ECD
Block	2	1.68	0.99	1.58	0.44
Light	1	2.78	12.81 #	0.00	0.14
Block*Light	2	0.85	0.27	0.40	0.04
Species	1	35.06 ****	5.01 *	0.22	14.81 ***
Species*Light	1	0.97	0.91	2.31	0.02
Nutrient	1	0.10	0.65	0.10	0.23
Species*Nutrient	1	0.26	0.86	0.11	0.29
Light*Nutrient	1	0.85	0.04	0.22	0.17
Species*Light*Nutrient	1	0.00	1.17	0.56	0.00
Defoliation	1	0.05	0.36	0.04	0.84
Species*Defoliation	1	0.03	0.62	2.73	2.93
Light*Defoliation	1	1.18	0.91	0.93	0.06
Species*Light*Defoliation	1	0.07	0.07	0.04	0.01
Nutrient*Defoliation	1	0.20	1.11	1.26	0.17
Species*Nutrient*Defoliation	1	0.38	0.09	1.76	1.01
Light*Nutrient*Defoliation	1	0.04	0.05	0.97	0.28
Species*Light*Nutrient*Defoliation	1	0.11	0.51	0.24	0.37
Error <i>df</i>		65	64	56	55

¹ # = $p \leq 0.10$, * = $p \leq 0.05$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

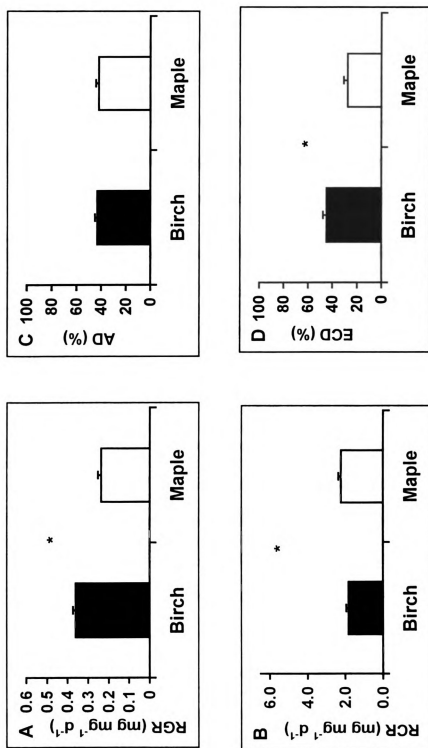


Figure 3.15. Relative growth rate (RGR) of forest tent caterpillar measured over 48 h during the fifth instar on paper birch and sugar maple in 1997 (A). Relative consumption rate (RCR) (B), approximate digestibility (AD) (C), and efficiency of conversion of digested food to biomass (ECD) (D), of fifth instar forest tent caterpillar measured over 48 h. Asterisks indicate significant species effects on insect growth or nutritional indices ($p \leq 0.05$).

defoliated by forest tent caterpillar (significant light*defoliation interaction, Table 3.9, Figure 3.10). Shading had no effect on RCR, AD, or ECD of whitemarked tussock moth (Table 3.9). Shading did increase the development time of male but not female whitemarked tussock moth by 4 d, but did not influence pupal mass of either sex (Table 3.10).

In 1997, prior seasons' shading did not affect growth rates of second instars (Table 3.11), or fourth instar growth, relative consumption rates, approximate digestibilities, or efficiency of conversion of digested food to biomass (Table 3.11).

Fertilization interacted with the light treatment to improve whitemarked tussock moth performance. In 1996, fertilization of maple increased survival by 14%, but the effect was only seen in the shade (significant species*light*nutrient interaction) (Table 3.4). Fertilization did not affect fourth instar growth or nutritional indices (Tables 3.9). However, fertilization did increase relative growth rate over the total larval period by 10%, but only when trees were grown in full sun (significant light*nutrient interaction, Table 3.9, Figure 3.11). Fertilization did not influence whitemarked tussock moth pupal mass or larval development time (Table 3.10). In 1997, fertilization did not affect second instar growth or fourth instar growth or nutritional indices (Table 3.11).

Early season defoliation of birch, but not maple, induced resistance to whitemarked tussock moth later during the same season. Defoliation did not have an effect on the survival of early instars in 1996 (Table 3.4). Fourth instar growth was not affected by defoliation, although in shaded birch defoliation was associated with a 40% reduction in digestive efficiency (ECD) (significant species*light*defoliation interaction,

