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INDUCED RESPONSES OF POPLARS TO DEFOLIATION AND THEIR EFFECTS ON LEAF-FEEDING LEPIDOPTERA

By

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ABSTRACT

INDUCED RESPONSES OF POPLARS TO DEFOLIATION AND THEIR EFFECTS ON LEAF-FEEDING LEPIDOPTERA

By

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An enduring hypothesis for explaining cyclical populations of forest Lepidoptera has been phytochemical resistance to herbivory elicited in trees subjected to defoliation. Severe defoliation of trees elicits both rapid and delayed changes in host quality for herbivores, known collectively as 'induced-resistance'. While rapid-induced resistance (RIR), which affects the generation of insects causing the damage, is thought to contribute to population stability, delayed-induced resistance (DIR), lasting a year or more, may lead to unstable dynamics and generate population cycles. DIR has typically been investigated in trees following a single defoliation event. However, many outbreak folivores defoliate trees for consecutive years and single year studies may not capture the full effects of DIR. Thus, cumulative effects of successive years of defoliation on tree physiology and insect herbivores are poorly understood.

I conducted two long-term studies designed to emulate the defoliation intensity and temporal scale of natural outbreaks. Chapter 2 describes an outbreak population of gypsy moth (*Lymantria dispar* L.) established experimentally in large-scale stands of hybrid poplar (*Populus sp.*), 1996-1999. In Chapter 3, I experimentally established forest tent caterpillar (*Malacosoma disstria* Hübner) populations and defoliated two stands of trembling aspen (*Populus tremuloides*) over a four-year period (1997-2000).

Gypsy moth defoliation increased total phenolics and condensed tannins in leaves while having only marginal effects on phenolic glycosides and other minor secondary

compounds (Chapter 2). There were strong within-year, rapid-induced effects on secondary metabolites, and condensed tannins and total phenolics remained high one year after the cessation of defoliation. Nitrogen was reduced within the year that defoliation occurred, especially in late season leaf samples. Effects on nitrogen did not extend to the following season and levels had recovered to predefoliation levels within a year of cessation of defoliation. Gypsy moth pupal mass, development time and fecundity were not affected by the treatments in 1996, whereas female pupal mass and fecundity were marginally reduced in 1997 and significantly reduced in 1998. Gypsy moth defoliation reduced the performance of competing species of Lepidoptera in 1998. Forest tent caterpillar, poplar tent maker (*Clostera inclusa*), and big poplar sphinx (*Pachysphinx modesta*) all suffering reduced pupal mass, fecundity, and/or increased development time. Fall webworm (*Hyphantria cunea*), was unaffected by defoliation or fertilization. Defoliation in previous years did not affect gypsy moth and three other species in 1999, suggesting that herbivore performance was independent of phenolic and tannin levels.

Chronic defoliation of aspen (Chapter 3) had larger effects on tent caterpillars than defoliation of poplars had on gypsy moth (Chapter 2). Relative growth rates of final instar females were significantly reduced although growth of male fifth instars was unaffected. Mean fecundity was reduced by 8-21% in the first year, 13-16% following three years, and 20-21% on trees with four consecutive years of defoliation. Development time and survival from hatch to adult was unaffected by the treatments. Defoliation affected parasitism rates by two tachinid flies. Parasitism appeared to be mediated by caterpillar density rather than induced host tree responses to defoliation, and also varied significantly among aspen clones. Intraspecific differences in phytochemistry among clones may alter host detection abilities in tachinids using olfactory cues from herbivore damaged foliage.

I dedicate this, with love and utmost and heartfelt thanks, to my mother, Ruth Powell, who has never wavered in her support for me, despite the many twists and turns of my chosen career path.

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INTRODUCTION

Outbreaks of forest Lepidoptera are a conspicuous feature of temperate and boreal forests. Of particular fascination for ecologists are the cyclical or nearly cyclical changes in density that characterize the population dynamics of many outbreaking species. The relative contributions of density-dependent and density-independent regulating mechanisms in the population dynamics of these insects, and indeed that of many other organisms, engaged researchers in fractious debate for several decades (e.g., Nicholson 1933, Andrewartha and Birch 1954, Dempster 1983, Hassell et al. 1989, Murdoch, 1994, Turchin 1995). Identification of delayed negative feedback in time series data from many forest insects (Turchin 1990, 1995) shifted the focus away from whether density dependence regulates populations, to questions regarding which of several potentially delayed-density dependent factors can best account for the observed cycles. While the autocorrelation analysis used by Turchin (1990) is sufficient to detect delayed-density dependent regulation, it can not determine causal agents. Thus, several factors including maternal effects, natural enemies, and defoliation-induced changes in host plant quality are all theoretically capable of producing second-order dynamics in forest insect populations (Berryman et al. 1987, 1996, Ginzburg and Taneyhill 1994, Underwood 1999).

More than two decades ago, investigators first recognized that reductions in survival, growth, and fecundity occurred in herbivorous insects feeding on the foliage of defoliated deciduous trees (e.g., Haukioja and Niemela 1977, Haukioja 1980). Because food quality is integral to the success of herbivores, defoliation-induced changes in foliage have been an appealing explanation for fluctuating population dynamics in insects that outbreak on hardwood trees. Any response by plants to herbivory that reduces the fitness of herbivores has been defined as 'induced-resistance' (Karban and Baldwin 1997). In deciduous trees, two types of induced-resistance have been recognized, based on both differences in temporal manifestation, and on their postulated effects on herbivore population dynamics (Haukioja and Niemela 1977, Haukioja 1980, 1982). Rapid-induced resistance (RIR) occurs within minutes or days of herbivory. Relatively little damage may be required to elicit RIR responses and the duration of the effects can be transient. Because of the short induction time, RIR affects the generation of herbivore responsible for the herbivory and thus is thought to exert a stabilizing effect on population dynamics (Haukioja 1982). In contrast to RIR, delayed-induced resistance (DIR) occurs in the year or years following herbivory and generally is elicited only by severe defoliation. Because of the time-lags in induction and relaxation, DIR acts on future generations of folivorous insects, and thus is hypothesized to have a destabilizing rather than a regulating effect on population dynamics (Haukioja 1982). The postulated effects of DIR on herbivore population dynamics has led a number of authors to propose similar host-quality driven models for explaining cycles in forest Lepidoptera (e.g., Benz 1974, Haukioja 1980, Rhoades 1983a).

The quality of foliage for herbivorous insects is determined by a surfeit of physical and biochemical traits including nitrogen, water content, carbohydrates, fiber, toughness, and a host of secondary compounds such as alkaloids, phenolics, and terpenoids (e.g., Feeny 1970, Mattson 1980, Scriber and Slansky 1981, Schultz 1988, Koricheva et al. 1998). These parameters are often altered quantitatively by defoliation thus changing the value of leaves as food for caterpillars (e.g., Bryant et al. 1988, Karban and Myers, 1989, Haukioja 1990). Identification of the individual leaf traits responsible for reductions in herbivore performance following defoliation has proven difficult, leading some to speculate that it is suites of characters rather than individual components that are responsible for the observed effects (Kause et al. 1999).

Rapid-induced resistance – direct effects on herbivorous insects

Herbivores ranging from mollusks to mammals have been used to investigate the effects of RIR. However, herbivorous insects, particularly the Lepidoptera, have been the organism of choice in most investigations. Studies have shown that RIR can reduce growth, fecundity, and survival of insects, as well as influence behaviors such as preference and foraging (e.g., Haukioja and Niemela 1977, Haukioja 1980, 1982, Haukioja and Hanhimaki 1985, Bergelson et al. 1986, Rossiter et al. 1988, Edwards et al. 1991, Hanhimaki and Senn 1992). Effects of RIR on herbivores have been variable (see Table 1) generating lengthy debate about the generality of RIR responses, as well as their effectiveness (e.g., Fowler and Lawton 1985, Hartley and Lawton 1991, Edwards et al. 1991, Karban and Baldwin 1997).

Table 1. Short-term effects of defoliation on the performance of folivorous Lepidoptera feeding on deciduous trees. Studies were selected if trees were partially or completely defoliated in the same season as the bioassays were conducted. In these studies, defoliation was applied either manually using scissors or tearing leaves, during natural insect outbreaks, or by experimental inoculation of trees with caterpillars. In some cases the defoliation was due to insect species other than the species assayed. Studies were only included in the table if a direct measure (fecundity or mortality) or surrogate measure (pupal mass, development time, growth rate) of herbivore fitness was recorded. * n/e = not estimated.

Defoliation type (severity %)	Tree genus	Insect species	Effects relative to controls	Reference
Manual (n/e)*	*Betula pendula*	*Coleophora serratella*	Development time increased by 3 days.	Bergelson et al. (1986)
Manual (n/e)	*Betula pubescens*	*Epirrita autumnata*	Reduction in survival, development time increased by 1-2 days	Haukioja & Niemela (1977)
Manual (>25% of leaves torn in each cluster)	*Betula pubescens*	*Epirrita autumnata*	0-20% reduction in pupal mass, 2-3 day increase in development time	Haukioja & Niemela (1979) Haukioja et al. (1983) Haukioja & Hanhimaki (1985)
Manual (n/e)	*Betula pubescens*	*Brephos parthenias* *Eriogaster lanestris*	No significant effect on pupal mass or development time of *B. parthenias*. Non-significant 3% decrease in final larval weight of *E. lanestris*.	Haukioja & Niemela 1979
Manual (n/e)	*Betula pubescens*	*Epirrita autumnata*	0-22% reduction on *Epirrita* population growth potential	Haukioja & Neuvonen (1987)

Cont'd

Table 1 cont'd

Manual - several treatments (n/e)	*Betula pubescens*	*Epirrita autumnata*	No significant decrease in 4th and 5th instar larvae among 3 defoliation treatments and controls.	Hanhimaki & Senn (1992)
Insect (~90%)	*Betula papyrifera*	*Lymantria dispar* *Orgyia leucostigma*	No effect of forest tent caterpillar defoliation on early instar gypsy moth growth. Defoliation reduced male white-marked tussock moth later in the season but had no effect on females.	Dankert et al. (1997)
Manual (5-25%)	*Betula pendula*	*Apocheima pilosaria*	Larval mass reduced 10-38%, mortality increased 11-16%.	Fowler & MacGarvin (1986)
Insect (30-50%)	*Quercus rubra*	*Lymantria dispar*	No effect of defoliation on the probability of mortality from NPV in multiple different experiments.	D'Amico et al. (1997)
Insect (7-58%)	*Quercus rubra*	*Lymantria dispar*	Female pupal mass negatively correlated with defoliation, phenolic, and tannin content of leaves.	Rossiter et al. (1988)
Insect (14-55%)	*Quercus*	*Lymantria dispar*	Decreased mortality from NPV when fed leaves from defoliated trees which had higher phenolic and tannin concentrations	Hunter & Schultz (1993)
Insect (>70%)	*Quercus rubra*	*Lymantria dispar*	Foliage from naturally defoliated red oak stands reduced female pupal mass by 22% with no change in development time when compared to undefoliated trees.	Lance et al. (1991)
Manual (100%)	*Quercus velutina* *Betula populifolia*	*Lymantria dispar*	Progressive defoliation emulating feeding reduced female pupal mass by 18% and 9% on oak. On gray birch, defoliation decreased female pupal mass by 10% in one year and increased it by 30% in another year.	Valentine et al. (1983)

Cont'd

Table 1 cont'd

Manual (100%)	*Quercus velutina* *Betula populifolia*	*Lymantria dispar*	Progressive defoliation emulating feeding reduced female pupal mass by ~8% and ~19% on oak and gray birch, respectively.	Wallner & Walton (1979)
Manual, Insect (partial, 100%)	*Quercus emoryi*	*Stilbosis sp.* *Camereria sp.*	Larval mortality increased in some studies, but decreased in others, depending on the amount of damage	Faeth (1986, 1988)
Insect (5-20%)	*Lupinus arboreus*	*Orgyia vetusta*	Early season defoliation reduced female pupal mass by 25% and fecundity by 48%.	Harrison & Karban (1986)
Insect (all leaves damaged – no % given)	*Alnus rubra*	*Hyphantria cunea*	No difference in pupal mass at one site, 8% increase in pupal mass at second site. No difference in survival at one site, 15% lower survival at second site	Williams & Myers (1984)
Insect (ca. 15%)	*Alnus rubra*	*Malacosoma californicum*	Mortality increased, growth rate decreased, fecundity decreased.	Rhoades (1983)
Insect (70%)	*Alnus rubra*	*Malacosoma californicum*	High defoliation caused an 8% reduction in female pupal mass and a 14% reduction in fecundity.	Rothman (1997)
Manual (n/e)	*Alnus rubra*	*Malacosoma californicum*	No effects on female pupal mass, survival reduced by 23% and 18% on damaged and adjacent to damaged leaves.	Myers & Williams (1987)
Insect (ca. 15%)	*Salix sitchensis*	*Hyphantria cunea* *Malacosoma californicum*	Webworm growth rate reduced, no effects on tent caterpillars in one year, tent caterpillar growth reduced in another year.	Rhoades (1983)

Cont'd

Table 1. cont'd

Insect (10-100%)	*Quercus garryana* *Malus sp.*	*Operophtera brumata*	Fecundity declines with increasing levels of defoliation. On apple maximal decrease is 56% and on oak 72%.	Roland & Myers (1987)
Insect (n/e)	*Acer saccharum* *Betula papyrifera*	*Malacosoma disstria* *Lymantria dispar*	Defoliation had no significant effects on growth or survival of 1st instar forest tent caterpillar and gypsy moth on birch or maple.	Dankert (1996)
Insect (0- 65%)	*Populus tremuloides*	*Papilio canadensis*	10% and 65% forest tent caterpillar defoliation reduced swallowtail growth by 10% and 24% respectively.	Dankert et al. (1997)
Insect (52% aspen and 27% maple)	*Populus tremuloides* *Acer saccharum*	*Malacosoma disstria*	No effect on 4th instar development time in aspen, 9% increase in maple. No effect on growth rate in aspen, ~20% decrease in maple.	Roth et al. (1998)
Manual (50%)	*Populus tremuloides* *Acer saccharum*	*Lymantria dispar*	Slight increase in development time on aspen, decrease on maple. Growth reduced on aspen, no effect on maple. Slight decrease in weight on aspen, slight increase on maple.	Lindroth & Kinney (1998)
Insect (2-20%)	*Populus spp.*	*Malacosoma disstria*	Second instars fed damged leaves from two clones had growth reduced by 66% and 68%.	Robison and Raffa (1997)
Insect, manual	*Populus spp.*	*Lymantria dispar*	The effects of various induction treatments were examined among 12 different clones. Gypsy moth growth was reduced by 0-71% in defoliation treatments depending on the clone.	Havill & Raffa (1999)
Insect (no % given)	*Populus spp.*	*Lymantria dispar*	13% increase in development time to 3rd instar, 25% decrease in 3rd instar mass.	Havill & Raffa (2000)

Criticism of the importance of RIR to herbivores has focused on inconsistency in results among experiments (e.g., Fowler and Lawton 1985). However, the plethora of methodologies used in this field, especially in early studies, has undoubtedly contributed to this variation. For example, experiments have used different scales (leaf, branch, tree), induction methods (scissors, leaf-punches, tearing, insect feeding), timing between induction and bioassays, tree species, herbivore species, types of assays, and levels of defoliation. Thus, many of these experiments are not directly comparable and using variable results as a criterion to dismiss the importance of induced-resistance is contrived. Given these well-documented sources of variation, it is not surprising that no consensus on the effects of RIR has been reached.

Three experimental approaches have been used to investigate the effects of RIR on insect herbivores. One direction of research has focused on behavioral responses by herbivores to feeding-induced changes in foliar quality, while another area of focus has been comparison of localized and systemic responses to herbivory. A third area of RIR research has addressed the effects of whole-tree defoliation on herbivore fitness as measured by growth, fecundity and survival. While there is overlap among these approaches, questions are asked at different scales (e.g., Edwards and Wratten 1983) and from differing perspectives (either plant-oriented or herbivore-oriented).

Dispersion of herbivory over an entire tree may have less impact on the tree than the same level of herbivory concentrated on individual leaf clusters or branches (Edwards and Wratten 1983). This suggests that induced-resistance may dissuade herbivores from feeding in proximity to prior leaf damage. Furthermore, increased herbivore movement may be correlated with higher mortality, presumably because it increases vulnerability to

natural enemies and dispersal losses (Edwards and Wratten 1983, Schultz 1983, although see Fowler and Lawton 1985 for alternative hypotheses). Several species of caterpillars assayed in laboratory and field experiments did not respond behaviorally to damaged leaves in any predictable way (Hartley and Lawton 1987, 1990). In some experiments, leaves partially damaged early in the season have reduced levels of herbivory later in the season (Hunter and Schultz 1995, Wold and Marquis 1997), suggesting that herbivorous insects may avoid previously damaged tissue. However, complete defoliation may have opposing effects, with some herbivores benefiting while populations or guilds of other species are reduced (Faeth 1988). Interpreting the results and identifying the relevant mechanisms in behavioral/preference experiments has proven difficult. Furthermore, the relevance of these processes to herbivore population dynamics remains to be demonstrated.