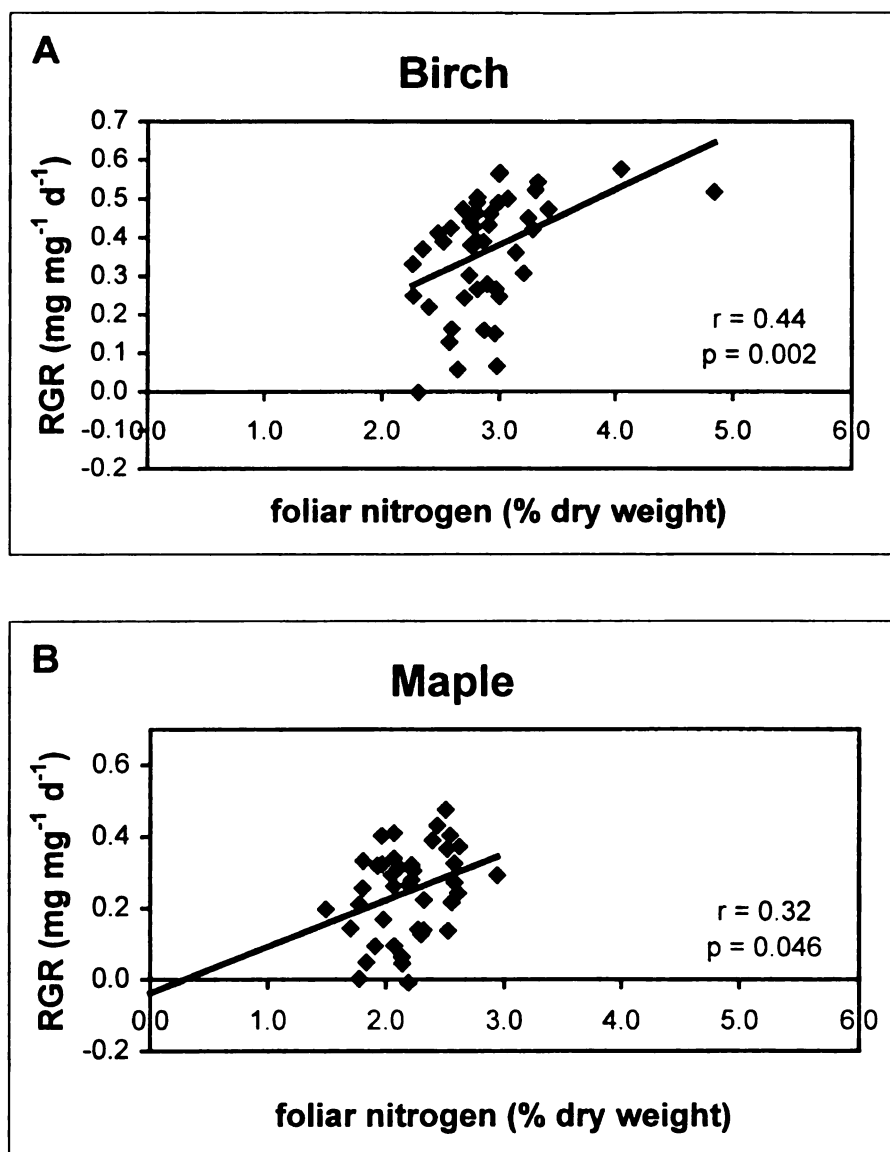


Figure 3.16. Relationship between foliar nitrogen content of paper birch (A) and sugar maple (B) and the relative growth rate (RGR) of fourth instar gypsy moth in 1997.

Table 3.5, Figure 3.12). Defoliation of birch reduced growth over the total larval period by 13%, bringing growth rates down to levels equal to those for maple-feeding larvae (Table 3.5, Figure 3.13). Correlation analyses also showed a negative relationship between percent defoliation and growth over the total larval period, and a positive relationship between percent defoliation and duration of the larval period (Table 3.8 B, Figure 3.14).

As with gypsy moth, there was no evidence of delayed induced resistance of birch to whitemarked tussock moth (Table 3.11). Prior season's defoliation was not correlated with second or fourth instar larval growth or nutritional indices in 1997 (Table 3.8 B).

Forest tent caterpillar.

As with gypsy moth and whitemarked tussock moth, birch was a better host for forest tent caterpillar than was maple. In 1997, digestive efficiency was 52% lower and consumption rate was 28% higher on maple relative to birch (Table 3.12, Figure 3.15). Increases in consumption were not adequate to compensate for poor host quality, and growth of fifth instar forest tent caterpillar was 35% slower on foliage of maple than birch (Table 3.12, Figure 3.15).

Light and fertilization treatments did not significantly influence the growth or nutritional indices of fifth instar forest tent caterpillar (Table 3.12). However, percent defoliation was positively correlated with digestive efficiency (ECD) of this species (Table 3.8 C). Consumption and relative growth rate were not correlated with percent defoliation of either host.