In early studies of RIR, a primary objective was to quantify the impact of localized and systemic responses to herbivory. These experiments used low levels of defoliation (often by tearing individual leaves) and comparisons were made among undamaged controls, damaged leaves, and intact leaves immediately adjacent to damaged foliage. Growth of the autumnal moth, *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch, *Betula pubescens*, was reduced when fed either damaged leaves (localized response) or intact leaves that were adjacent to damaged leaves (systemic response) (Haukioja and Niemela 1977, Haukioja and Hanhimaki 1985). In an experiment addressing systemic induction caused by leaf-tearing, autumnal moth pupal masses were reduced by 0-14% and survival was markedly lower (Haukioja and Niemela 1979).

More recently, Havill and Raffa (1999) compared RIR induced by low levels of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) feeding damage, jasmonic acid (a well defined biochemical elicitor of induced-responses), and mechanical damage (scissors) among 12 poplar, *Populus* spp., cultivars. Not only did the type of damage influence induction, the strength of the induced resistance on gypsy moth varied by as much as 72% between different cultivars. Follow-up studies showed that defoliation reduced the mass of third instars by 25% when larvae were fed foliage from clones found to be most inducible (Havill and Raffa 2000). Similar effects on growth have been found in early instar forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), where partial defoliation of poplar reduced growth by three fold (Robison and Raffa 1997). Results of these experiments indicate that systemic induction occurs in poplars as a response to real or simulated herbivory, and that the impact on herbivores can be relatively strong. Similar research on other species of trees is needed to determine the generality of these responses. As with the behavior/preference experiments above, however, the significance of these results to natural populations of insects is not known.

Many investigations of plant-herbivore interactions have used short-term (one instar or less) bioassays as surrogates for labor intensive bioassays encompassing the entire larval stage. While growth rate is generally considered to be correlated with pupal mass (Ayres et al. 1997), and hence fecundity in outbreak Lepidoptera with non-feeding adults, this may not always be the case. Some instars are more sensitive to foliar quality than others (Hanhimaki and Senn 1992, personal observation) and furthermore, critical periods in foliar development or induction may be missed with short-term bioassays. While these studies suggest possible consequences of RIR on fitness, caution should be used in

extrapolating population effects from the results unless accompanied by corroborating full-length larval assays.

Experiments designed to simulate the more intense herbivory associated with outbreak species have also yielded variable results. Manual defoliation (50%) of trembling aspen, *Populus tremuloides*, slightly increased development time and reduced growth of gypsy moth, whereas the same treatment applied to sugar maple, *Acer saccharum*, had no effect (Lindroth and Kinney 1998). In contrast, defoliation did not affect growth of forest tent caterpillar on aspen but reduced its growth by 20% on sugar maple (Roth et al. 1998). Defoliation of sugar maple and paper birch, *Betula papyrifera*, had no significant effect on early instar growth of either forest tent caterpillar or gypsy moth (Dankert 1996). These results highlight the variability of experiments assessing the effects of RIR on caterpillar growth.

The effects of RIR on pupal mass and fecundity have been quantified in only a few studies. Rossiter et al. (1988) used gypsy moth larvae to generate a 10-58% gradient in defoliation of red oak, *Quercus rubra*, and then assessed the effects of the treatments by rearing gypsy moth larvae in sleeve cages on the trees from hatch to pupation. Female pupal mass was negatively correlated with defoliation and was reduced by as much as 20%. Progressive, manual defoliation of black oak, *Quercus velutina*, and gray birch, *Betula populifolia*, to mimic gypsy moth feeding over the duration of the larval period, reduced female gypsy moth pupal mass by as much as 18% on oak (Wallner and Walton 1979, Valentine et al. 1983). On birch, effects of defoliation on pupal mass were more variable and both increases and decreases were recorded depending on the year. In a recent study, defoliation (>70%) of red alder, *Alnus rubra*, using western tent caterpillar,

Malacosoma californicum (Lepidoptera: Lasiocampidae), reduced fecundity by more than 14% (Rothman 1997). Variability in the effects of RIR among studies may be in part due to differences in the levels of defoliation used (see Table 1). Roland and Myers (1987) found that reductions in pupal mass of winter moth, *Operophtera brumata* (Lepidoptera: Geometridae) appeared to be correlated linearly with increasing defoliation in apple, *Malus* sp. and Garry oak, *Quercus garryana*.

Data from natural populations of gypsy moth also suggest that RIR can reduce pupal mass and fecundity while lengthening development times. Relative to an undefoliated site, pupal masses were reduced by 22% for laboratory reared caterpillars fed foliage from red oaks with a single year of defoliation and 32% from a site with one year of previous and one year of current defoliation (Lance et al. 1991). In similar studies of natural populations, Myers and Williams (1984, 1987) found that RIR had little or no effect on pupal mass of western tent caterpillar. However, as Neuvonen and Haukioja (1985) pointed out, this type of experiment may be inherently confounded because the insects and not the experimenters selected the trees. Furthermore, pathogens or stress factors such as crowding may also reduce fecundity in outbreak populations, and the effects may not easily be separated from those of the host tree. These types of natural experiment should be interpreted with caution.

The role of RIR in population dynamics of outbreak forest insects has often been downplayed or ignored. This is unfortunate because, although RIR does not function in a delayed density-dependent manner and thus can not directly contribute to population cycles, it still may play a role in population dynamics. For example, RIR can act to slow population growth, allowing other factors to exert greater negative pressure on population

growth. Temporal variability in RIR could also be important in population processes because annual fluctuations in its strength may allow populations to escape regulation. Variability in RIR across landscapes could contribute to spatial heterogeneity in population densities, contributing to changes in natural enemy activity. For these reasons, research on the effects of RIR remains valuable and should be continued.

Delayed induced resistance – direct effects on herbivorous insects

In contrast to the variable effects of RIR on caterpillars, DIR has had generally negative effects on caterpillar fitness (Table 2). Large contributions to the understanding of DIR have come from research on birches and their defoliators, particularly mountain birch and autumnal moth in Fennoscandia. Ruohomaki et al. (1992) summarized 24 separate experiments conducted in this system over a 15-year period. Although not always statistically significant, their review showed that relative to control trees, pupal mass was reduced in more than 90% of the experiments following a single year of defoliation. There was considerable annual variation in the strength of DIR with pupal mass reductions ranging from 0 to 44% depending on the year. Interestingly, the largest DIR effects occurred in years where larvae grew the best on control trees. Similar long-term declines in the quality of foliage following defoliation have been recorded from paper birch in Alaska. Experiments designed to simulate severe outbreaks of the black-marked spear moth, *Rheumaptera hastata*, an important defoliator in this system showed that in the year following defoliation, survival was reduced by 19% (Werner 1979). In another experiment, a ca. 28% decrease in pupal mass was recorded for *R. hastata* caterpillars fed foliage from birch trees completely defoliated in the previous year (Bryant et al. 1993).

Table 2. Long-term effects of defoliation on the performance of folivorous Lepidoptera feeding on deciduous trees. Studies were selected if trees were partially or completely defoliated in the growing year or years prior to the bioassays being conducted. Defoliation was applied either manually using scissors or tearing leaves, by natural insect outbreaks, or by experimental inoculation of trees with caterpillars. In some cases the defoliation was due to insect species other than the species assayed. Studies were only included in the table if a direct measure (fecundity or mortality) or surrogate measure (pupal mass, development time, growth rate) of herbivore fitness was recorded. *n/e = not estimated.

Year(s) previous defoliation	Type (severity %)	Tree species	Insect species	Effect relative to controls	Reference
1	Insect, manual (20-100%)	*Betula pubescens*	*Epirrita autumnata*	Review of 24 different experiments 1979-1992. Pupal masses were reduced 0-44% depending on year and experiment.	Ruohomaki et al. (1992)
1	Manual (100%)	*Betula papyrifera*	*Rheumaptera hastata*	Pupal mass reduced by 24% and survival by 45% in unfertilized treatments.	Bryant et al. (1993)
1	Insect (10-100%)	*Quercus garryana* *Malus spp.*	*Operophtera brumata*	Defoliation levels <50% in previous year increased pupal mass, levels >50% decreased pupal mass.	Roland & Myers (1987)
1	Insect (10-100%)	*Lupinus arboreus*	*Orgyia vetusta*	No effects on pupal mass or fecundity in year following defoliation	Harrison (1995)

Cont'd

Table 2 cont'd

1	Insect (>70%)	*Quercus rubra*	*Lymantria dispar*	5-11% reduction in female pupal mass in year following defoliation. Insects selected trees – confounded design.	Lance et al. 1991.
1	Manual (50%)	*Betula pubescens*	*Epirrita autumnata*	No effect of defoliation on growth rates in unfertilized treatments, pupal mass increased slightly on previously defoliated trees.	Muitikainan et al. (1997)
1	Insect (90%)	*Betula papyrifera*	*Lymantria dispar*	Defoliation by forest tent caterpillar reduced gypsy moth growth by 15% in the following year.	Dankert et al. (1997)
1	Insect (0-70%)	*Alnus rubra*	*Malacosoma californicum*	No effects on pupal mass and fecundity in year following defoliation.	Rothman (1997)
1 and 2	Manual (100%)	*Fagus crenata*	*Quadracalcarifera punctatella*	12% reduction in female pupal mass after 1 year, 26% after 2 years. Survival significantly reduced.	Kamata et al. (1996)
3	Insect (n/e)	*Alnus rubra*	*Malacosoma californicum*	Female pupal mass reduced by 0-15% on trees defoliated in previous years.	Myers & Williams (1984)
2	Insect (20-80%)	*Betula pubescens*	*Epirrita autumnata*	Pupal masses assayed in year following natural outbreak of 2 years were reduced by 0-10%.	Kaitaniemi et al. 1999a
1, 2, and 3	Manual (50-100%)	*Betula papyrifera*	*Rheumaptera hastata*	Survival reduced by 19, 41, and 70% following 1, 2, and 3 consecutive years of 100% defoliation.	Werner (1979)
1, 2, 3, and 4	Manual (100%)	*Quercus velutina* *Betula populifolia*	*Lymantria dispar*	Successive defoliations resulted in cumulative declines in female pupal mass on black oak but not on gray birch.	Valentine et al. (1983)

Cont'd

Table 2 cont'd

1 and 2	Manual (25-75%)	*Betula pubescens*	*Epirrita autumnata*	Pupal mass of autumnal moth reduced 11-17% and winter moth 10-20%. Cumulative effects not significantly different from single year effects.	Kaitaniemi et al 1999b
			Operophtera brumata		
1 and 2	Manual (100%)	*Quercus velutina*	*Lymantria dispar*	Cumulative reductions in female pupal mass after 1 and 2 years defoliation of black oak, female pupal mass reduced after defoliation of gray birch but no cumulative effect.	Wallner and Walton 1979
		Betula populifolia			
3	Insect (n/e)	*Alnus rubra*	*Malacosoma californicum*	14% reduction in female pupal mass in one year, 17% reduction in adult female mass in second year.	Myers and Williams 1987
1, 2, and 3	Manual (100%)	*Populus tremuloides*	*Choristoneura conflictana*	Growth, pupal mass, and survival reduced by 18-75%. Effects cumulative, largest reductions in 3rd year. Bioassay did not correspond with natural phenology of insect.	Clausen et al. 1991

Traditionally, experimental tests of DIR have consisted of a single year's defoliation followed by phytochemical analyses and/or bioassays in the following year or years. This approach may underestimate the strength of DIR because outbreaks of many defoliators are at least two years in duration (e.g., Mattson et al. 1991). Haukioja et al. (1988) hypothesized that consecutive defoliations may have cumulative effects on herbivore performance. Despite this speculation, the effects of cumulative defoliation on trees and herbivores have received only limited attention in the literature (Kaitaniemi et al. 1999b).

Some studies have shown that there may indeed be cumulative negative effects of successive years of defoliation on herbivores. Werner (1979) found that each additional year of defoliation increased the mortality of spear-marked black moth feeding on paper birch. In similar studies, Wallner and Walton (1979) and Valentine et al. (1983) defoliated black oak and gray birch for several successive years. Gypsy moth pupal mass progressively decreased with each additional year of defoliation. Clausen et al. (1991) defoliated trembling aspen for three years in succession and found that 18-75% decreases in pupal mass of large aspen tortrix, *Choristoneura conflictana* (Lepidoptera: Tortricidae), with the largest losses in the third year. More recently, Kamata et al (1996) found that pupal mass of beech caterpillar, *Quadracalcarifera punctatella* (Lepidoptera: Notodontidae), was reduced by 12% and 26% following one and two years of complete defoliation, respectively.

Not all studies have shown additive effects of consecutive defoliations. Kaitaniemi et al. (1999b) found that two years of 75% defoliation on mountain birch did not have greater effects on winter moth and autumnal moth than a single year of 75% defoliation. In a unique experiment, Kaitaniemi et al (1999a) maintained undefoliated control trees

using insecticide throughout a natural outbreak of autumnal moth. Unprotected trees suffered two consecutive years of defoliation although unlike previous outbreaks, the amount of foliage removed, 15-25% in 1992 and 30-45% in 1993, was not high. Bioassays indicated that DIR was weak, reducing pupal mass by only 2-11% relative to control trees following two years of defoliation. These researchers also used regression to convert the mass of adult males captured in pheromone traps to female equivalents to determine the reductions in fecundity present in natural populations. They found that fecundity was decreased maximally by 18% in the year following peak density (two consecutive years of defoliation). Results from these natural and experimental studies are well within the range of DIR effects recorded from single year defoliations (Ruohomaki et al. 1992) suggesting that there were no cumulative effects of defoliation, or alternatively, that the levels of defoliation were too low to elicit maximal DIR responses.

Delayed induced resistance has been postulated to be a general mechanism driving the cyclic dynamics of forest insects (e.g., Haukioja 1980, Rhoades 1983). However, a number of studies have suggested that DIR may be more system specific than first thought. Roland and Myers (1987) found that moderate levels of defoliation (<50%) in the previous season actually increased pupal mass of winter moth in the subsequent season on both apple and oak whereas on apple, higher defoliation (>50%) in the previous season reduced pupal mass. In some systems, even severe defoliation does not elicit DIR. Rothman (1997) found that severe defoliation of red alder had no effect on the mass of western tent caterpillar pupae in the year following defoliation. Similarly, Harrison (1995) showed that defoliation in the previous year did not affect fecundity of western tussock moth, *Orgyia vetusta* (Lepidoptera: Lymantriidae) feeding on bush

lupine, *Lupinus arboreus*. It is interesting to note that both red alder and bush lupine ar nitrogen-fixing species. Species which fix nitrogen may be buffered from the nitroge losses that follow defoliation, or may more rapidly recover to predefoliation levels (se Tuomi et al. 1990).

Since studies of DIR have been confined to a few systems, it is unknown if th results can be generalized to other species. Among the 10 species with the larges outbreaks on deciduous trees in North America (Mattson et al. 1991), research on DI has been conducted for just three species (gypsy moth, large aspen tortrix, black-marke spear moth). In addition, the paucity of studies that examined DIR over time scales tha characterize natural outbreaks suggest that we have only a rudimentary understanding o the role of DIR in population dynamics.

Mechanisms underlying induced responses in trees

Variability in the effects of RIR and DIR on herbivorous insects may in part stem fro the effects of underlying environmental heterogeneity on host trees. A number o mechanisms have been proposed to explain the induction of resistance followin defoliation. These can be broadly grouped into 'supply-side' hypotheses, focusing on th partitioning of resources among primary and secondary functions and 'demand-sid hypotheses, focusing on wound-induced demand for secondary compounds (Lerdau et a 1994, Karban and Baldwin 1997). With respect to deciduous trees, the carbon-nutrie balance (CNB) hypothesis (Bryant et al. 1983), a subset of predictions under the mo globally encompassing growth-differentiation balance hypothesis (Herms and Mattso 1992), has received the most attention of the supply-side hypotheses. The major premi

of the CNB is that growth is more sensitive to changes in nutrients than is photosynthesis. When growth is limited by nutrients, excess carbon from storage or photosynthesis can be allocated to production of carbon-based secondary compounds, hence the 'supply-side' moniker. Rather than a passive allocation of carbon, demand side hypotheses view resistance as a hormonally mediated response system to various stress factors such as wounding (Karban and Baldwin 1997).

Baldwin and Karban (1997) suggest that the majority of rapidly induced chemical responses of plants to herbivory can be explained by the demand-side active response theories. However, both passive 'supply' hypotheses and active 'demand' hypotheses will likely be required to explain completely the full temporal range of induced-resistance responses in trees (e.g., Tuomi et al. 1990, Herms and Mattson 1992, Koricheva et al. 1998). Integration of different hypotheses across temporal and hierarchical scales has been hampered by a dichotomy in the research focus and methodology of ecologists investigating DIR, and plant physiologists and molecular biologists investigating RIR.