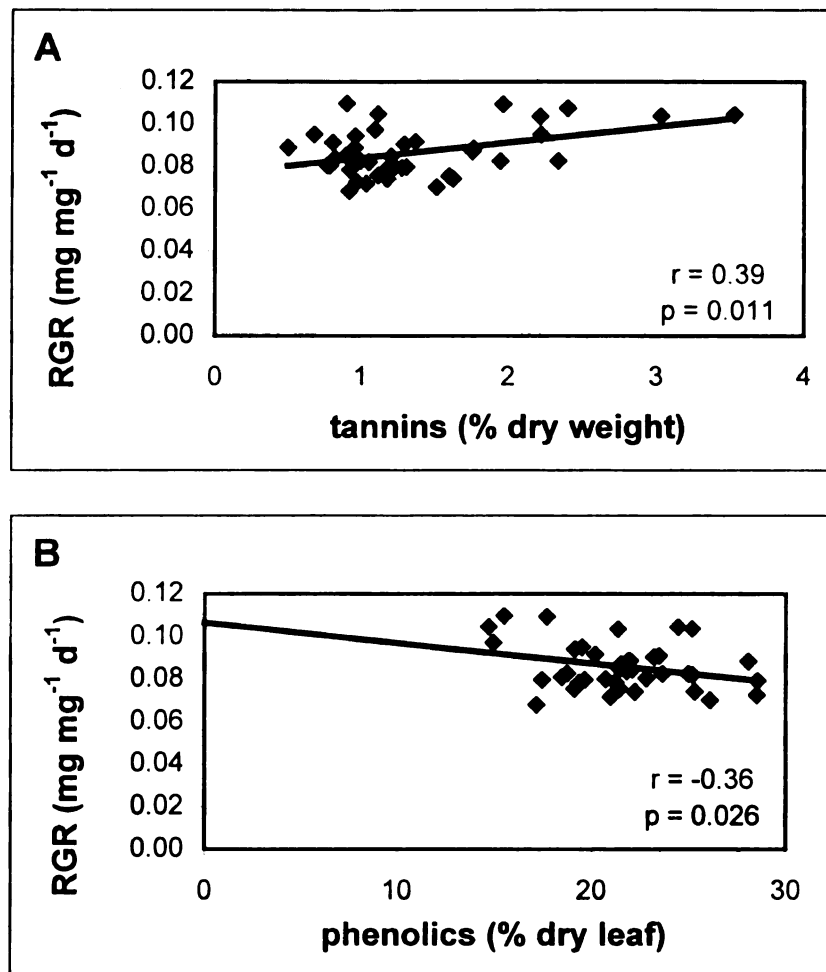


Figure 3.17. Relationship of maple condensed tannins (A) and total phenolic concentrations (B) with whitemarked tussock moth relative growth rate measured over the total larval period in 1996 (RGR).

Insect Performance in Relation to Foliar Traits.

Gypsy moth.

There was no significant correlation between foliar nitrogen and gypsy moth performance in 1996, but the quality of birch foliage was improved as total phenolic and condensed tannin concentrations increased. In 1996, efficiency of conversion of digested birch foliage to biomass (ECD) was positively correlated with total phenolic and condensed tannin content of foliage (Table 3.8 A). In maple, gypsy moth pupal mass was positively correlated with specific leaf mass in 1996 (Table 3.8 A).

In 1997, second instar relative growth rates were negatively correlated with foliar condensed tannin content of maple, but not birch (Table 3.8 A). Fourth instar growth rate was positively correlated with foliar nitrogen content in both birch and maple (Table 3.8 A, Figure 3.16), and negatively correlated with C/N ratios in birch foliage (Table 3.8 A). Digestive efficiencies (ECD) were negatively correlated with specific leaf mass of birch (Table 3.8 A).

Whitemarked Tussock Moth.

In 1996, there were no significant correlations between birch foliar traits and whitemarked tussock moth performance. However, in 1997, as foliar nitrogen content increased, the quality of birch to whitemarked tussock moth also increased as indicated by positive correlations between foliar nitrogen and efficiency of conversion of digested food to biomass and relative growth rate of fourth instars (Table 3.8 B). Correspondingly, C/N ratios in birch were negatively correlated with both fourth instar

relative growth rate and efficiency of conversion of digested food to biomass (Table 3.8 B).

In maple in 1996, larval growth as measured from egg hatch to pupation was positively correlated with condensed tannin levels (Table 3.8 B, Figure 3.17) and specific leaf mass, and negatively correlated with total phenolic levels of foliage (Table 3.8 B, Figure 3.17). Duration of the larval period was positively correlated with both condensed tannin and total phenolic levels of maple (Table 3.8 B). There were no significant correlations between maple foliar traits and whitemarked tussock moth performance in 1997.

Forest Tent Caterpillar.

In birch, neither nitrogen content nor concentrations of foliar phenolics were correlated with forest tent caterpillar performance; however, larval consumption of birch foliage was positively correlated with foliar C/N ratios in 1997 (Table 3.8 C). In maple, increases in foliar nitrogen improved host quality; efficiency of conversion of digested foliage to biomass was positively correlated with foliar nitrogen content (Table 3.8 C). Neither fifth instar growth nor approximate digestibilities were correlated with foliar traits.

DISCUSSION

Light Effects on Host Quality.