Mechanistically, the CNB is predicated on the availability of nitrogen, which is generally regarded as the growth-limiting nutrient in temperate and boreal forests. Defoliation, especially early in the growing season, decreases nitrogen stores in deciduous trees (Harper 1977, Chapin and Kedrowski 1983) and may damage fine roots, reducing nitrogen acquisition from soil, and exacerbating the nitrogen deficit (Tuomi et al. 1990). Thus, until a tree can recover or compensate for the nitrogen lost when defoliated, production of photosynthate will continue to exceed the carbon needs of growth processes, maintaining elevated levels of carbon-based secondary compounds.

Decreases in foliar nitrogen following extensive defoliation appear to be a genera phenomenon in deciduous trees (e.g., Tuomi et al. 1991, Bryant et al. 1991). Nitroge decreases can occur within damaged leaves following herbivory, and if the defoliation i extensive, may carry over into the following growing season. Following defoliation within-season declines in nitrogen have been found in various cultivars of poplars, aspen sugar maple and a number of other tree species (e.g., Robison and Raffa 1997, Lindroth and Kinney 1998, Roth et al. 1998). The mechanisms behind within-season nitroge decreases in damaged leaves are not well understood. Some nitrogen may be leache from the leaves or the tree may withdraw nitrogen from the leaf as has been shown with other nutrients (Chapin 1980, Nef 1988). Trees that are severely defoliated produce second set of leaves in the same growing season from dormant buds. Stored nitroge pools that are available for leaf expansion during bud break in the spring are not available when trees produce a second set of leaves. Thus, following refoliation, late season leaves will be significantly lower in nitrogen than leaves on trees that were not defoliated.

Long-term, across-year depression of foliar nitrogen following defoliation is more easily explained than within season changes. Following severe defoliation, recovery o nitrogen may require a year or more in trees growing in nutrient poor soil. Several years were required for foliar nitrogen to return to predefoliation levels in defoliated mountain birch in Fennoscandia (Tuomi et al. 1984). Recovery was shorter for paper birch in Alaska (Bryant et al. 1991) and willow, *Salix sunnamen,* in Finland (Tuomi et al. 1991) although two years were still required to reach predefoliation levels. Lowe concentrations of foliar nitrogen in the year following artificial or natural defoliation have also been documented in black oak, gray birch, and red alder (Valentine et al 1983

Myers and Williams 1987), suggesting that it may be a general consequence o defoliation.

The CNB predicts that reductions in nitrogen should lead to increases in carbon-base secondary compounds. Congruent with prediction, foliar nitrogen levels followin defoliation have been found repeatedly to be negatively correlated with concentrations o phenolics (Tuomi et al 1990, Bryant et al 1991, 1993). Under the CNB hypothesis nitrogen fertilization is predicted to attenuate levels of phenolics. In some studies application of nitrogen fertilizer to defoliated deciduous trees reduced levels of phenolic while other studies did not show this pattern (e.g., Haukioja and Neuvonen 1985, Bryan et al. 1993). The actual response of secondary metabolites to fertilization in defoliate trees may be dependent on both the ambient soil fertility (Herms and Mattson 1992) an the specific phenolic compounds measured (Koricheva et al. 1998).

Following complete defoliation, the phenolic concentrations in reflush foliage ma revert to levels similar to that found after bud break. Baldwin and Schultz (1982) foun only low levels of condensed tannins in the reflush leaves of red oak following complet defoliation by gypsy moth, and Faeth (1988) observed a similar pattern in *Quercu emoryi*. In contrast, damaged leaves remaining on the tree and sampled at the same tim as reflushed leaves had very high levels of condensed tannins in both studies. Becaus new leaves are major carbon sinks, growth should take precedence for available carbo leaving little for production of condensed tannins.

The CNB hypothesis does not predict some types of rapidly induced changes in tree including rapid systemic responses induced by low levels of herbivory. In herbaceou plants, several wound-responsive, signal-transduction pathways have been identifie

(e.g., Bergey et al. 1996, Karban and Baldwin 1997, Constabel 1999). In response t herbivory, specific elicitors stimulate secondary metabolism and/or translocation o secondary compounds from storage sites. Several studies have shown that insect feedin damage to the leaves of woody plants elicits different responses than equivalent amount of tissue removed mechanically (e.g., Haukioja and Neuvonen 1985, Hartley and Lawto 1987, Krause and Raffa 1992, Havill and Raffa 1999). This suggests that leaves o woody plants have insect-specific wound responses, similar to those demonstrated i numerous herbaceous plants. Furthermore, application of the generalized elicito compound, jasmonic acid, has been shown to induce RIR in hybrid poplars in the absenc of tissue damage (Havill and Raffa 1999), indicating that a signal-transduction pathwa may be operating. The role of phenotypic variation in rapidly-induced systemic woun responses in the leaves of trees has not been investigated.

Indirect effects of induced resistance - competition among folivores

Historically, interspecific competition has not been regarded as an importan component structuring phytophagous insect communities (e.g., Hairston et al. 196C Schoener 1983, Strong et al. 1984). Considerable evidence has accumulated suggestin that this view was inaccurate. In a comprehensive review, evidence for interspecifi competition was found in more than 75% of the cases examined (Denno et al 1995 Among chewing folivores, plant-mediated, indirect competitive interactions, such a those driven by induced-resistance, accounted for more than half of reported negativ interactions between species. Thus, changes in plant quality elicited by caterpillar feedin

can have significant effects on other members of the phytophagous insect commun utilizing the same hosts.

Because outbreak species have such a profound impact on the foliage resource, th may have particularly large effects on the fitness of competing folivores. T vulnerability of folivorous insects to indirect competition from outbreak species mediat by plant-quality may depend on several different factors. These include, the timing defoliation in relation to the phenology of competing folivores, life-history attributes su as leaf-rolling, leaf-mining, or open feeding, and the degree of dietary specializati (Hartley and Lawton 1987, Bowers and Stamp 1993, Denno et al. 1995, Dankert et 1997, Agarwal and Karban 1999). Non-outbreak species may be less adapted to lar changes in nitrogen, water, leaf toughness, or damage-induced secondary compour than outbreak species.

Experiments have demonstrated that host-plant mediated indirect competition do occur and can have significant effects. Defoliation of paper birch, sugar maple, and asp by forest tent caterpillars induced both RIR and DIR, reducing the performance of otl outbreak species [gypsy moth and white-marked tussock moth, *Orgyia leucostigr* Lepidoptera: Lymantriidae)] as well as tiger swallowtail, *Papilio canaden* (Lepidoptera: Papilionidae), a non-outbreak species (Dankert et al. 1997). Similar Harrison and Karban (1986) found that early season feeding by an arctiid caterpillar bush lupine reduced the quality of foliage for a later feeding tussock moth speci affecting its growth significantly.

Early season feeding damage may induce-effects that continue for the remainder the growing season. Both manually applied damage and naturally occurring gypsy mo

defoliation reduced the probability of subsequent damage by late season feeding ins
(Hunter and Schultz 1995, Wold and Marquis 1997). In forests, gypsy moth outbre
may reduce the population densities and/or species diversity of other lepidopte
(Sample et al. 1996, Work and McCullough 2000), although the effects documented w
small and restricted to a few taxa. However, not all studies have shown detrime
effects of early season damage. Spring defoliation of *Quercus emoryi* resulted in hig
densities of some leaf-mining species on late season leaves presumably because w
content was higher in the refoliated leaves (Faeth 1988). The mechanisms underly
competitive interactions between outbreak folivores and other species are not kno
Using wild radish, Agrawal (2000) demonstrated that the effects of induced-resista
may vary with different species of herbivores and in turn, plants may perceive and r
to herbivory by different species idiosyncratically. It is not known if trees and t
associated herbivores respond similarly, particularly to defoliation. In f
comprehensive evaluation of the competitive effects of outbreak species on o
folivores are lacking in most systems and methodological problems in estimating den
and species abundance confound interpretation of existing studies.

Indirect effects of induced resistance – interaction with the third trophic level.

Twenty years ago, Price et al. (1980) published an influential paper suggesting that
relationship between herbivorous insects and their host plants need to be considere
context with higher trophic levels. The importance of tritrophic interactions in ecol
was not immediately recognized. Indeed, a search of ISI Citation Abstracts revealed o
three papers in the 1980's using the keyword 'tritrophic', although a larger numbe

papers were published covering this subject area. In the decade following, there ha a veritable explosion of papers on tritrophic interactions, 200 papers publishe 1990, with an additional 30 published through July of 2000 alone. The ra interactions falling under the tritrophic umbrella is extremely broad. More literature searches suggest that while knowledge on the influence of plant- infochemicals on host seeking behavior in parasitic wasps is growing exponentiall areas including interactions between plant chemistry and pathogens, as well a chemistry and entomophagous predators, have lagged behind.

The effects of induced resistance in trees on insect pathogens have not bee studied. Most research to date has focused on interactions between gypsy moth, moth nuclear polyhedrosis virus (LdMNPV), and the secondary chemistry of o outbreaks of gypsy moth, NPV is generally the dominant source of mortality integral to the collapse of many high density populations. Oaks contain hydro tannins that may precipitate proteins. Gypsy moth defoliation increases concentrations in oaks (Schultz and Baldwin 1982, Rossiter et al. 1988), ar induction is thought to reduce the susceptibility of larvae to mortality from Laboratory experiments have confirmed that higher tannin levels in defoliated t inhibit baculovirus infection (Hunter and Schultz 1993). However, in field D'Amico et al. (1998) found no evidence that NPV activity was affected by defo on two species of oak. They attributed their findings to the lack of RIR during the time period early in the season when most NPV transmission between larvae occur

Research to date has concentrated primarily on the effects of within season cha phytochemistry on pathogen interactions. Only two studies have examin

interaction between defoliation in the previous year and herbivore susceptibility to pathogens. Wallner (1983) found that gypsy moth were more susceptible to NPV when fed leaves from oak trees which had been severely defoliated one year previously than on undefoliated controls. Conversely, Rothman (1997) found no interactive effect of western tent caterpillar NPV with previous defoliation, although there was no direct effect of DIR on the caterpillars either. Phytochemicals may exhibit multiple modes of action when interacting with other biochemical processes and pathogens, and these interactions may be context dependent. Thus, the effects of induced-resistance on mediating insect susceptibility to pathogens remain to be determined.

Very few studies have specifically addressed possible relationships between predators and either RIR or DIR in trees. A recent study examining constitutive resistance in willows and the influence of fertility regimes on the impact of predators indicated that this may be a fruitful area of research. Sipura (1999) found that bird predation of phytophagous insects was elevated on unfertilized willows of the genotype with highest constitutive resistance. Invertebrate predators can also interact with induced-responses in trees. Pear trees infested with psyllids were found to release volatiles attractive to anthocorid predators (Drukker et al. 1995). Further study indicated that the predators were not cueing on the insects themselves but rather on volatiles released by the tree in response to their feeding (Scutareneau et al. 1997). These recent developments may herald an expanded interest in predators as a component of tritrophic interactions.

A number of studies have shown that herbivore damaged plants emit signals that are attractive to parasitoids of caterpillars (e.g., Eller et al. 1988, Turlings et al. 1993, Thaler 1997, Dicke 1999). Thus, increasing emphasis has been placed on the study of plant

attributes such as nutritional status, production of secondary compounds, and physi structure that may enhance or interfere with parasitoid activity (Agrawal 2000). Wh the focus of such research has generally been with agricultural plants, there is increasi evidence that similar tritrophic interactions occur in trees (e.g., Havill and Raffa 2000).

Although hymenopterans have been the focus of research in agricultural systems, relationship between tachinids and induced-responses have received more attention trees. It has been recognized for more than 60 years that leaves damaged by feedi caterpillars are attractive to some tachinids (Bess 1936). In some cases, speci compounds released by plants and used as cues by tachinids searching for hosts ha been identified (Roland et al. 1995). Studies addressing the effects of RIR and DIR on host location of parasitoids in trees are for the most part lacking. In a recent contributi Havill and Raffa (2000) found that poplar leaves damaged by gypsy moth were more th three times more attractive to females of the gypsy moth parasitoid *Glyptapante flavicoxis* (Hymenoptera: Braconidae), than undamaged control leaves. Damaged leav also significantly reduced the growth of gypsy moth caterpillars, indicating that RIR m have a synergistic interaction with this parasitoid.

Induced-resistance and population dynamics

Simulation models have suggested that RIR can regulate herbivore populations and t DIR can create cyclical population dynamics in the absence of other density-depend factors (Edelstein-Keshet and Rausher 1989, Underwood 1999). In Underwood's (19 model, outcomes were contingent upon both the strength and timing of induc resistance. Whether populations were regulated or cycled depended on both the dec

rate of the resistance and the lag time to maximum strength of the induced-response date, the effects of RIR on a variety of forest insects have not been large. W simulation models do not specify absolute values required for regulation, in fo systems at least, the weak RIR recorded in experiments appears insufficient to regu insect populations.

Only one study has looked at the effects of induced-resistance on the popula growth rates of an outbreak folivore. In the absence of natural enemies, Haukioja e (1987) calculated that for autumnal moth feeding on undefoliated trees, $r = 2.9$ where was $r = 1.1$ on trees defoliated in the previous year, where r is the intrinsic rate of nat increase for the population. While DIR had a substantive effect on r, acting alone, it incapable of preventing this population from continuing to grow. In natural systems, is not operating independently of other factors and its effects may be sufficient to s population growth to the point where other time-lagged factors can drive popula density down.

Currently, synthetic studies are lacking where the effects of DIR are measu relative to the contributions of other top-down and bottom up regulating factors. U primarily historical data, Bylund (1995) demonstrated that delayed density-depen parasitism was the most important factor correlated with fluctuations of autumnal m populations in Sweden while other factors including induced-resistance contributed m less to population regulation. In another study, Virtanen and Neuvonen (1999) estim the relative contribution of altitude, climate, host quality, and parasitoids on autur moth in Finland over a three-year period. Like Byland (1995), they concluded parasitoids were more important than host plant quality in determining population tre

These studies suggest that induced resistance, which has long been thought to drive
system, may in fact play a lesser role in determining population dynamics.

Overview of dissertation

My dissertation is focused on four general themes: (1) What are the consequences
several consecutive years of defoliation on growth and phytochemistry of trees and
these variables affect fitness parameters of folivorous Lepidoptera using these trees
host plants? (2) Can DIR account for the large reductions in fecundity that characteri
the declining phase of outbreaks in many outbreak Lepidoptera? Population density of
decreases concurrently with fecundity, suggesting that some delayed-density depend
factor is driving the change. I was interested in whether DIR can account for all or at le
a substantial portion of this fecundity loss. (3) Does defoliation by an outbreak spec
have significant indirect, host-tree-mediated effects on other folivorous species belong
to different seasonal guilds and exhibiting different life-history strategies? (4) Can F
and DIR enhance or impair the success of parasitoids attacking outbreak folivores?

To address these questions, I used two poplar systems, one in a natural for
environment (*Populus tremuloides*) and one in replicated blocks of hybrid pop
(*Populus* × *euramericana*). The use of poplars offers several advantages for address
questions about plant-herbivore interactions. First, both of my experimental syste
consisted of well-defined clones. Using clones greatly reduces the inter-tree variabi
that has plagued earlier studies of RIR and DIR. Secondly, the phytochemistry of popl
is reasonably well known and less complex than other species frequently used in sim
experiments such as birches and oaks. Third, trees in the genus *Populus* are prefer

hosts for two dominant outbreak species, forest tent caterpillar and gypsy moth, all for experiments in which the experimental trees are defoliated naturally.

In my dissertation, I describe two long-term studies where trees were defoliat caterpillars to simulate natural outbreak events. In Chapter 2, the results of a study experimental outbreaks of gypsy moth were established in a hybrid poplar plantati described. Half of the plots were protected from defoliation. In addition, ni fertilizer was applied to half of the defoliated and undefoliated plots to exami effects of fertility on tree and herbivore responses to defoliation. The experiment w for four years and emulated the rise (1 year of light defoliation), outbreak (one y severe and one year of moderate defoliation), and collapse (one year of ver defoliation) in population densities that characterize natural gypsy moth outbrea quantified the effects of the defoliation and fertilization treatments on pupal fecundity, and development time of gypsy moth and five other lepidopterans found poplar plots. The species represented a diverse range of life history strategies a free-feeding Lepidoptera.

In Chapter 3, I describe an experiment where trees belonging to two different clones growing in a natural forest environment, were inoculated with high densi forest tent caterpillar. Ten trees in each clone were defoliated for three consecutive while another ten trees were undefoliated. In the fourth year, five of the prev defoliated trees were left undefoliated and five of the control trees were defoliated first time. This provided a set of four treatments allowing comparison of the effe RIR (first time defoliated), DIR (three previous years of defoliation), and the con effects of RIR and DIR (three previous and one current year). I compared fore

caterpillar growth, pupal mass, fecundity and survival across these 4 treatments. In addition, I examined the effects of RIR and DIR on the probability of parasitism by two species of tachinids, *Leschenaultia exul* and *Patelloa pachypyga*, that specialize on forest tent caterpillar.

LITERATURE CITED

Agrawal, A.A. 2000. Specificity of induced-resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos* **89**: 493-500.

Agrawal, A.A. and R. Karban. 1999. Why induced defenses may be favored over constitutive strategies in plants. Pp. 45-61 in R. Tollrian and C.D. Harvell, eds, The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, New Jersey.

Andrewartha, H.G. and L.C. Birch. 1954. The Distribution and Abundance of Animals. University of Chicago Press, Chicago, Illinois

Ayres, M.P., T.P. Clausen, S.F. MacLean, Jr., A.M. Redman and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78**: 1696-1712.

Benz, G. 1974. Negative feedback by competition for food and space, and by cyclic induced changes in the nutritional base as regulatory principles in the population dynamics of the larch budmoth, *Zieraphera dineana* (Guenee) (Lepidoptera: Tortricidae*). Zietschrift fur Angewandte Entomologie* **76**: 196-228.

Bergelson, J.M. and J.H. Lawton. 1986. The effects of foliage damage on case-bearing moth larvae, *Coleophora serratella*, feeding on birch. *Ecological Entomology* **11**: 241-250.

Bergey, D.R., G.A. Hoi and C.A. Ryan. 1996. Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. *Proceedings of the National Academy of Science* **93**: 12053-12058

Berryman, A.A., N.C. Stenseth and A.S. Isaev. 1987. Natural regulation of herbivorous forest insect populations. *Oecologia* **71**: 174-184.

Berryman, A.A. 1996. What causes population cycles of forest Lepidoptera? *Trends in Ecology and Evolution* **11**: 28-32.

Bess, H.A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. *Annals of the Entomological Society of America* **29**: 593-613.

Bowers, M.D. and N.E. Stamp. 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. *Ecology* **74**: 1778-1791.

Bryant, J.P., F.S. Chapin, D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**: 357-368.

Bryant, J.P. J. Tuomi and P. Niemela. 1988. Environmental constraints of constitutive and long-term inducible defenses in woody plants. Pp. 367-389 in K.G. Spencer, ed., Chemical Mediation of Coevolution. Academic Press, San Diego, CA.

Bryant, J.P., K. Danell, F. Provenza, P.B. Riechardt, T.A. Clausen and R.A. Werner. 1991. Effects of mammal browsing on the chemistry of deciduous woody plants.

Pp. 135-154 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Bryant, J.P., P.B. Reichardt, T.P. Clausen and R.A.Werner. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. *Ecology* **74**: 2072-2084.

Byland, H. 1995. Long-term interactions between the autumnal moth and mountain birch: the role of resources, competitors, natural enemies, and weather. PhD Dissertation. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Chapin, F.S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233-260.

Chapin, FS and R.A. Kedrowski. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology* **64**: 376-391.

Clausen, T.P., P.B. Reichardt, J.P. Bryant and R.A. Werner. 1991. Long-term and short-term induction in quaking aspen: related phenomena? Pp. 71-83 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Constabel, P. 1999. A survey of herbivore inducible proteins and phytochemicals. Pp. 137-166 in A.A. Agrawal, S. Tuzin and E. Bent, eds, Induced Plant Defenses Against Pathogens and Herbivores. APS Press, St. Paul, Minnesota.

D'Amico, V., J.S. Elkinton, G. Dwyer, R.B. Willis and M.E. Montgomery. 1998. Foliage damage does not affect within-season transmission of an insect virus. *Ecology* **79**: 1104-1110.

Dankert, B.A. 1996. Paper birch and sugar maple resistance to insect folivores as influenced by defoliation, drought, and fertilization. MS thesis. Michigan State University, East Lansing, Michigan.

Dankert, B.A., D.A. Herms, D. Parry, J.M. Scriber and L.A. Haas. 1997. Mediation of interspecific competition between folivores through defoliation induced changes in host quality. Pp. 71-88 in A. Raman, ed. Ecology and Evolution of Plant Feeding Insects in Natural and Man-Made Environments. International Scientific Publications. New Delhi, India

Dempster, J.P. 1983. The natural control of butterflies and moths. *Biological Reviews of the Cambridge Philosophical Society* **58**: 461-481.

Denno, R.F., M.S. McClure and J.R. Ott. 1995. Interspecific interactions in phytophagous insects: competition revisited and resurrected. *Annual Review of Entomology* **40**: 297-331.

Dicke, M. 1999. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology* **28**: 601-617.

Drukker, B., P. Scutareanu and M.W. Sabelis. 1995. Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? *Entomologia Experimentalis et Applicata* **77**: 193-203.

Edelstein-Keshet, L. and M.D. Rausher. 1989. The effects of inducible plant defenses on herbivore populations. I. Mobile herbivores in continuous time. *The American Naturalist* **133**: 787-810.

Edwards, P.J. and S.D. Wratten. 1983. Wound-induced defences in plants and their consequences for patterns of insect grazing. *Oecologia* **59**: 88-93.

Edwards, P.J., S.D. Wratten and R.M. Gibberd. 1991. The impact of inducible phytochemicals on food selection by insect herbivores and its consequences for the distribution of grazing damage. Pp. 205-221 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Eller, F.J., J.H. Tumlinson and W.J. Lewis.1988. Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environmental Entomology* **17**: 745-753.

Faeth, S.H. 1986. Indirect interactions between temporally separated herbivores mediated by the host plant. *Ecology* **67**: 479-494.

Faeth, S.H. 1988. Plant mediated interactions between seasonal herbivores: Enough for evolution or coevolution. Pp. 391-414 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Feeny, P.P. 1970. Changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**: 565-581.

Fowler, S.V. and J. H. Lawton.1985. Rapidly–induced defenses and talking trees: the devil's advocate position. *The American Naturalist* **126**: 181-195.

Fowler, S. and M. MacGarvin. 1986. The effects of leaf damage on the performance of insect herbivores on birch, *Betula pubescens*. *Journal of Animal Ecology* **55**: 565-573.

Ginzburg, L.R. and D.E. Taneyhill. 1994. Population-cycles of forest Lepidoptera – a maternal effects hypothesis. *Journal of Animal Ecology* **63**: 79-92.

Hanhimaki, S. and J. Senn. 1992. Sources of variation in rapidly inducible responses to leaf damage in the mountain birch-insect herbivore system. *Oecologia* **91**: 318-331.

Hairston, N.G., F.E. Smith and L.B. Slobodkin. 1960. Community structure, population control and competition. *The American Naturalist* **115**: 354-366.

Harper, J.L. 1977. Population Biology of Plants. Academic Press, San Diego.

Harrison, S. 1995. Lack of strong induced or maternal effects in tussock moths (*Orgyia vetusta*) on bush lupine (*Lupinus arboreus*). *Oecologia* **103**: 343-348.

Harrison, S. and R. Karban. 1986. Effects of an early season folivorous moth on the success of a later-season species mediated by a change in the quality of a shared host, *Lupinus arboreus*. *Oecologia* **69**: 354-359.

Hartley, S.E. and J.H. Lawton. 1991. Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. Pp. 105-132 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Hassell, M.P., J. Latto and R.M. May. 1989. Seeing the wood for the trees: Detecting density dependence from existing life-table studies. *Journal of Animal Ecology* **58**: 883-892.

Haukioja, E. 1980. On the role of plant defenses in the fluctuation of herbivore populations. *Oikos* **35**:202-213.

Haukioja, E. 1982. Inducible defenses of white birch to a geometrid defoliator, *Epirrita autumnata*. Proceedings of the 5th International Symposium on Insect-Plant Relationships, Wageningen.

Haukioja, E. 1990. Induction of defenses in trees. *Annual Review of Entomology* **36**: 25-42.

Haukioja, E. and S. Hanhimaki. 1985. Rapid wound resistance in white birch foliage to the geometrid *Epirrita autumnata*: A comparison of trees and moths from within and outside the outbreak range of the moth. *Oecologia* **65**: 223-228.

Haukioja, E. and P. Niemela. 1977. Retarded growth of a geometrid larvae after mechanical damage of leaves of its host tree. *Annales Zoologici Fennici* **14**: 48-52.

Haukioja, E. and P. Niemela 1979. Birch leaves as a resource for herbivores: Seasonal occurrence of increased resistance in foliage after mechanical damage of adjacent leaves. *Oecologia* **39**:151-159.

Haukioja, E. and S. Neuvonen. 1985. Induced-long term resistance of birch foliage against defoliators: defensive or incidental. *Ecology* **66**: 1303-1308.

Haukioja, E. and S. Neuvonen. 1987. Insect population dynamics and the induction of plant resistance: the testing of hypotheses. Pp. 411-432 in P. Barbosa and J.C. Schultz, eds, Insect Outbreaks. Academic Press, San Diego.

Haukioja, E., S. Neuvonen, S. Hanhimaki and P. Niemela. 1988. The autumnal moth in Fennoscandia. Pp. 163-178 in A.A. Berryman, ed, Dynamics of Forest Insect Populations. Patterns, Causes, and Implications. Plenum, New York.

Havill, N.P. and K.F. Raffa. 1999. Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior. *Oecologia* **120**: 295-305.

Havill, N.P. and K.F. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecological Entomology* **25**: 171-179.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants – to grow or defend. *Quarterly Review of Biology* **67**: 283-335.

Hunter, M.D. and J.C. Schultz. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia* **94**: 195-203.

Hunter, M.D. and J.C. Schultz. 1995. Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. *Ecology* **76**: 1226-1232.

Kaitaniemi, P., K. Ruohomaki, T. Tammaru and E. Haukioja. 1999a. Induced resistance of host tree foliage during and after natural insect outbreak. *Journal of Animal Ecology* **68**: 382-389.

Kaitaniemi, P., S. Neuvonen and T. Nyyssonen.1999b. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. *Ecology* **80**: 524-532.

Kamata, N. Y. Igarashi and S. Ohara. 1996. Defoliation of *Fagus crenata* affects the population dynamics of the beech caterpillar *Quadricalcarifera punctatella*. Pp. 68-85 in W.J. Mattson, P. Niemela, M. Rousi, eds, Dynamics of Forest Herbivory: Quest for Pattern and Principle. USDA Forest Service, North-Central Forest Experiment Station General Technical Report NC-183

Karban, R. and I.T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago, Illinois.

Karban, R. and J.H. Myers. 1989. Induced plant response to herbivory. *Annual Review of Ecology and Systematics* **20**: 331-348.

Kause A, V. Ossipov, E. Haukioja, K. Lempa, S. Hanhimaki and S. Ossipova. 1999. Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia* **120**: 102-112.

Koricheva J, S. Larsson, E. Haukioja and M. Keinanen. 1998. Regulation of woody plant secondary metabolism by resource availability: Hypothesis testing by means of meta-analysis. *Oikos* **83**: 212-226.

Krause, S.C. and K.F. Raffa. 1992. Comparison of insect, fungal, and mechanically induced defoliation of larch – effects on plant productivity and subsequent host susceptibility. *Oecologia* **90**: 411-416.

Lance, D.R., J.S. Elkinton and C.P. Schwalbe. 1991. Responses of gypsy moth larvae (Lepidoptera: Lymantriidae) to foliage of oaks from naturally infested sites on Cape Cod, Massachusetts. *Journal of Entomological Science* **26**: 214-222.

Lerdau, M., M. Litvak and R. Monson. 1994. Plant chemical defense: Monoterpenes and the growth-differentiation balance hypothesis. *Trends in Ecology and Evolution* **9**: 58-61.

Lindroth, R.L and K.K. Kinney. 1998. Consequences of enriched atmospheric CO_2 and defoliation for foliar chemistry and gypsy moth performance. *Journal of Chemical Ecology* **24**:1677-1695.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen-content. *Annual Review of Ecology and Systematics* **11**: 119-161.

Mattson, W.J., D.A. Herms, J.A. Witter and D.C. Allen. 1991. Woody plant grazing systems: North American outbreak folivores and their host plants. Pp. 53-84 in Y.N. Baranchikov, W.J. Mattson, F.P. Hain and T.L. Payne, eds, Forest Insect Guilds: Patterns of Interactions with Host Trees. United States Department of Agriculture, Forest Service. General Technical Report NE-153.

Mutikainen, P. M. Walls, J. Ovaska, M. Keinanen, R. Julkenen-Tiitto and E. Vapaavuori. 2000. Herbivore resistance in *Betula pendula*: Effect of fertilization, defoliation, and plant genotype. *Ecology* **81**: 49-65.

Myers, J.H. and K.S. Williams. 1984. Does tent caterpillar attack reduce the food quality of red alder foliage? *Oecologia* **62**: 74-79.

Myers, J.H. and K.S. Williams. 1987. Lack of short or long term inducible defenses in the red alder- western tent caterpillar system. *Oecologia* **48**: 73-78.

Murdoch, W.W. 1994. Population regulation in theory and practice. *Ecology* **75**: 271-287.

Nef, L. 1988. Interactions between the leaf miner, *Phyllocnitis suffusella*, and poplars. Pp. 239-251 in W.J. Mattson, J. Levieux and C. Bernard-Dagan, eds, Mechanisms of Woody Plant Defenses against Insects. Springer, New York.

Nicholson, A.J. 1933. The balance of animal populations. *Journal of Animal Ecology* **2**: 132-178.

Price, P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.N. Thompson and A.E. Weis. 1980. Interactions among three trophic levels. *Annual Review of Ecology and Systematics* **11**: 41-65.

Rhoades, DF. 1983a. Herbivore population dynamics and plant chemistry. Pp. 155-220 in R.F. Denno and M.S. McClure, eds, Variable plants and Herbivores in Natural and Managed Systems. Academic Press, San Diego.

Rhoades, D.F. 1983b. Response of alder and willow to attack by tent caterpillars and fall webworms: Evidence for pheromonal sensitivity of willows. Pp. 55-68 in P. Hedin, ed, Plant Resistance to Insects. Symposium Series 208. American Chemical Society, Washington, DC.

Robison, D.J. and K.F. Raffa.1997. Effects of constitutive and inducible plant traits of hybrid poplars on forest tent caterpillar feeding and population ecology. *Forest Science* **43**: 252-267.

Roland, J. and J.H. Myers 1987. Improved insect performance from host-plant defoliation – winter moth on oak and apple. *Ecological Entomology*. **12**: 409-414.

Roland, J. K.E. Denford and L. Jimenz. 1995. Borneol as an attractant for *Cyzenis albicans*, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae). *The Canadian Entomologist* **127**: 413-421.

Rossiter, M.-C., J.C. Schultz, I.T. Baldwin. 1988. Relationships among defoliation, red oak phenolics and gypsy moth growth and reproduction. *Ecology* **69**: 267-277.

Roth, S., R.L. Lindroth, J.C. Volin and E.L. Kruger. 1998. Enriched atmospheric CO2 and defoliation: effects on tree chemistry and insect performance. *Global Change Biology* **4**: 419-430.

Rothman, L.D. 1997. Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. *Ecology* **78**: 1481-1493.

Ruohomaki, K., S. Hanhimaki, E. Haukioja, L. Iso-Iivari, S. Neuvonen, P. Niemela and S. Suomela. 1992. Variability in the efficacy of delayed-inducible resistance in mountain birch. *Entomologia Experimentalis et Applicata* **62**: 107-115.

Sample BE, L. Butler, C. Zivkovich, R.C. Whitmore and R. Reardon. 1996. Effects of *Bacillus thuringiensis* Berliner var Kurstaki and defoliation by the gypsy moth [*Lymantria dispar* (L)(Lepidoptera: Lymantriidae)] on native arthropods in West Virginia. *The Canadian Entomologist* **128**: 573-592.

Schoener, T.W. 1983. Field experiments on interspecific competition. *The American Naturalist* **122**: 240-285.

Schultz, J.C. 1983. Impact of variable plant defensive chemistry on susceptibility of insects to natural enemies. Pp. 37-54 in P. Hedin, ed, Plant Resistance to Insects. Symposium Series 208. American Chemical Society, Washington, DC.

Schultz, J.C. 1988. Plant responses induced by herbivores. *Trends in Ecology and Evolution*. 3: 45-49.

Schultz, J.C. and I.T. Baldwin. 1982. Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science* **217**: 149-151.

Scriber, J.M. and F. Slansky. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology* **26**: 183-211.

Scutareneau, P., B. Drukker, J. Bruin, M.A. Posthumus and M.W. Sabelis. 1997. Volatiles from *Psylla*-infested pear trees and their possible involvement in attraction of anthocorid predators. *Journal of Chemical Ecology* **23**: 2241-2260.