The effects of shade on host quality were dependent upon host species, insect species, and other environmental conditions. Shade was important in influencing the effect of other environmental stresses on host quality (see below). Because shading reduced levels of total phenolics and condensed tannins in hosts, it was predicted to improve the quality of both birch and maple foliage for folivores in 1996. However, predictions for birch were based solely on reductions of condensed tannins in the shade. Considering that condensed tannins were not correlated with insect performance in this study (see Relative Host Quality of Paper Birch and Sugar Maple, below), the prediction is refuted. Even so, shade did in some cases have an effect on host quality. Shading reduced digestive efficiencies of gypsy moth fed birch in 1996, and decreased female pupal masses of gypsy moth reared on both birch and maple. In whitemarked tussock moth, growth was reduced and duration of larval period lengthened by shade in 1996.

In agreement with predictions based upon treatment effects on foliage chemistry (Tables 3.1 and 3.2), there was no effect of prior shading of either host species on insect performance in 1997.

It is important to note that in the present study insects were fed detached foliage under uniform laboratory conditions such that the effects of shade on insects are due only to effects of shade on foliage quality, and do not include direct physiological and/ or behavioral effects of light on the insects. In natural environments these direct and

indirect effects would be operating simultaneously. Light may affect herbivores strongly through direct effects on behavior rather than indirect effects mediated by the host plant. Both forest tent caterpillar and early instar gypsy moth are strongly photopositive and feed predominately in areas of the canopy with maximum light (Dylan Parry, personal communication). This study provides evidence that low-light can negatively affect insect performance through changes in host-quality, but these effects were not overwhelmingly strong or consistent. More severe levels of shading can be expected to lead to more extreme changes in foliar traits, and therefore potentially in insect performance. The mechanisms of observed effects remain unclear, but lack of significant correlations indicate that they do not appear to be linked to nitrogen, carbon, condensed tannins, total phenolics, or specific mass of leaves. Some foliar traits other than those measured, perhaps water content, alkaloids or extent of lignification, may be responsible for the indirect effects of shade observed on gypsy moth and whitemarked tussock moth. Carbon/ nutrient balance hypothesis predictions for nitrogen-based compounds (increasing in foliage of shaded trees) are consistent with observed reductions of insect performance in shade.

Nutrient Effects on Host Quality.

Host and herbivore responses to fertilization were species-specific and were affected by light environment and defoliation. Based on effects on foliar nitrogen, fertilization was predicted to have no effect in 1996 and to correspond to improved insect performance in maple but not birch in 1997 (Tables 3.1 and 3.2). However, fertilization decreased growth of gypsy moth in shaded treatments in 1996, also consistent with

predictions of the carbon/ nutrient balance hypothesis for nitrogen-based defenses. In full sun, though, fertilization increased gypsy moth performance (survival, digestive efficiencies) in 1996. In 1997, fertilization increased second instar gypsy moth growth on maple. Growth of second instars was greatest on fertilized, non-defoliated hosts, indicating that defoliation may influence host quality by depleting foliar nutrients (see below), consistent with carbon/ nutrient balance hypothesis predictions for delayed induced resistance. The effect of fertilization was greatest in shaded treatments, further suggesting the potential for environmental factors to interact.

Because second but not fourth instar gypsy moth larvae were affected by fertilization in 1997, I cannot say for certain that fertilization has a biologically significant effect on this insect species. As discussed above, second instar gypsy moth would not naturally feed on foliage of the age used in the 1997 study.

The effects of fertilization on whitemarked tussock moth were also dependent upon light environment. Fertilization increased tussock moth performance in 1996 by increasing growth in sun treatments and increasing survival on shaded maple. Fertilization effects on foliage nitrogen content were also dependent on the shade treatment (Chapter 2). Fertilization increased foliar nitrogen in birch in full sun but not in shade (Chapter 2). Correspondingly, fertilization increased whitemarked tussock moth performance in 1996 as reflected in increased relative growth rates. Although correlations between whitemarked tussock moth growth and birch nitrogen content were not significant in 1996, there was a positive correlation between these traits in 1997.

As with shade, fertilization did not affect forest tent caterpillar performance in 1997. Fifth instars may be impervious to subtle changes in foliar traits that would influence performance of younger instars.

Defoliation Effects on Host Quality.

As with shade and fertilization, effects of forest tent caterpillar defoliation on host quality were dependent upon host species, insect species, and interactions with other treatments. Because maple received such low levels of defoliation ($8 \pm 3\%$ in the defoliated treatment), any observed effects are likely not representative of those that would be observed during natural outbreaks when trees are severely defoliated.

Defoliation generally increased the quality of birch and maple to gypsy moth in the season of defoliation as indicated by improved digestive efficiencies. However, defoliation reduced growth of both males and females over the total larval period, and increased larval duration of females. These observations cannot be attributed to changes in foliar nitrogen, carbon, C/N ratios, condensed tannins, total phenolics, or specific leaf mass as there were no defoliation-induced effects on these traits (Chapter 2, Tables 3.1 and 3.2). Ruohomaki et al. (1996), examining defoliation-induced resistance of mountain birch to a geometrid larvae, were also unable to attribute insect response to defoliation to corresponding changes in foliar defensive compounds. They examined resistance in three groups of trees: control, shaded, or fertilized. Insect performance was the same in all three groups, although shaded trees and fertilized trees had lower levels of foliar condensed tannins and total phenolics than controls.