Sipura, M. 1999. Tritrophic interactions: Willows, herbivorous insects, and birds. *Oecologia* **121**: 537-535.

Strong, D.R., J.H. Lawton and TRF Southwood. 1984. Insects on Plants: Community Patterns and Mechanisms. Harvard University Press, Cambridge.

Thaler, J.S. 1997, Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* **399**: 686-688

Tuomi, J., P. Niemela, E. Haukioja, S. Siren and S. Neuvonen. 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia* **61**: 208-210.

Tuomi, J., P. Niemela and S. Siren.1990. The Panglossian paradigm and delayed-inducible accumulation of foliar phenolics in mountain birch. *Oikos* **59**:399-410.

Tuomi, J., T. Fagerstrom and P. Niemela. 1991. Carbon allocation, phenotypic plasticity, and induced defenses. Pp. 85-104 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Turchin, P. 1990. Rarity of density-dependence or population regulation with lags? *Nature* **344**: 660-663.

Turchin, P. 1995. Population regulation: Old arguments and a new synthesis. Pp. 19-40 in N. Cappuccino and P.W. Price, eds, Population Dynamics: New Approaches and Synthesis. Academic Press, New York.

Turlings, T.C.J., P.J. McCall, H.T. Alborn and J.H. Tumlinson. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* **19**: 411-425.

Underwood, N. 1999. The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist* **153**: 283-294.

Valentine, H.T., W.E. Wallner and P.M. Wargo. Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia* **57**: 298-302.

Virtanen, T. and S. Neuvonen (1999). Performance of moth larvae on birch in relation to altitude, climate, host quality, and parasitoids. *Oecologia* **120**: 92-101.

Wallner, W.E. 1983. Gypsy moth host interactions: a concept of room and board. Pp 5-8 in R.L. Talerco and M. Montgomery, eds, Proceedings: Forest Defoliator –Host Interactions: a Comparison between Gypsy Moth and Spruce Budworms. USDA Forest Service, Northeastern Station General Technical Report NE-85.

Wallner, W.E. and G.S. Walton. 1979. Host defoliation: a possible determinant of gypsy moth population quality. *Annals of the Entomological Society of America* **72**: 62-67.

Werner, R.A. 1979. Influence of host foliage on development, survival, fecundity and oviposition of the spear-marked black moth, *Rheumaptera hastata* (Lepidoptera: Geometridae). *The Canadian Entomologist* **111**: 317-322.

Wold, E.N. and R.J. Marquis. 1997. Induced defense in white oak: effects on herbivores and consequences for the plant. *Ecology* **78**: 1356-1369.

Work, T.T. and D.G. McCullough. 2000. Lepidopteran communities in two forest ecosystems during the first gypsy moth outbreaks in northern Michigan. *Environmental Entomology* **29**: 884-900.

CHAPTER 2:

EMULATING INSECT OUTBREAK: EXPERIMENTALLY TESTING THE EFFECTS OF CUMULATIVE DEFOLIATIONS ON INDUCED-RESISTANCE AND CATERPILLAR HERBIVORY

INTRODUCTION

Insect outbreaks are a conspicuous component of boreal and temperate forest ecosystems throughout the world. During outbreaks, high levels of defoliation for one or more years in succession can significantly lower concentrations of leaf nitrogen, water content, and sugars while elevating fiber, toughness and secondary compounds, attributes that determine the quality of leaves as food for caterpillars (e.g., Feeny 1970, Mattson 1980, Scriber and Slansky 1981, Schultz 1988, Neuvonen and Haukioja 1991, Kaitaniemi et al. 1998, Kause et al. 1999). Changes in host plant quality elicited by feeding damage that have negative consequences on herbivores are defined as induced-resistance (Karban and Baldwin 1997). Two types of induced resistance have been identified in trees (Haukioja and Niemela 1977, Haukioja 1980, 1982); rapid-induced resistance (RIR), which occurs within hours or days of feeding damage, and delayed-induced resistance (DIR), which has its greatest effects in the year or years subsequent to the defoliation event and usually requires severe herbivory to be elicited. In simulation models (e.g., Edelstien-Keshet and Rausher 1989, Underwood 1999), RIR when sufficiently strong, can stabilize herbivorous insect populations because it acts instantaneously on population growth. Conversely, DIR functions in a delayed-density dependent manner, which tends to destabilize insect populations (May 1973, Berryman et al. 1987) and can create cyclic population dynamics

under some conditions (Underwood 1999). Several factors including maternal effects, natural enemies, and induced-resistance are capable of producing the delayed-negative feedbacks (Berryman et al. 1987, 1996, Ginzburg and Taneyhill 1994), identified in time series data from many forest insect populations (Turchin 1990, 1995).

Much of our understanding of DIR has come from research on birches (*Betula spp.)* and a few other species of deciduous trees. Defoliation of birch elevates levels of foliar phenolic compounds including hydrolyzable and condensed tannins, and reduces primary nutrients such as nitrogen and water (Haukioja et al. 1985, Tuomi et al. 1984, 1990, Bryant et al. 1988, 1993, Kaitaniemi et al. 1998). Caterpillars feeding on foliage from previously defoliated trees suffer reduced growth and fecundity, and protracted development times relative to larvae feeding on undefoliated trees (e.g., Haukioja and Neuvonen 1985, Ruohomaki et al. 1992, Bryant et al. 1993, Kaitaniemi et al 1999a). Because changes in foliar biochemistry can persist for several years after the cessation of defoliation (Bryant et al. 1991), insect fitness may continue to suffer long after an outbreak has ended (Haukioja and Neuvonen 1987). Long term reductions in foliar quality following defoliation have also been shown to have significant negative effects on larch bud moth, *Zieraphera diniana,* feeding on alpine larch in Switzerland (Benz 1974, Baltensweiler and Fischlin 1988).

Traditionally, experimental tests of DIR have consisted of defoliation in a single year followed by phytochemical analyses and/or bioassays in the following year or years. This approach may underestimate the strength of DIR because outbreaks of many defoliators are at least two years in duration (e.g., Mattson et al. 1991), leading Haukioja et al. (1988) to hypothesize that consecutive defoliations may have cumulative effects on

herbivore performance. Despite this speculation, the effects of successive years of defoliation on trees and their herbivores have received only limited attention in the literature (Kaitaniemi et al. 1999a). In concordance with the hypothesis of Haukioja et al. (1988), several studies have shown increasingly negative effects on insect performance with each successive year of defoliation (Werner 1979, Wallner and Walton 1979, Valentine et al. 1983, Clausen et al. 1991). In contrast, successive years of defoliation were shown to have only minor cumulative effects on phytochemistry and insect performance in experiments with mountain birch (*Betula pubescens*) (Kaitaniemi et al. 1999a, 1999b) and trembling aspen (*Populus tremuloides*) (Parry et al. 2000).

The role of DIR in insect population dynamics may not be as general as first thought (e.g., Haukioja 1980, Rhoades 1983). In some cases defoliation may increase herbivore performance while in others, there may be no effect. Roland and Myers (1987) compared the performance of winter moths on apple (*Malus* sp.) defoliated from 0-100% and Garry oak (*Quercus garryana*) defoliated from 0-50% in the previous year. They found that moderate levels of defoliation (<50%) in the previous season were associated with increased pupal masses in the subsequent season on both apple and oak. Higher levels of defoliation (>50%) in the previous season was associated with reduced pupal masses. Rothman (1997) found no effect of high defoliation of red alder (*Alnus rubra*) on western tent caterpillar (*Malacosoma californicum*) pupal masses in the year following defoliation that exceeded 70%. Similarly, Harrison (1995) found no effects of previous severe defoliation of bush lupine (*Lupinus arboreus*) on fecundity of western tussock moth (*Orgyia vetusta*) despite evidence for RIR in the previous year.

Defoliation induced changes in plant quality extend beyond the interaction between trees and the herbivore eliciting the response to include other member of the phytophagous community. The magnitude and direction of host-plant mediated competition between an outbreak species and other phytophages depends on several different factors including the timing of defoliation in relation to the phenology of competing folivores, the habitat or life history strategy of competitors, and the degree of feeding specialization by the herbivore (Denno et al. 1995, Dankert et al. 1997). Outbreak species may be more adapted to defoliation-induced changes in nitrogen, water, or leaf toughness than species whose populations remain perpetually below the carrying capacity of the environment. Conversely, generalists may be more susceptible to the effects of toxic secondary phytochemicals than adapted specialists. In factorial combinations, Agrawal (2000) showed that induction of wild radish by one species of caterpillar had variable effects on three other species. The reciprocal effects were interesting in that the plant perceived damage from each of the four species of caterpillars differently. Such variation in plant perception of herbivores and variability in herbivore response to induced-resistance may account for the diversity and sometimes conflicting experimental results within and among tree species (see Karban and Baldwin 1997 for review).

Divergent effects of induced-resistance on herbivores suggest that responses to defoliation may be system specific or are mediated by environmental factors, underscoring the incomplete understanding of mechanisms driving the long-term consequences of sustained insect outbreak on forest trees. Adding to the complexity, several mechanisms operating at different hierarchical and temporal scales may be required to completely explain induced-resistance in trees (e.g., Tuomi et al. 1990, Herms

and Mattson 1992, Karban and Baldwin 1997, Koricheva et al. 1998). In deciduous trees, much focus has been on the carbon-nutrient balance hypothesis (CNB) as a mechanism explaining the induction of carbon-based secondary compounds. The CNB, a subset of predictions under the more globally encompassing growth-differentiation balance hypothesis (GDB), postulates that when nutrients limit growth more than photosynthesis, excess carbon can be allocated to the production of carbon-based secondary metabolites (Bryant et al 1983, Tuomi et al. 1984, 1990, Herms and Mattson 1992). Tuomi et al. (1984, 1990) extended the CNB to encompass changes in nutrient/carbon balance following defoliation. Defoliation, especially early in the growing season, decreases nitrogen stores in deciduous trees (Harper 1977, Chapin and Kedrowski 1983) and may damage fine roots, reducing acquisition from soil, and exacerbating nitrogen deficits (Tuomi et al. 1990). Thus, until the tree can recover or compensate for the nitrogen lost when defoliated, photosynthesis will generate carbon in excess of growth requirements; this carbon is available for allocation to secondary metabolism. The majority of studies testing predictions of the CNB in trees have focused on the year following defoliation. Recently, Hunter and Schultz (1995) demonstrated that the CNB could also account for within year changes of some phenolic compounds.

The domain of the CNB hypothesis does not address rapidly induced systemic changes in trees. These responses occur over very short-term periods of hours or days and are often invoked by relatively low levels of herbivory, spreading rapidly to undamaged leaves. Several wound-responsive, signal-transduction pathways have been identified in herbaceous plants (e.g., Bergey et al. 1996, Karban and Baldwin 1997, Constabel 1999). In these pathways, specific elicitors stimulate secondary metabolism and/or translocation

of secondary compounds from storage sites following herbivory. To my knowledge, similar pathways have not yet been identified in trees, although there are no compelling reasons to suggest that they do not occur. Several studies have shown that insect herbivory elicits different responses in woody plants than equivalent amounts of tissue removed mechanically (e.g., Haukioja and Neuvonen 1985, Hartley and Lawton 1987, Krause and Raffa 1992, Havill and Raffa 1999). This suggests that leaves of trees have insect-specific wound responses much like those identified in herbaceous plants. Furthermore, application of the generalized elicitor compound, jasmonic acid, has been shown to induce RIR in hybrid poplars in the absence of tissue damage (Havill and Raffa 1999), indicating that a signal-transduction pathway may be operating.

Reported here are the results of a four-year study in which I experimentally established populations of gypsy moth [*Lymantria dispar* L., (Lepidoptera Lymantriidae)] in replicated blocks of poplar to emulate the increase, outbreak, and decline in densities characteristic of natural outbreaks. Several aspects of this study are unique. The poplar plantation consisted of ontogenetically mature trees (ca. 15 m high, 9 years old) rather than saplings, and was planted on a relatively large spatial scale (four ha). Furthermore, insects were used to defoliate the trees, control trees had realistic background levels of herbivory, and the temporal scale of the study was designed to mimic the duration of a natural outbreak (one year of light defoliation followed by a severe year, a moderate year, and a year with very low defoliation). I extended my observations beyond the classically studied relationship between defoliator and host tree to include five other generalist and specialist lepidopteran species belonging to three different seasonal feeding guilds to test for host-plant mediated indirect competition. My

main objective was to assess the consequences of long-term defoliation on trees and the reciprocal effects on their associated herbivores. Specifically I predicted that: (1) successive years of defoliation would cause greater changes to tree physiology than a single defoliation event, and that nitrogen fertilization would mitigate the response as predicted by the Carbon-Nutrient Balance hypothesis. (2) The quality of leaves would be reduced for herbivores following defoliation (RIR and/or DIR) and that the magnitude of the effects would increase with each successive year of defoliation. (3) That phytochemical changes induced by an outbreak species would have significant detrimental effects on the growth of other members of the leaf-feeding lepidopteran community, and that the magnitude of the effects would be greater for oligophagous, non-outbreaking species than for polyphagous species and species characterized by outbreak population dynamics.

MATERIALS AND METHODS

Experimental design

My study was conducted in four one-ha stands of a single clone of *Populus x euramericana* c.v. "Eugeneii" growing on the Long Term Ecological Research site at Kellogg Biological Station in southwestern Michigan, USA (see Marino and Gross 1988 for detailed site description). The trees were planted in 1987 as root cuttings in a 1m × 2 m array in fields that had been under agricultural cultivation for nearly 100 years. The soil is a Kalamazoo sandy loam (Typic Hapludalf). Poplars were nine-years-old, had diameters-at-breast-height (DBH) of 7.7 ± 0.53 cm (mean ± SE), and were 12.1 ± 0.49 m

(mean ± SE) in height when the study was initiated in 1996. These very fast-growing trees were ontogenetically mature (annual flower production) at the beginning of the study.

Within each one ha stand of poplar, I placed a 0.25 ha block in the northeast corner, which was divided into four plots, each separated by a buffer strip of two rows of trees, which were included in the treatments but not used in the experiment. Each of the four plots within a block was randomly assigned one of four treatments: (1) undefoliated and unfertilized, (2) undefoliated and fertilized (100 kg ha^{-1} N as NH_4NO_3), (3) defoliated and unfertilized, or (4) defoliated and fertilized. Fertilizer was applied in spring shortly after bud break in each year of the study. Undergrowth vegetation was suppressed from the time of planting and throughout my experiment by twice-annual applications of glyphosate (2% v/v, Roundup, Monsanto Corporation, St. Louis, MO). Before randomization of plots, a minor restriction was placed on the location of defoliation treatments within a block. Because I was relying solely on physical barriers (insecticides were not used) to keep caterpillars from colonizing the control plots, the possibility of the random assignment of defoliation plots diagonally from each other was eliminated. Locating the defoliation plots diagonally would have doubled the edge exposure of the control plots increasing substantially the difficulty of maintaining low herbivory levels in controls. Initially all plots had the same probability of being assigned to a defoliation treatment. Once the first plot assignment had been made, the second defoliation treatment could be assigned to only two of the three remaining plots because of this restriction. I felt that the practical benefits of employing a restricted design far outweighed the loss of fully random deployment of treatments within each block.

Establishing the gypsy moth outbreak – A number of studies have shown that artificial removal of leaves by tearing or using scissors elicits different physiological responses than does leaf removal by caterpillars (e.g., Haukioja and Neuvonen 1985, Hartley and Lawton 1987, Krause and Raffa 1992, Havill and Raffa, 1999). To more closely emulate processes experienced by trees in natural outbreaks, defoliation was applied by inoculating the plots with large numbers of caterpillars. I chose gypsy moth because many trees in the genus *Populus* are a preferred hosts (Liebhold et al. 1995, Havill and Raffa 1999), egg masses and larvae were already present in some of the plots, and I could obtain large numbers of eggs from several high-density populations elsewhere in southern Michigan. Prior to the initiation of the study, gypsy moth populations in my plots and in the surrounding oak/hickory/maple forests were at low but detectable levels and had been present in the area since at least 1991 (S. Gage, personal communication). In the spring of 1996, I collected large numbers of gypsy moth eggs from high-density populations in Ottawa County, MI, and in 1997, from Wayne and Kalamazoo Counties, MI for innoculative release in the experimental plots.