Defoliation by forest tent caterpillar did induce resistance of birch to whitemarked tussock moth. Percent defoliation of birch early in the season was correlated with decreased growth and increased larval duration of whitemarked tussock moth feeding later in the season of defoliation. This adds to the growing body of literature supporting the observation that early season defoliation can affect the performance of species feeding later in the same season (Hunter 1987, Hanhimaki 1989, Dankert et al. 1997, Denno et al. 1995, Wold and Marquis 1997).

Effects of defoliation are important to consider when attempting to predict susceptibility of forest stands to insect outbreaks. Many researchers have hypothesized that stressed plants are more susceptible to herbivores than healthy plants (reviewed in Waring and Cobb 1989, Koricheva et al. 1998). The results of the present study indicated that, in general, defoliation-stressed birch are lower quality hosts for gypsy moth and whitemarked tussock moth than non-defoliated birch, but only when trees are shaded. When growing in full sun, as they likely would be in nature, birch quality increased with defoliation. Gypsy moth had increased digestive efficiency and decreased consumption rates on defoliated birch in the sun and whitemarked tussock moth were not effected by defoliation of birch grown in the sun. Therefore, defoliated birch may be more susceptible to attack by gypsy moth and whitemarked tussock moth during outbreaks of forest tent caterpillar. This study addresses only indirect effects of defoliation, mediated through changes in host quality. Density dependent effects of defoliation on behavior might substantially alter the performance of gypsy moth and whitemarked tussock moth on defoliated birch in natural settings.

Neither gypsy moth nor whitemarked tussock moth were affected by prior season's defoliation in 1997, indicating no evidence for delayed induced resistance at the levels of defoliation achieved in this experiment. Moreover, forest tent caterpillar performance in 1997 was not correlated with prior season's defoliation, indicating little potential for self-regulation of this insect population through delayed density dependent effects of defoliation on host quality (Haukioja 1980), at least at defoliation levels observed here. Dankert et al. (1997) did observe delayed induced resistance in another group of birch trees belonging to the same cohort used in this experiment when defoliation levels were more severe (80-90%).

Relative host quality of paper birch and sugar maple.

Sugar maple was a lower quality host for gypsy moth, whitemarked tussock moth, and forest tent caterpillar than was paper birch. These findings are in agreement with previous observations that slow-growing, shade-tolerant plants such as sugar maple are more resistant to herbivory than fast-growing, shade-intolerant species such as paper birch (Coley 1983, Dudt and Shure 1994). This is consistent with the hypothesis that slow-growing species, limited in their ability to replace lost tissues, have higher levels of constitutive defenses (Coley et al. 1985).

Total phenolics and condensed tannins were not consistently implicated as determinants of host quality. Maple did have higher levels of total foliar phenolics (but lower levels of condensed tannins) than birch (Chapter 2), which were negatively correlated with growth rate and positively correlated with larval development time for

whitemarked tussock moth in 1996 but not 1997. Conversely, condensed tannin concentrations of maple, one component of total phenolic measures, were positively correlated with whitemarked tussock moth relative growth rates measured over the total larval duration in 1996, suggesting that maple condensed tannins do not play a defensive role against this species. Prior research has indicated that whitemarked tussock moth is not affected by hydrolyzable tannins in the diet (Karowe 1989) due to the protective role of the peritrophic membrane of the midgut (Barbehenn and Martin 1992).

The negative correlation between whitemarked tussock moth growth rate and total phenolics, and the positive correlation with maple condensed tannins observed in this study generally agrees with the idea that whitemarked tussock moth is a tannin tolerant species. However other compounds active in the total phenolic assay are apparently having a detrimental effect on whitemarked tussock moth. If one such compound is a hydrolyzable tannin, it must not be any of those present in the tannic acid mixture used in the diet studies of Karowe or Barbehenn and Martin. Alternatively, it may be a hydrolyzable tannin present in both diet studies and in the maple foliage, but may remain inert unless in the presence of some foliar enzymes or other compounds found in maple leaves but not in artificial diet. It is also possible that the detrimental compound is not a phenolic at all. Other defensive compounds (e.g. alkaloids) or foliar traits (e.g. water content) may actually be the causal agents of maple resistance and may covary with total phenolic content. There was also no correlation between birch condensed tannins or total phenolics and larval growth rates, providing further evidence that whitemarked tussock moth is a tannin-tolerant species.

Gypsy moth also performed worse on maple than on birch. However, there is no indication that condensed tannin or total phenolic concentrations are responsible for this variation in host quality. In fact, gypsy moth digestive efficiency was positively correlated with total phenolic concentrations. This is not altogether surprising, for although gypsy moth can exhibit reduced growth on high tannin diets, it is not uncommon to find a positive relationship between phenolic levels and larval feeding, larval mass, and pupal masses (Kleiner and Montgomery 1994), at least in laboratory studies. In the field, there is a limit to this tolerance, as strong negative correlations between poplar condensed tannin levels and female pupal masses have been observed (Dylan Parry, unpublished data). Similarly, Rossiter et al. (1988) found defoliation-induced increases in foliar phenolics of natural red oak populations to be negatively correlated to gypsy moth pupal mass, fecundity, and egg mass.