In the laboratory, egg masses were separated manually to release the eggs from the hair matrix in which they are embedded. I submerged eggs for 1 hour in a 10% formalin solution (Bell et al. 1981), which was agitated periodically. Formalin removes nuclear polyhedrosis virus, critical because this pathogen is the major source of mortality in high-density gypsy moth populations and is spread from contaminated egg surfaces to emerging neonates (Woods and Elkinton 1987). After the formalin treatment, eggs were placed on a mesh screen and rinsed under running water for 1 h, then dried overnight at room temperature under a fume hood. When dry, eggs were weighed and 100g allotments

were placed in cheesecloth packages and secured with twist ties. Eggs were kept at 4°C in an environmental chamber until bud break approached. To synchronize hatch of collected eggs with the natural phenology of gypsy moth, *in situ* egg masses in the poplar stands were monitored for hatch as bud break approached. Once natural hatching was observed in the field, egg packages were placed in an environmental chamber at 27°C until the first eggs began hatching within a few days. The cheesecloth packages were then stapled to the bark of 10-12 trees in the center of each defoliation treatment plot. Emerging caterpillars climbed from the cloth bags and ascended the trees.

In 1996, I increased gypsy moth densities from background levels in the defoliation treatment plots by adding ca. 248,000 eggs to each of the 8 defoliation plots (150 grams of eggs/plot or 8×10^3 egg masses/ha assuming 500 eggs/mass). A further ca. 3.1 million eggs (2.25 kg /plot or the equivalent of 1×10^5 egg masses/ha assuming 500 eggs/mass) were added to each defoliation plot in 1997 to bolster egg masses from the previous year's population. Egg density in the release packages was estimated from the total mass of washed eggs divided by the average weight of individual gypsy moth eggs, 0.65 mg (from Diss et al. 1996, and D.P., unpublished). I reduced the estimated amount by 10% to account for eggs parasitized by *Ooencyrtis kuwaniae* (Hymenoptera: Encyrtidae), and those that were infertile, calculated from randomly drawn subsamples of eggs. Densities of egg masses in the poplars surrounding the plots increased very rapidly following my introductions in 1996, likely driven by large numbers of caterpillars wandering out of the defoliation treatment plots. By 1997, the entire one ha stands of poplar surrounding my research blocks contained densities of gypsy moth sufficient to severely defoliate most of the trees that were not protected. Because these larvae were used only to defoliate the

trees and were not part of any experiment, I made no attempt to control them, nor did I distinguish between those originating within or outside the defoliation plots. It was not necessary to add any gypsy moth egg masses in 1998 because egg mass densities remained very high within the plots and surrounding poplar stands. Progeny of these egg masses were sufficient to cause moderate to severe defoliation in 1998. To my knowledge, this is the first instance of a self-sustaining, experimental outbreak population of gypsy moth.

Following the 1998 season, gypsy moth populations crashed in my plots apparently due to a variety of contemporaneous mortality sources. These included larval parasitoids (primarily the tachinid *Compsilura concinnata*), pupal parasitism [likely *Brachymeria intermedia* (Hymenoptera: Chalcididae)], extensive predation by flocks of starlings (*Sturnus vulgaris*), black-billed cuckoos (*Coccyzus erythropthalmus*), as well as moderate levels of NPV. I did not quantify sources of mortality but combined, they were very effective and reduced populations to low levels. No mortality from the fungus *Entomophaga maimaiga*, responsible for devastating epizootics in gypsy moth populations elsewhere in North America, was detected in my plots. Few egg masses were laid in 1998 and as a result, larval populations were very sparse in 1999. Because I was interested in the recovery of trees after the collapse of an outbreak, gypsy moth eggs were not added to the existing low densities in 1999.

Gypsy moth neonates are highly mobile and may wander extensively before settling and beginning to feed (Mason and McManus, 1981). Following release, neonates readily dispersed throughout the canopy of my plots by ballooning on silk threads or by crawling from tree to tree. To minimize colonization of control trees, tree wrap coated with

Tangletrap was placed on the trunk of each control tree ca. 0.5 m above the ground. I trimmed back all branches and other vegetation that could provide a conduit for caterpillar incursion from defoliation plots to control plots. To remove caterpillars that circumvented my barriers or ballooned onto the control trees, I used a combination of manual and mechanical methods. I accessed the canopy using ladders and a truck mounted 'cherry picker' and used a pressure water sprayer to dislodge small caterpillars from the treetops. Third and later instar gypsy moth caterpillars descend the trees in the morning to seek daytime hiding places in low density populations so I placed burlap bands on each control plot tree above the sticky barriers. Burlap bands were checked daily and any caterpillars found were returned to the defoliation plots. With the exception of one plot in 1997, I was very successful in maintaining very low densities of gypsy moth in the control plots throughout the course of the experiment. I did not actively attempt to remove any other herbivorous insects from the control trees and other than gypsy moth, no other species of insects were particularly abundant in the plots although there was considerable diversity of other herbivorous insects present. In natural forests, estimates indicate that between 5-20% of the foliage is removed by non-outbreak populations of herbivorous insects (e.g., Mattson and Addy 1975, Reynolds and Crossley 1997). Thus, to assess the effects of defoliation induced changes in host plant quality on herbivores, I felt that trees subjected to background herbivory were a more appropriate control than trees maintained free of insect damage.

To estimate defoliation in 1997 and 1998, I relied upon visual estimates recorded independently by three observers for each tree in the plots. These estimates were averaged to obtain mean plot defoliation levels. Defoliation was partitioned into four

classes: 1. 0-25%, 2. 25-50%, 3. 50-75%, and 4. 75-100%. In 1996 I collected insect frass in 50 cm diameter traps. Two traps were placed in each plot. Each trap consisted of a bucket with a screen funnel on the open end that directed falling frass into collection cups. Frass was dried and weighed. I used the same frass traps in 1997 and discerned that frass capture was highly correlated with visual estimations of defoliation ($r^2 = 0.75$). This regression was used to estimate defoliation in 1996 because the relatively low level of defoliation was difficult to estimate visually. These data showed that in the treatment plots, light to moderate levels of defoliation were achieved in 1996, uniformly severe levels of defoliation in 1997, and moderate to severe levels in 1998 (Figure 1). I did not quantify defoliation in 1999 because caterpillar density was so low that all trees were well below the threshold where any defoliation could be detected visually (overall, less than 1 egg mass per plot was found in the spring of 1999 as opposed to several hundred per plot in the spring of 1998).

Tree responses to defoliation

Tree growth – To ascertain the effects of treatments on whole tree processes, diameter was recorded annually from each living tree in the plots. Two measurements of diameter were recorded 90° apart at 1.4 m above the ground using digital calipers. I permanently marked the measurement points on each tree so that diameter could be recorded from the same point each year.

Phytochemistry – Several primary and secondary biochemical constituents of leaves were recorded each year. I based the timing of leaf samples on the phenology of gypsy moth so as to standardize collections among different years. From 1996-1999, samples of leaves were collected in June, which corresponded to the presence of final instar caterpillars in

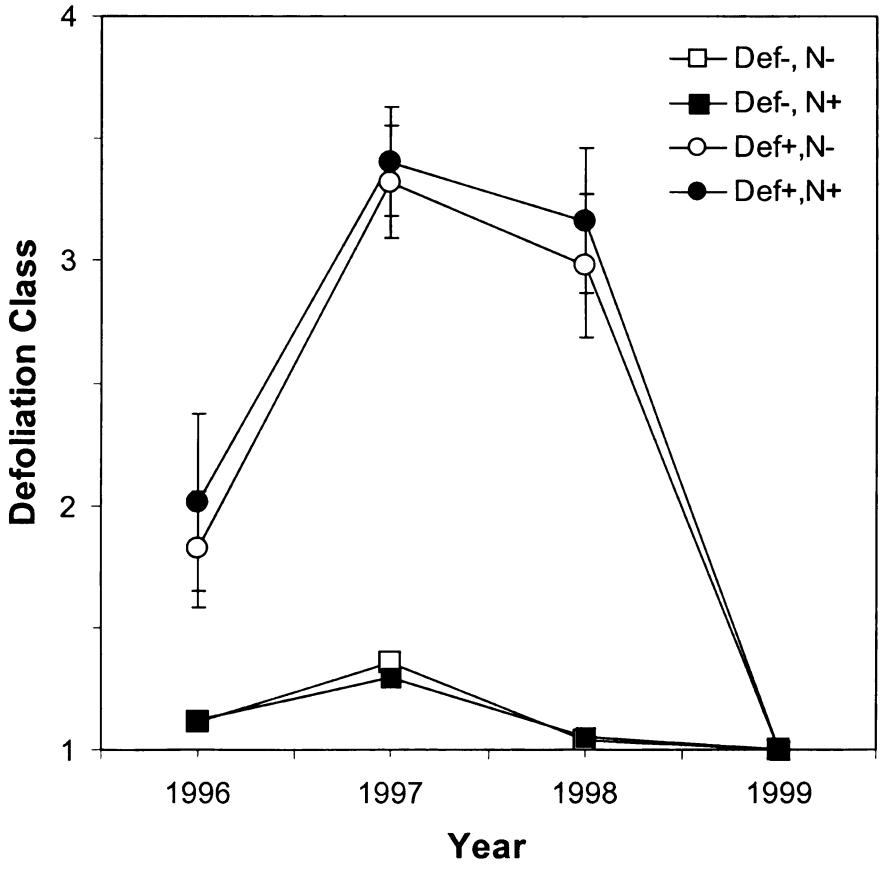


Figure 1. Defoliation levels (mean ± SE) in treatment and control plots 1996-1999. Means (± SE) were estimated from the four plots in each treatment. Defoliation was estimated visually using four classes: Class 1 – 0-25%, Class 2 – 25-50%, Class 3 - 50-75%, Class 4- 75-100%. Treatment codes are undefoliated = Def -, defoliated = Def+, unfertilized = N-, and fertilized = N+.

the field. In 1997 and 1998, I also sampled foliage from the plots one month after gypsy moth feeding was complete, and defoliated trees had completely refoliated, in early to mid July. Additionally, in 1998, I collected an early season sample two weeks after bud break and the hatch of gypsy moth eggs.

To sample leaves, I randomly selected healthy trees (no dead tops or other significant dieback) in each plot. June leaf samples were taken from 10-14 trees/plot in 1996-1998 and from 4 trees per plot in 1999. July samples in 1997 and May and July samples in 1998 were collected from four trees per plot. Leaf sampling was very labor intensive, therefore the number of trees sampled in any given collection was dictated by the availability of help. To sample, I used extendable pole-pruners to cut two branches with approximately equal light exposure from the upper canopy of each tree. Poplar leaves exhibit considerable variability in phytochemistry that is dependent on the position of the leaf on a shoot (Meyer and Montgomery 1987, Robison and Raffa 1997). To standardize sampling, the first six expanded leaves (corresponding to a plastochron index of 4-11, see Meyer and Montgomery 1987) on each branch, were detached, placed in small paper envelopes and immediately flash frozen in liquid nitrogen. Frozen leaves were packed in dry ice for transport to the laboratory and were held at –40°C until lyophilized. Following lyophylization, leaves were ground in a miniature Wiley mill through an #40 mesh screen and stored at –20°C over silica until used in analyses.

Levels of total phenolics and condensed tannins were quantified in 1996. In 1997 and 1998, I quantified foliar nitrogen, water, total phenolics, condensed tannins, and six other secondary phenolic compounds (phenolic glycosides, cinnamic acid, catechins, luteolin,

quercetin-glycoside, and myricetin-glycoside). In 1999, levels of nitrogen, water, total phenolics, condensed tannins were determined.

To quantify levels of foliar nitrogen, 9-11 mg of leaf powder was weighed into 8 × 5 mm tin capsules, the edges were folded over and the capsule compressed to remove air. These tin pellets, along with acetanilide standards, were then placed in an autosampler of a Carlo-Erba model NA-1500 NC analyzer. Water content of leaves was determined gravimetrically. At each sampling date, leaves were collected from the trees, weighed on an electronic balance and placed in paper envelopes in a drying oven at 45°C for several days. Samples were removed and reweighed to determine water content.

To determine concentrations of foliar total phenolics and condensed tannins, leaf powder was weighed on an electronic balance (70-80 mg) and placed in 15-ml polypropylene screw-top centrifuge tubes. Aqueous methanol (50%) was added (100:1 ml/g). Tubes were tightly capped, and extractions were conducted in the dark at 25° C on a shaker table. Samples were then centrifuged at 2500 rpm for 10 min, and the supernatant filtered through disposable filter columns (200-300 μm pore size; Fisher Scientific) into 3.5 ml polystyrene sample cups.

The Folin-Denis method for measuring total phenolics (Swain and Hillis 1959) was modified for use in an air-segmented, continuous flow analyzer (RFA-300 Rapid Flow Analyzer and 301 autosampler; Astoria-Pacific International, Clackamas, Oregon) (Nitao et al., in review). The analyzer was configured to run at room temperature, and to dilute the phenolic samples by a factor of 78.3 before mixing with the Folin-Denis reagent in a ratio of 1:13.8 (F-D reagent : diluted sample) for 0.3 min at room temperature. Sodium carbonate solution was added in a ratio of 1:14.8 (sodium carbonate: sample stream) and

mixed for 0.6 min before being measured at 750 nm using a standard curve based on tannic acid (Fisher Scientific). The autosampler, standard curve construction (polynomial fit), quantification and data capture were all controlled through RFA-PC software.

The same sample preparation and extraction procedure was used for condensed tannins. The sulfuric acid method for measuring condensed tannins (Bate-Smith and Rašper, 1969; Bae et al.1993) was adapted for use with the autosampler above (Nitao et al., in review). The analyzer was configured to mix 43% sulfuric acid diluted with methanol (v/v) with tannin samples in a ratio of 1:6.3 (sample:sulfuric acid) for 0.4 min at room temperature, heated to 50° C for 5.0 min, and allowed to mix for an additional 1.2 min at room temperature. Condensed tannins were measured at 580 nm using a standard curve based on quaking aspen tannin standard (provided by Karl Kleiner, University of Wisconsin, Madison, Wisconsin).

To quantify other phenolic components of poplar foliage, lyophilized leaf powder (8-10 mg) was placed in an Eppendorf vial and 0.7 ml of methanol added. Vials were left standing for 15 min in a ice-bath. Samples were removed from ice and homogenized with an Ultra-Turrax homogenizer for 2 min and then centrifuged at 10000 rpm for 3 min. Solvent was taken into 6 ml glass-vial (in a cold-bath) and evaporated under nitrogen. The extraction was repeated three times with washing. Prior to HPLC, the sample was dissolved into H2O:methanol (1:1, 600 µl :600 µl). The HPLC-runs followed the procedure of Julkunen-Tiitto et al. (1996). Despite my best efforts, HPLC revealed considerable quantities of salicin and salicin derivatives, generally regarded as hydrolytic breakdown products of unstable phenolic glycosides such as salicortin and tremulacin (Lindroth and Pajutee 1987, Lindroth and Koss, 1996). Because of this degradation, I

pooled salicin, tremulacin, and salicortin (appreciable levels of tremuloiden were not detected) to get an estimate of total phenolic glycosides as was done in Lindroth and Pujutee (1987).

In addition to the direct measures of foliar chemistry, I estimated the mean area and toughness of individual leaves. For these measures, leaves were sampled from two branches on each tree and standardized for plastochron position. Leaves were photocopied shortly after being clipped to provide a permanent record. The photocopies were placed on a flatbed scanner and the area calculated by a digital image analysis program (CI400 Computer Image Analysis System, Version 2.0, Jandel Scientific, San Rafael, CA). Leaf toughness was determined using a digital force meter attached to a penetrometer (Chatillon DFM2, Chatillon Inc., Greensboro, NC). Two measurements were taken on each leaf, one on the right and one on the left of the midrib. To standardize my measurements, force data were collected from leaf lamina between the first and second lateral veins.

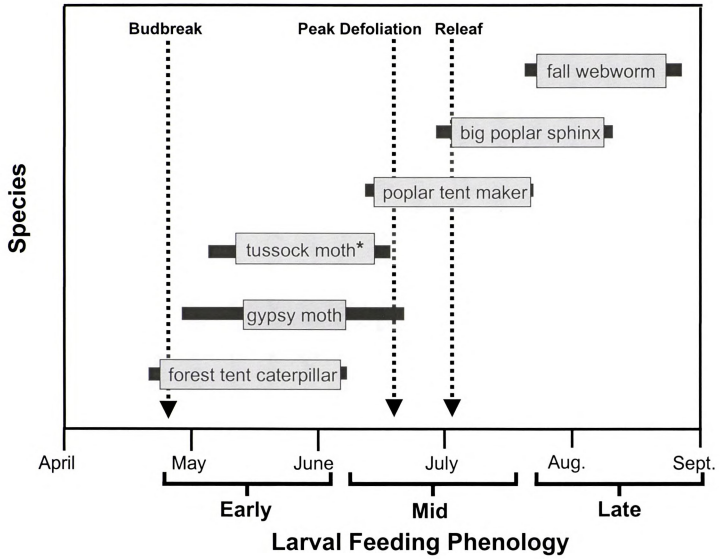
Effects of defoliation on caterpillars

Bioassay insects – To assay for changes in foliage quality following treatments, I utilized six species of Lepidoptera. The caterpillars used, gypsy moth, forest tent caterpillar, *Malacosoma disstria* Hübner (Lasiocampidae), white-marked tussock moth, *Orgyia leucostigma* (J.E. Smith) (Lymantriidae), poplar tent maker *Clostera inclusa* (Hübner) (Notodontidae), big-poplar sphinx, *Pachysphinx modesta* (Harris) (Sphingidae), and fall webworm, *Hyphantria cunea* (Drury) (Arctiidae), were selected because they occurred naturally in the poplar plantation. These species represent a broad cross section of life history strategies for exposed leaf-feeding caterpillars. Three species are highly

polyphagous generalists (gypsy moth, fall webworm, white-marked tussock moth), one species is moderately polyphagous (forest tent caterpillar), two are poplar-feeding oligophages (poplar tent maker, big poplar sphinx), and one species (white-marked tussock moth) is bivoltine in Michigan although I assayed only first generation larvae. The four polyphagous species are characterized by outbreak population dynamics whereas the two oligophagous species exhibit stable dynamics in natural systems. Three species (forest tent caterpillar, poplar tent maker, and fall webworm) are highly gregarious and live in colonies whereas the other three species are solitary. Using feeding phenology (e.g., Hunter 1995), these lepidopterans can be broadly partitioned into three seasonal guilds (Figure 2). Gypsy moth, forest tent caterpillar, and first generation white-marked tussock moth are spring feeders initiating feeding at or shortly after bud break, poplar tent maker and big poplar sphinx are mid-season feeders, and in Michigan, the fall webworm is a late season feeder, hatching in mid to late July.