Forest tent caterpillar is a tannin-sensitive species (Karowe 1989, Barbehenn and Martin 1994). However, I found no relationship between condensed tannins or total phenolics. The reduced performance on maple relative to birch observed here is contrary to findings of Dankert (1995) who found lower survival and growth on paper birch relative to sugar maple in a study using trees from the same cohort as those used in this experiment. These conflicting findings are difficult to explain as insects were reared under identical environmental conditions. However, Dankert's population of tent caterpillars were collected in New Foundland, while those in the present study were collected in Ontario. While sugar maple grows in New Foundland, it does not grow in the region of Ontario where the forest tent caterpillar eggs were collected for this study.

It is possible that the relatively higher resistance of maple to the forest tent caterpillar used in this study reflect the lack of an evolutionary history of this insect population with maple.

Maple resistance to gypsy moth and forest tent caterpillar also may be linked to lower levels of nitrogen in maple than in birch foliage (Chapter 2). In 1997, growth rate of fourth instar gypsy moth on birch and maple was positively correlated with foliar nitrogen content, as was growth of fifth instar forest tent caterpillar on maple.

Compensatory Consumption.

Although relative growth rates of gypsy moth measured from egg hatch to pupation were reduced on maple in both 1996 and 1997, in 1996 fourth instars were able to compensate for decreased host quality by increasing consumption. Failure of fourth instars to exhibit compensatory feeding in 1997 may have resulted from the fact that in 1996 larvae were reared from egg hatch on experimental trees and in 1997 they were field collected from poplars, trees dominated by secondary metabolites (phenolic glycosides) not found in birch and maple. Dietary switching can reduce the performance of some herbivores on novel hosts (Joseph and Kelsey 1994). The timing of a host switch in the course of the insect's lifecycle is important for determining the effect of the switch on the insect (Stoyenoff et al. 1994). Early instars, although more sensitive to host quality than later instars (Table 3.8) may have been able to adapt to foliage quality in 1996 so that by the fourth instar they had made behavioral (e.g., consumption) and /or physiological (e.g.,

induction of digestive enzymes) adjustments enabling them to maintain relatively high growth rates even on non-preferred hosts. Alternatively, it may be that in 1997, fourth instars switched from poplar to maple were not sufficiently able to adjust their consumption rates within the 2 d period of growth measurement to compensate for decreased host quality.

Second instar gypsy moth and whitemarked tussock moth were more sensitive to foliage quality than were conspecific fourth instars in 1997 (as indicated by higher numbers of significant treatment effects on second relative to fourth instars). These results were expected as 1) second instars naturally feed on younger foliage than do fourth instars, and young foliage is expected to have higher water and nutrient contents relative to older foliage (Scriber and Slansky 1981), and 2) larvae experimentally tested on maple underwent a host switch. While it is not unusual for lepidopteran larvae to switch hosts in natural settings, this switch does not occur until larvae have completed early development. In the case of gypsy moth, dispersal from the original host occurs after larvae reach the fourth stadium (Lance and Barbosa 1979, Leonard 1981, as cited in Stoyenoff et al. 1994). Therefore, younger larvae may be more affected by a change in host than older instars. These findings emphasize the importance of mimicking natural conditions as closely as possible in laboratory studies.

Second instar whitemarked tussock moth were less sensitive to foliage quality than were second instar gypsy moth in 1997, even though both species exhibited reduced growth on maple. This is indicated by a higher number of significant treatment effects on second instar gypsy moth relative to second instar whitemarked tussock moth. In 1997,

all feeding studies were conducted in mid-June. This corresponded with natural host-herbivore phenology of fourth instar gypsy moth and fifth instar forest tent caterpillar, and with the second instars of the second whitemarked tussock moth generation.

However, second instar gypsy moth would not naturally be feeding on foliage of such an advanced phenological stage. This phenological asynchronicity may explain the greater sensitivity of second instar gypsy moth to foliage quality. Thus, the results should be interpreted with some caution.

Conclusions.

Host species was the most important factor affecting the performance of all three folivores studied. Shade, fertilization, and defoliation also were influential in affecting insect performance. However, effects of these factors never influenced all insect species equally, and effects were often interactive. The mechanistic basis of the effects of shade, fertilization, and defoliation were not made clear by my results. Previous work has demonstrated that shade and fertilization can lower C/N ratios and carbon-based defensive compounds in foliage, thereby improving insect performance (Bryant et al. 1983). However, neither condensed tannin nor total phenolic concentrations were good predictors of insect performance in this study. Presence of nitrogen-based defensive compounds, (which occur in maple but not birch), may be driving observed reductions of insect performance in shaded trees, as these compounds are predicted to be increased by shade and fertilization. Nutritional value of foliage also affects host quality (Mattson 1980, Scriber and Slansky 1981, Tuomi et al. 1984). While there was limited evidence of an association between foliar nitrogen content and insect performance in some cases, the

majority of effects of treatments on insect performance could not be explained by changes in foliar nitrogen levels. This does not negate the importance of foliar defensive compounds or nitrogen content on insect performance; rather it implies that effects of light, nutrient, and defoliation treatments in this study are not acting by a simple mechanism which influences foliar nitrogen or carbon-based defensive compounds. The interactive nature of environmental stresses on host quality and differential responses of various insect species to host foliage complicate the process of devising models with strong power to predict forest susceptibility to pest species.