Long term bioassays – Life-long bioassays (egg to adult) were conducted for gypsy moth each year from 1996 to 1999. The height of the trees and the propensity of poplars to maintain branches only in the upper third of the tree precluded on-tree bioassays so all larvae were reared in the laboratory. Caterpillars were obtained from eggs collected in a hybrid poplar stand near the plots but not used as part of the experiment. Gypsy moth caterpillars were reared in pairs in 150 × 25 mm petri dishes in 1996 and 1997. Thereafter, bioassays for all species were done with groups of 10 in 5.6 L clear plastic boxes with ventilation holes in the lids. Four trees in each plot were used to supply foliage in 1996 and 6-8 trees were used from 1997-1999. Trees were randomly selected on each foliage collection date with the stipulation that the same tree could not be used on

Figure 2. Approximate larval feeding periods of the six species of Lepidoptera used in the study in relation to important phenological events during the study. * White-marked tussock moth has a spring and a summer generation in southern Michigan, I used only spring generation larvae in the bioassays.


Budbreak
Peak Defoliation
Releaf
fall webworm
big poplar sphinx
poplar tent maker
tussock moth*
gypsy moth
forest tent caterpillar
Species
April
May
June
July
Aug.
Sept.
Early
Mid
Late
Larval Feeding Phenology

consecutive dates to avoid changing tree physiology through excessive sampling. Tw
with attached leaves were clipped from the selected trees using pole-pruners and retur
to the laboratory in plastic bags on ice. The stem of each twig was cut under water a
inserted into water-filled aqua-picks to maintain leaf turgor and placed in the caterpi
rearing containers. This method allowed caterpillars to choose from ~15-30 leaves
which to feed. Foliage was never allowed to become depleted and fresh leaves w
provided every second or third day as needed through to pupation. In 1996 and 19
caterpillars were reared in environmental chambers at 25:18°C and 16:8 (light:da
photoperiod. Thereafter, a naturally lit and ventilated room was used and temperatu
were allowed to fluctuate daily with ambient conditions.

In 1998, in addition to gypsy moth, 20 forest tent caterpillar, 20 poplar tent maker
big poplar sphinx, and 20 fall webworm were reared on foliage from each plot. For
tent caterpillars were obtained from egg masses collected from the hybrid poplar plo
Big poplar sphinx eggs were obtained from adults collected in the plots and mated
captivity. Poplar tent maker and fall webworm were collected from poplars as larvae
the year prior to the bioassay, reared to maturity in the laboratory, and mated in captiv
to produce the eggs used in the 1998 bioassay. Groups of ten insects were reared in
plastic boxes described above with the exception of big poplar sphinx. Caterpillars
this species were very aggressive to conspecifics, necessitating individual rearing in p
dishes. Overall, caterpillar phenology in the bioassays was similar to that of lar
observed in the field in 1996-1998 and slightly faster in 1999. I again reared 20 for
tent caterpillar and 20 poplar tent maker larvae in 1999 and also used 20 first generat
white-marked tussock moth.

Following pupation, the pupae were weighed on an electronic balance, and pla individually in 25-ml plastic cups. The species used in my study have non-feeding adu and female mass and fecundity are generally highly correlated for species exhibiting t life-history strategy (Tammaru and Haukioja 1996). Pupae were checked daily emergence, which was recorded along with the sex of each individual adult.

To estimate fecundity, gypsy moth, forest tent caterpillar, and poplar tent maker w reared in cages on poplar foliage collected from outside of the experimental plots. Pu were weighed and at emergence, females were dissected and the number of eggs count allowing me to develop a regression equation that describes the relationship betw pupal mass and fecundity for each species. I derived the following equations for gypsy moth, $y = 475.56x - 106.23$, $r^2 = 0.90$, n = 18, (2) forest tent caterpillar, y $376.74x + 41.92$, $r^2 = 0.74$, n = 44 and (3) poplar tent maker, $y = 1147.0x + 56.77$, r 0.65, n = 29, where y = fecundity and x = pupal mass. Sufficient numbers of wh marked tussock moth, big poplar sphinx, and fall webworm were available only for bioassays, so it was not possible to generate fecundity regression equations independen of the experimental larvae.

Short-term bioassays - I conducted short term, within instar bioassays using gypsy m in 1997 and 1998. Late fourth instar caterpillars that were preparing to molt w collected from poplars surrounding my plots and allowed to molt in the laborato Within 24 hours of molting, I placed fifth instar caterpillars individually in petri dis and assigned them randomly to each plot. Leaves collected from each plot were th allocated to the appropriate petri dish. Seven caterpillars per plot were reared in 1997 a 10 caterpillars per plot were reared in 1998. In 1997 I used a leaf area meter to determ

the amount of foliage consumed by subtracting the final area from the initial. Lea were then placed in a drying oven and the wet/dry conversion ratio used to estim initial weight. After 48 hours in 1997 and 72 hours in 1998, the caterpillars w removed, frozen, and placed in a drying oven at 40°C for 5 days along with the frass a remaining leaf material. To obtain initial dry weights, a regression equation w calculated from the mass of newly molted caterpillars before and after drying. Beca there was no association with any of the treatments, caterpillars that died or did not f were not included in any analyses. Relative growth rate was calculated as RGR $\ln(\text{weight}_f) - \ln(\text{weight}_i)/T$ where ln is the natural logarithm, weight_f = final weig weight_i = initial weight, and T is the elapsed time in days (Gordon 1968). Measures efficiency of conversion of ingested food (ECI), approximate digestibility (AD), a efficiency of conversion of digested food (ECD) were calculated using the stand formulas of Waldbauer (1968). In 1998, rather than calculate total consumption from area of leaf consumed, gravimetric techniques were used.

Statistical analyses

I analyzed the effects of my treatments as a randomized block design. I treated 'block', 'defoliation', and 'fertilization' terms as fixed effects and F-tests were made o the mean square error. The poplar plots at Kellogg Biological Station are unique a were not chosen at random from some greater pool of poplar stands, leading me to ass them as a fixed term, recognizing the limitations that this assignation has on generality of my conclusions. Both defoliation and fertilization represent spec treatment levels and were also assigned as fixed terms. All data collection was done us subsamples from within each plot. These were averaged and statistics performed us

the plot as the sampling unit. To test the effects of leaf quality variables (nitrogen, wa allelochemicals, physical properties) on insect performance (growth, pupal mass, development time), I used Pearson product-moment correlation. As above, indivic plots were considered the sampling unit and all analyses were performed using the means.

RESULTS

Effects of defoliation on trees

Growth – Defoliation significantly reduced the diameter growth of trees in 1997 1998 (Figure 3, Table 3). In 1996 there was no effect of defoliation on growth and 1999, previous defoliation did not effect the growth of trees. Fertilizer positively affec growth in every year although the effects were not significant in 1996. Previ fertilization continued to enhance growth in 1999 although the effect was of c marginal significance.

Phytochemistry - I found that the defoliation levels achieved in 1996, the first year of study, were sufficient to significantly increase concentrations of total phenolics condensed tannins (Figure 4, Table 4). There was no main effect of fertilizer treatm on either condensed tannins or total phenolics but defoliation and fertilizer exhib marginally significant, interactive effects on condensed tannin concentration. Tann increased in response to fertilization in undefoliated plots but decreased in defolia fertilized plots.

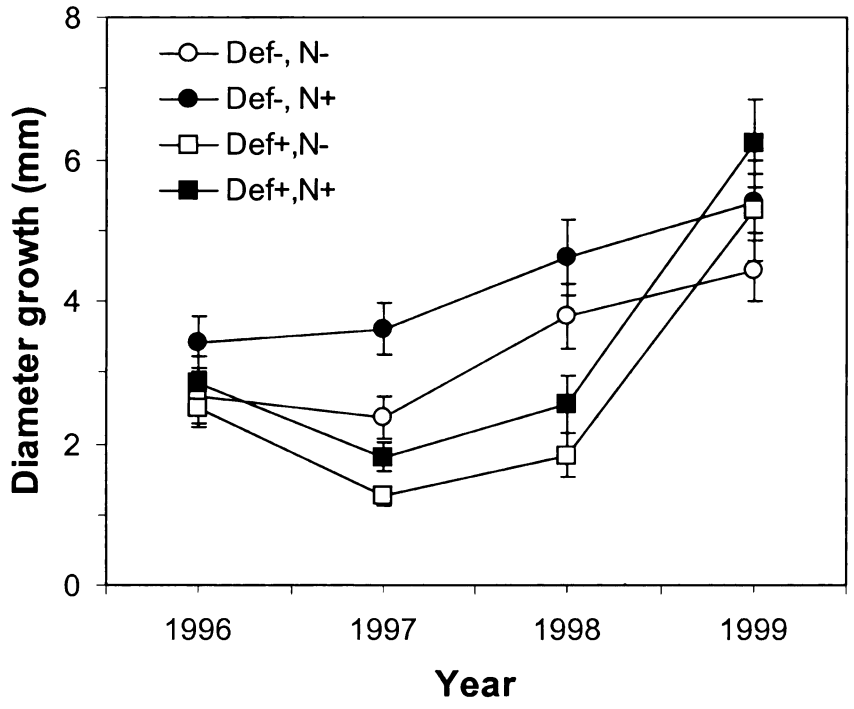


Figure 3. Annual diameter growth (mean ± SE) for poplars subjected to defoliation and fertilization treatments over the course of the study. Means (± SE) were calculated from the four plots within each treatment category. Treatment codes are undefoliated = Def -, defoliated = Def+, unfertilized = N-, and fertilized = N+.

Table 3. The effects of defoliation and fertilization treatments on tree diameter growth over the 4 year study (1996-1999). Treatments were applied in 1996-1998. Effects in 1999 are from treatments applied in previous years. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

	1996			1997			1998			1999		
Source	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
Block	3	1.30	2.8 †	3	0.05	0.2	3	0.88	2.0	3	1.43	1.3
Defoliation	1	0.46	1.0	1	8.53	33.3 ***	1	17.92	39.9 ***	1	3.02	2.8
Fertilization	1	1.02	2.3	1	3.15	12.3 *	1	2.96	6.6 *	1	4.77	4.4 †
Defol × Fert	1	0.11	0.2	1	0.49	1.9	1	0.01	0.0	1	0.10	0.1
Error	9	0.45		9	0.26		9	0.45		9	1.08	

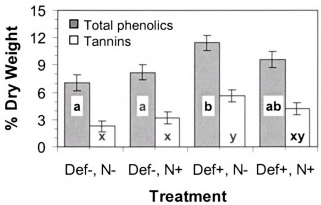


Figure 4. The effects of defoliation and fertilization treatments on concentrations (mean ± SE) of total phenolics and condensed tannins in June 1996. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 4. *The effects of defoliation and fertilization* treatments on major phenolic compounds in 1996. Compounds were extracted and quantified from leaves sampled shortly before peak defoliation (June). Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		Total phenolics			Condensed Tannins		
Sample	Source	df	MS	*F*	df	MS	*F*
June	Block	3	8.35	2.9 †	3	5.35	3.5 †
	Defoliation	1	33.05	11.6 **	1	18.84	12.2 **
	Fertilizer	1	0.47	0.2	1	0.28	0.2
	Defol × Fert	1	8.96	3.2	1	5.59	3.6 †
	Error	9	25.60		9	13.92	

In June 1997, concentrations of foliar nitrogen, total phenolics, condensed tannins, and six other phenolic compounds were measured shortly before peak defoliation. I found that defoliation significantly reduced the concentration of nitrogen by 10% and 15% in unfertilized and fertilized plots, respectively. Defoliation increased levels of total phenolics by 15% in unfertilized plots and by 11% in fertilized plots (Figure 5, Table 5). Tannins also were increased by defoliation (24% in unfertilized plots, 15% in fertilized plots). Fertilizer had no significant effects on the concentration of foliar nitrogen nor did it influence total phenolics and condensed tannins. One control plot was removed from the analysis because large numbers of gypsy moth larvae moved into it from the adjoining defoliation plot. Although I removed these caterpillars, analysis indicated that concentrations of total phenolics and condensed tannins increased dramatically and were as high or higher than in the defoliation plots.

Reflush leaves sampled from defoliation plots in July 1997 had marginally lower nitrogen levels (-9% and -10% in unfertilized and fertilized plots, respectively), whereas nitrogen was significantly higher in fertilized trees (11% in undefoliated plots, 12% in defoliated plots; Figure 5, Table 5). Total phenolics (-11% in both unfertilized and fertilized plots) and condensed tannins (-20% and –42% in unfertilized and fertilized plots, respectively) were significantly lower in the July foliage of trees that had been completely defoliated earlier in the season (Fig 5, Table 5). There was no main or interactive effect of fertilizer on levels of total phenolics or condensed tannins in July leaves. Interestingly, the condensed tannin and total phenolic concentrations in the control plot that had experienced significant gypsy moth herbivory had returned to levels found in the other control plots by late season.

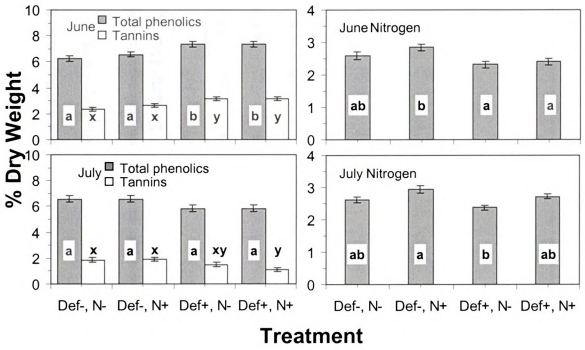


Figure 5. The effects of 1997 defoliation and fertilization treatments on concentrations (mean ± SE) of total phenolics, condensed tannins, and foliar nitrogen in June and July. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+

Table 5. The effects of defoliation and fertilization treatments on major phenolics and nitrogen in 1997. Leaves were sampled shortly before peak defoliation (June) and one month after (July). Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		Total phenolics			Condensed tannins			Nitrogen		
Sample	Source	df	MS	*F*	df	MS	*F*	df	MS	*F*
June	Block	3	2.51	15.5 **	3	0.81	9.8 **	3	0.10	2.3
	Defoliation	1	2.84	17.6 **	1	1.22	14.8 **	1	0.45	10.7 *
	Fertilization	1	0.03	0.2	1	0.05	0.6	1	0.11	2.5
	Defol x Fert	1	0.03	0.1	1	0.02	0.3	1	0.02	0.6
	Error	8	1.29		8	0.66		8	0.34	
July	Block	3	0.40	1.6	3	0.03	0.9	3	0.07	1.2
	Defoliation	1	2.16	8.5 *	1	1.38	0.0	1	0.22	3.9 †
	Fertilization	1	0.00	0.0	1	0.15	0.3	1	0.44	7.9 *
	Defol x Fert	1	0.00	0.0	1	0.18	0.2	1	0.00	0.0
	Error	9	2.30		9	0.94		9	0.50	

The treatment effects on minor phenolic constituents were variable (Table 6, Table 7). Defoliation significantly increased the levels of phenolic glycosides but had no main effects on any of the other compounds measured. Fertilizer significantly reduced levels of quercetin and marginally reduced myricetin concentration. With the exception of luteolin, concentrations of all minor phenolic compounds were altered by defoliation earlier in the season. In refoliated leaves, phenolic glycosides, cinnamic acid, and quercetin increased whereas levels of catechins and myricetin were decreased. Fertilizer had marginally significant effects on phenolic glycosides and there was a marginally significant interactive effect of defoliation and fertilizer on cinnamic acid.