Understanding of mechanisms of forest susceptibility will be aided by future research examining foliar and insect responses across a range of nutrient, light, and defoliation levels. Such experimental designs will enable regression analysis, which can better expose the relative importance of individual foliar traits affected by environmental stress that in turn influence herbivore performance.

CHAPTER 4:

CONCLUSIONS

Light availability, nutrients, and defoliation all influenced patterns of resource acquisition, resource allocation and herbivore resistance in paper birch and sugar maple. Tree responses to these stresses were influenced by species-specific characteristics, and therefore were not often the same in both species. Moreover, the effects of one stress on tree responses were often dependent upon simultaneous exposure to other stresses. Herbivore responses to environmentally stressed host-foliage were also species specific. The mechanistic basis of insect responses to host quality were not clear, but did not appear to be strictly a function of foliar nutrient content or levels of carbon based defenses.

Consistent with life history theory, paper birch was generally faster-growing, had higher light-saturated photosynthetic rates, and was more responsive to environmental variation than was sugar maple. Birch assimilated carbon more rapidly at all light levels, even in shaded environments. Therefore, although maple is considered to be a more shade tolerant species than birch, this tolerance could not be attributed to a superior photosynthetic capacity of maple over birch in low light. Furthermore, the species had equivalent respiration rates implying that their basic requirements for photosynthate were similar, at least at the leaf level. However, the shade tolerance of maple was evidenced by its photosynthetic rates being less responsive to decreased light levels than birch's photosynthetic rates.

Corresponding to its higher photosynthetic rates and adaptation to high light environments, birch had higher foliar nitrogen content and greater specific leaf mass than maple. Total phenolic concentrations were also greater in birch, although the compositional structure of those phenolics differed between the species as indicated by maple having higher concentrations of condensed tannins than birch.

In agreement with Life History Theory, paper birch was a superior host for gypsy moth, whitemarked tussock moth, and forest tent caterpillar than was sugar maple. Although levels of foliar nutrients and defensive compounds did influence the performance of folivores, each species of insect responded differently to these traits. In maple, total phenolics were correlated with reduced host quality for whitemarked tussock moth in 1996 but not 1997. Conversely, condensed tannin concentrations of maple were positively correlated with whitemarked tussock moth performance, indicating that condensed tannins of maple do not play a defensive role against this species. Gypsy moth growth on birch and maple in 1997 was positively correlated to foliar nitrogen content, as was forest tent caterpillar growth on maple.

Shading had a large impact on tree growth and herbivore resistance. Trees did not appear to acclimate photosynthetically to two years of shade, as development under reduced light conditions did not affect light-saturated photosynthetic rates in either species. However, the photosynthetic rate of birch in low light was significantly less than that in full sun. Trees did respond to temporal shifts in light availability in 1996 with decreased specific leaf mass. Decreased specific leaf mass may result from increased leaf size or from compositional differences in sun versus shade-grown foliage. By spreading out photosynthetic apparatus over a larger area leaves may more efficiently harvest the

limited incident radiation in shaded environments. However, in this study, increased leaf areas were evidenced only in immature foliage collected in August from non-defoliated birch trees. Shade increased foliar nitrogen per unit mass in maple, suggesting that decreases in other foliar components may have occurred. The shade treatment was not implemented in 1997; therefore, previously shaded trees experienced ambient light conditions mimicking gap formation. Foliage on trees that were previously shaded had a specific leaf mass equivalent to that of foliage on trees which had developed in full-sun. This indicates that birch and maple trees may quickly adjust to gap formation.

Both birch and maple exhibited reduced growth in shade. Height growth of both species was not affected by light availability during the first year of shading (1995), while diameter growth was decreased by shade. In the following year (1996), height growth was decreased by shade, while there was no influence of light on diameter growth.

Although shading had no strong effect on foliar carbon or C/N ratios, it reduced carbon-based defenses in both birch and maple in 1996 as predicted by the carbon/nutrient balance hypothesis. However, birch and maple carbon-based secondary metabolites did not respond to shade in the same way. Total phenolics of birch remained the same, but the composition of these phenolics changed, as condensed tannin content was reduced. In maple, shade also induced compositional changes in phenolics but in this case shade was associated with a reduction in total phenols and maintenance of condensed tannin levels.

The light treatment did not generally have a strong effect on insect performance, but was important in influencing the effect of other environmental stresses on host

quality. When shade did affect insect performance, it reduced host quality. Shading reduced digestive efficiencies of gypsy moth fed birch in 1996, and decreased female pupal masses of gypsy moth reared on both birch and maple. The growth of whitemarked tussock moth was reduced and duration of larval period lengthened by shade in 1996. However, there was no effect of prior shading of either host species on insect performance in 1997. This study provides evidence that low-light can negatively affect insect performance through changes in host-quality, but that these effects are not strong or consistent. More severe levels of shading can be expected to lead to more extreme changes in foliar traits and therefore potentially in insect performance. The mechanisms of observed effects remain unclear, but they are linked in some cases to nitrogen, carbon, condensed tannins, total phenolics, and specific leaf mass of leaves.

Effects of fertilization on tree physiology and herbivore resistance were not as strong as expected. Fertilization did not affect the photosynthetic rates of birch or maple. Fertilization also had no effect on tree growth rates with the exception that it did reduce the height growth of both species in 1995. These findings were unexpected, and indicate that nitrogen was not limiting in non-fertilized treatments. Reductions in growth in 1995 may indicate that a nitrogen surplus was reached in fertilized cells such that some other nutrient became limiting. It would be necessary to analyse soil samples to determine if this was indeed the case.

Fertilization decreased levels of total phenolics in immature birch foliage harvested in August of 1996. This response was in agreement with predictions of the carbon/ nutrient balance hypothesis, although it was not accompanied by significant

increases in foliar C/N ratios. In contrast to the August response, fertilization had no effect on C-based defenses of either species when sampled in June.

Although fertility had no effect on foliar defenses of birch or maple in June of 1996, herbivores did respond to fertilization. These responses were host and insect species-specific and were affected by light environment and defoliation. Fertilization decreased the growth of gypsy moth on shaded trees in 1996, but increased growth on trees in full sun. Fertilization also increased second instar gypsy moth growth on maple in 1997. The effect of fertilization was greatest in shaded treatments, further indicating the potential for environmental factors to interact. Fertilization increased whitemarked tussock moth performance in 1996 by increasing growth in sun treatments and increasing survival on shaded maple. Although correlations between whitemarked tussock moth growth and birch nitrogen content were not significant in 1996, there was a positive correlation between these traits in 1997. These results suggest that increased soil nutrient levels do not necessarily need to stimulate plant growth in order to influence herbivore resistance.

As with shade and fertilization, effects of defoliation on foliar quality were dependent upon host species, insect species, and the other treatments. Because maple received such low levels of defoliation, any observed effects are not likely to be representative of those that would be observed during natural outbreaks.

Effects of defoliation on birch physiology mimicked those of nutrient deficiency in that fertilization often mitigated the effects of defoliation. Defoliation decreased foliar nitrogen levels in non-fertilized birch. Correspondingly, defoliation also increased C/N ratios, but this was true for both fertilized and non-fertilized trees. Defoliation can

increase the C/N ratio because the majority of the plant's nitrogen supply is contained in the canopy and is lost when the tree is defoliated. However, carbon is available from stored reserves in other plant parts. Defoliation reduced height growth in both species; however, there was no evidence of fertilization mitigating this effect.

The effects of forest tent caterpillar defoliation on the quality of birch as a host for whitemarked tussock moth and gypsy moth were environmentally dependent. Although foliar condensed tannin levels were lower in all defoliated birch trees in June of 1996, defoliated trees were lower quality hosts for gypsy moth and whitemarked tussock moth than non-defoliated birch, but only when trees were shaded. There were no delayed effects of defoliation on birch resistance to gypsy moth or whitemarked tussock moth, indicating that forest tent caterpillar defoliation at the observed levels will not mediate interspecific competition among these species. Moreover, forest tent caterpillar performance the year following defoliation was not affected by the defoliation treatment, indicating no evidence for delayed density dependent regulation of this insect population.

APPENDICES

APPENDIX 1

Record of Deposition of Voucher Specimens

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.: 1998-10

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

Light, Nutrient Availability, and Defoliation Effects on Resource Acquisition,
Resource Allocation, and Herbivore Resistance of Paper Birch and Sugar Maple

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)

Heather L. Govenor

Date 11/16/1998

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

Deposit as follows:

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

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

APPENDIX 1.1

Voucher Specimen Data

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
<u>Lymantiria dispar</u> L.	Canadian Forest Service, Insect Rearing Facility Sault Ste. Marie, Ontario 20 May 1996		3					MSU
<u>Orygia leucostigma</u> Smith	Canadian Forest Service, Insect Rearing Facility Sault Ste. Marie, Ontario 17 July 1996		3					MSU
<u>Malacosoma disstria</u> Hubner	Same population as Dylan Parry, Voucher No. 2000-?							

(Use additional sheets if necessary)

Investigator's Name(s)

Heather L. Governor

Voucher No. 1998-10

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



Curator

Date

16 Nov-1998

Date 16 November 1998

LITERATURE CITED

LITERATURE CITED

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