Leaves were sampled at three intervals in 1998. Despite significantly lower nitrogen in the late season leaves in 1997, there was no effect of either defoliation or fertilization treatments on foliar nitrogen concentrations following bud break in May 1998 (Figure 6, Table 8). Herbivory was minimal at this time because caterpillars were still very small indicating that the effects of severe defoliation and fertilization treatments in the previous season did not carry over to 1998. Conversely, the previous year's treatments did alter concentrations of secondary compounds in the following spring. May 1998 samples had significantly higher levels of total phenolics (10% in unfertilized plots, 23% in fertilized plots) and condensed tannins (21% and 31% in unfertilized and fertilized plots, respectively) were marginally higher in plots defoliated in 1997 (Figure 6, Table 8). Fertilizer did not have an effect on concentrations of secondary compounds in spring.

Defoliation and fertilization treatments had significant effects on nitrogen in leaves sampled in June 1998 shortly before peak defoliation. Nitrogen was lower in defoliated plots (-3% in unfertilized plots, -10% in fertilized plots) and elevated in plots that had

Table 6. The effects of defoliation and fertilization treatments on minor phenolic compounds (mean ± SE) sampled in 1997. Compounds were quantified from leaves sampled shortly before peak defoliation (June) and 1 month after refoliation (July). Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

		Treatment			
Sample	Compound	DEF-, N- (Mean ± SE)	DEF-, N+ (Mean ± SE)	DEF+, N- (Mean ± SE)	DEF+, N+ (Mean ± SE)
June	Phenolic glycosides	0.157 ± 0.019	0.163 ± 0.017	0.552 ± 0.153	0.678 ± 0.213
	Cinnamic acid	0.136 ± 0.028	0.191± 0.054	0.227 ± 0.053	0.197 ± 0.057
	Catechin	0.771 ± 0.059	0.896 ± 0.040	0.871 ± 0.092	0.752 ± 0.065
	Luteolin	0.242 ± 0.017	0.205 ± 0.029	0.204 ± 0.029	0.214 ± 0.024
	Quercetin-glycoside	0.461 ± 0.017	0.388 ± 0.029	0.423 ± 0.028	0.378 ± 0.024
	Myricetin-glycoside	0.042 ± 0.014	0.015 ± 0.003	0.067 ± 0.032	0.018 ± 0.004
July	Phenolic glycosides	1.433 ± 0.096	1.566 ± 0.130	1.761 ± 0.141	2.333 ± 0.140
	Cinnamic Acid	0.291 ± 0.039	0.177 ± 0.033	0.390 ± 0.046	0.372 ± 0.062

Cont'd

Table 6 cont'd

Catechin	0.635 ± 0.045	0.538 ± 0.057	0.454 ± 0.022	0.419 ± 0.043
Luteolin	0.214 ± 0.022	0.195 ± 0.020	0.213 ± 0.010	0.226 ± 0.017
Quercetin-glycoside	0.525 ± 0.033	0.477 ± 0.023	0.578 ± 0.032	0.577 ± 0.028
Myricetin-glycoside	0.032 ± 0.005	0.015 ± 0.004	0.013 ± 0.004	0.013 ± 0.004

Table 7. Effects of defoliation and fertilization treatments on concentrations of minor phenolic compounds in 1997. Compounds were quantified from leaves sampled shortly before peak defoliation (late June), and following refoliation (late July). Plot means with all factors treated as fixed were used in the ANOVA. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

Source	Phenolic glycosides			Cinnamic Acid			Catechins			Luteolin			Quercetin-glycoside			Myricetin-glycoside		
June	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
Block	3	0.20	3.3 †	3	0.01	1.5	3	0.01	0.9	3	0.002	1.3	3	0.002	0.8	3	0.000	0.7
Defoliation	1	0.77	12.6 **	1	0.01	1.1	1	0.00	0.1	1	0.001	0.7	1	0.003	1.1	1	0.000	0.6
Fertilization	1	0.01	0.2	1	0.00	0.1	1	0.00	0.0	1	0.000	0.6	1	0.016	5.7 *	1	0.005	4.7 †
Defol × Fert	1	0.01	0.1	1	0.01	1.2	1	0.06	4.1 †	1	0.002	1.3	1	0.001	0.3	1	0.000	0.3
Error	9	0.55		9	0.12		9	0.22		9	0.026		9	0.025		9	0.011	
July																		
Block	3	0.08	0.8	3	0.02	2.4	3	0.01	0.6	3	0.005	2.4	3	0.025	6.4 *	3	0.000	0.3
Defoliation	1	1.23	11.8 **	1	0.06	6.2 *	1	0.09	9.5 *	1	0.001	0.5	1	0.023	5.8 *	1	0.001	5.2 *
Fertilization	1	0.52	5.0 †	1	0.02	2.0	1	0.02	1.8	1	0.000	0.0	1	0.003	0.6	1	0.000	3.3
Defol × Fert	1	0.21	2.0	1	0.05	4.7 †	1	0.00	0.4	1	0.001	0.5	1	0.002	0.5	1	0.000	3.1
Error	9	3.14		9	0.29		9	0.21		9	0.033		9	0.035		9	0.001	

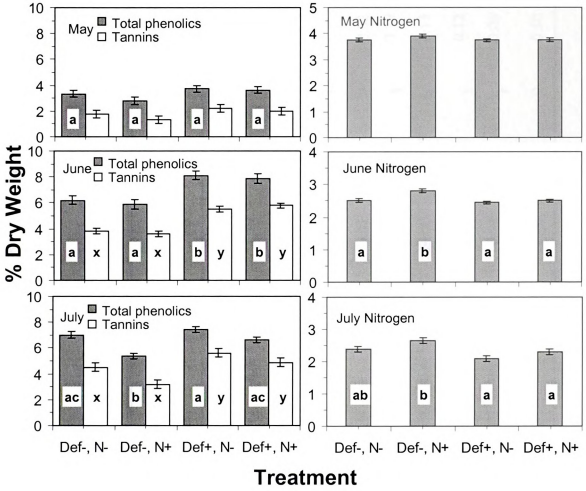


Figure 6. The effects of 1998 defoliation and fertilization treatments on foliar concentrations (mean ± SE) of total phenolics, condensed tannins, and nitrogen in May, June, and July. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 8. The effects of defoliation and fertilization treatments on major phenolics and nitrogen in 1998. Leaves were sampled in early spring (May), shortly before peak defoliation (June) and one month after (July). Analysis of variance was done using plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		Total phenolics			Condensed tannins			Nitrogen		
Sample	Source	df	MS	*F*	df	MS	*F*	df	MS	*F*
May	Block	3	0.53	1.6	3	0.29	0.9	3	0.08	4.06 *
	Defoliation	1	2.12	6.3 *	1	1.19	3.9 †	1	0.04	2.02
	Fertilization	1	0.14	0.4	1	0.45	1.5	1	0.02	1.22
	Defol × Fert	1	0.03	0.1	1	0.02	0.1	1	0.02	1.07
	Error	9	0.34		9	0.31		9	0.02	
June	Block	3	0.21	0.4	3	5.16	11.1 **	3	0.19	19.9 **
	Defoliation	1	15.47	30.4 ***	1	24.50	53.0 ***	1	0.13	13.7 **
	Fertilization	1	0.17	0.3	1	0.05	0.1	1	0.12	13.0 **
	Defol × Fert	1	0.03	0.1	1	0.72	1.6	1	0.06	5.78 *
	Error	9	0.51		9	0.46		9	0.50	

Cont'd

Table 8 cont'd										
July	Block	3	0.75	3.7†	3	0.95	1.6	3	0.02	0.6
	Defoliation	1	2.94	14.4**	1	12.32	20.9**	1	0.40	12.2**
	Fertilization	1	6.07	29.7***	1	6.59	11.2 **	1	0.23	7.2*
	Defol × Fert	1	0.81	4.0†	1	0.55	0.9	1	0.01	0.2
	Error	9	0.20		9	0.59		9	0.03	

been fertilized (Figure 6, Table 8). There was a significant interaction between defoliation and fertilizer. Defoliation increased total phenolics (24% in unfertilized plots, 26% in fertilized plots) and condensed tannins (31% in unfertilized plots, 37% in fertilized plots) but fertilizer itself did not change levels of either compound significantly (Figure 6, Table 8).

Samples collected from refoliated trees in defoliation plots in July 1998, approximately one month after peak defoliation had significantly lower nitrogen (-12% in unfertilized plots, -14% in fertilized plots) than trees in control plots (Figure 6, Table 8). Fertilizer increased nitrogen levels in both undefoliated and defoliated plots. In contrast to 1997, leaves sampled from plots defoliated earlier in the season had elevated levels of total phenolics (7% in unfertilized plots, 20% in fertilized plots) and condensed tannins (20% and 35% in unfertilized and fertilized plots, respectively; Figure 6, Table 8). There was a significant reduction in phenolics and tannins in fertilized plots and a marginally significant interaction between defoliation and fertilization on total phenolic levels.

Of the minor phenolics quantified in May 1998, quercetin concentrations were reduced in plots defoliated in the previous year while fertilizer had no measurable effect on any compound (Table 9, Table 10). Concentrations of catechin, luteolin, and myricetin were all increased by defoliation in June leaf samples (Table 9, Table 10) whereas fertilizer significantly decreased luteolin and marginally increased catechins, but had no effects on other minor phenolics. In July leaves, defoliation had significant effects on levels of luteolin and in addition, there was an interaction between fertilization and defoliation. Myricetin-glycosides were significantly higher in the plots defoliated earlier in the season.

Table 9. Effects of defoliation and fertilization treatments on foliar concentrations of minor phenolic compounds in 1998. Compounds were quantified from leaves sampled 2 weeks after bud break and gypsy moth egg hatch (May), shortly before peak defoliation (late June), and following refoliation (late July). Plot means with all factors treated as fixed were used in the ANOVA. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		Treatment			
Sample	Compound	DEF-, N- (mean ± SE)	DEF-, N+ (mean ± SE)	DEF+, N- (mean ± SE)	DEF+, N+ (mean ± SE)
May	Phenolic glycosides	0.462 ± 0.088	0.508 ± 0.060	0.464 ± 0.115	0.478 ± 0.086
	Cinnamic Acid	0.258 ± 0.039	0.250 ± 0.022	0.238 ± 0.050	0.269 ± 0.047
	Catechin	0.721 ± 0.117	0.657 ± 0.067	0.685 ± 0.108	0.717 ± 0.090
	Luteolin	0.159 ± 0.004	0.142 ± 0.006	0.140 ± 0.012	0.144 ± 0.014
	Quercetin	0.405 ± 0.019	0.381 ± 0.019	0.333 ± 0.028	0.364 ± 0.040
	Myricetin	0.029 ± 0.003	0.027 ± 0.001	0.028 ± 0.004	0.029 ± 0.004

Cont'd

Table 9 cont'd

June	Phenolic glycosides	0.338 ± 0.183	0.534 ± 0.219	0.315 ± 0.187	0.368 ± 0.213
	Cinnamic Acid	0.154 ± 0.023	0.214 ± 0.031	0.245 ± 0.072	0.268 ± 0.050
	Catechin	0.032 ± 0.006	0.044 ± 0.011	0.085 ± 0.014	0.086 ± 0.044
	Luteolin	0.356 ± 0.019	0.330 ± 0.028	0.492 ± 0.030	0.407 ± 0.032
	Quercetin	0.420 ±0.161	0.545 ± 0.155	0.603 ± 0.188	0.973 ± 0.218
	Myricetin	0.621 ± 0.090	0.558 ± 0.089	0.713 ± 0.058	0.627 ± 0.048
July	Phenolic glycosides	1.232 ± 0.289	1.075 ± 0.179	1.172 ± 0.248	1.074 ± 0.209
	Cinnamic Acid	0.175 ± 0.037	0.188 ± 0.049	0.148 ± 0.036	0.226 ± 0.035
	Catechin	0.799 ± 0.104	0.564 ± 0.056	0.655 ± 0.053	0.759 ± 0.053
	Luteolin	0.461 ± 0.058	0.324 ± 0.020	0.403 ± 0.019	0.407 ± 0.024
	Quercetin	0.782 ± 0.064	0.679 ± 0.035	0.714 ± 0.033	0.766 ± 0.028
	Myricetin	0.057 ± 0.021	0.012 ± 0.005	0.022 ± 0.007	0.022 ± 0.008

Table 10. The effects of defoliation and fertilization treatments on foliar concentrations of minor phenolic compounds in 1998. Compounds were quantified from leaves sampled 2 weeks after bud break and gypsy moth hatch (May), shortly before peak defoliation (late June), and following expansion of refoliated leaves (late July). Analysis of variance was done using the plot means with all factors treated as fixed. Plot means with all factors treated as fixed were used in the ANOVA. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

Source	Phenolic glycosides			Cinnamic Acid			Catechins			Luteolin			Quercetin-glycoside			Myricetin-glycoside		
May	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
Block	3	0.01	10.4 **	3	0.016	4.3 *	3	0.013	0.3	3	0.001	7.0 **	3	0.009	6.9 *	3	0.0001	2.3
Defoliation	1	0.00	0.1	1	0.000	0.0	1	0.001	0.0	1	0.000	1.6	1	0.008	6.3 *	1	0.0000	0.0
Fertilization	1	0.00	0.4	1	0.001	0.7	1	0.001	0.0	1	0.000	0.9	1	0.000	0.0	1	0.0001	0.1
Defol × Fert	1	0.00	0.1	1	0.002	0.4	1	0.009	0.2	1	0.000	2.3	1	0.003	2.4	1	0.0000	0.3
Error	9	0.09		9	0.034		9	0.415		9	0.001		9	0.012		9	0.0003	
June																		
Block	3	0.29	2.5	3	0.012	1.4	3	0.311	4.3 *	3	0.006	3.3 †	3	0.063	8.1 **	3	0.0015	0.6

Cont'd

Table 10 cont'd

Defoliation	1	0.03	0.3	1	0.021	2.5	1	0.373	5.1 *	1	0.046	23.4 ***	1	0.026	3.3	1	0.0089	3.5 †
Fertilization	1	0.06	0.5	1	0.007	0.8	1	0.246	3.4 †	1	0.012	6.3 *	1	0.022	2.8	1	0.0001	0.1
Defol × Fert	1	0.02	0.2	1	0.001	0.2	1	0.060	0.8	1	0.004	1.8	1	0.001	0.1	1	0.0001	0.0
Error	9	1.07		9	0.076		9	0.655		9	0.089		9	0.309		9	0.0367	
July																		
Block	3	0.42	1.7	3	0.013	1.9	3	0.031	2.3	3	0.008	6.4 *	3	0.014	2.9 †	3	0.0002	1.5
Defoliation	1	0.06	0.2	1	0.006	0.9	1	0.040	2.9	1	0.010	8.1 *	1	0.012	2.5	1	0.0012	10.5 *
Fertilization	1	0.06	0.3	1	0.007	1.0	1	0.000	0.0	1	0.002	1.8	1	0.003	0.7	1	0.0003	2.6
Defol × Fert	1	0.02	0.1	1	0.009	1.3	1	0.038	2.8	1	0.009	7.4 *	1	0.016	3.4 †	1	0.0000	0.2
Error	9	2.94		9	0.061		9	0.124		9	0.011		9	0.043		9	0.0010	

Water concentration was significantly reduced in the defoliation plots in June 1998 (Figure 7, Table 11), but was not affected by fertilizer. There was no significant effect of either defoliation or fertilizer on July water content although the among-plot variability was much higher than earlier in the season (Figure 7, Table 11). Water content was 5-8% higher in plots that had been severely defoliated and produced a second set of leaves. Conversely, it was lower than controls in moderately defoliated plots where trees retained their damaged leaves. As well, the area of intact leaves was reduced in defoliation plots but was not affected by fertilizer (Figure 7, Table 11). Leaf toughness increased in defoliation treatment plots but was not altered by fertilizer (Figure 7, Table 11).

Leaves were sampled only once in 1999, corresponding with the presence of late instar gypsy moth. Because gypsy moth develops more slowly in low-density populations due primarily to shifts in feeding behavior (Lance et al. 1986), the samples were taken at a point phenologically later in the season than in the previous three years. No defoliation was evident and fertilizer was not applied in 1999. Although there was trend toward lower nitrogen in plots defoliated over the three previous years, the difference was not significant (Figure 8, Table 12). Conversely, nitrogen was elevated in plots that had been fertilized in the three preceding years (8% in undefoliated plots, 5% in defoliated plots). Concentrations of total phenolics (19% in both unfertilized and fertilized plots) and condensed tannins (15% in unfertilized plots, 27% in fertilized plots) were higher in previously defoliated plots (Figure 8, Table 12). Prior fertilization marginally reduced total phenolics and significantly reduced condensed tannins. Treatment history was also reflected in foliar water content with reduced levels in the defoliation plots, although

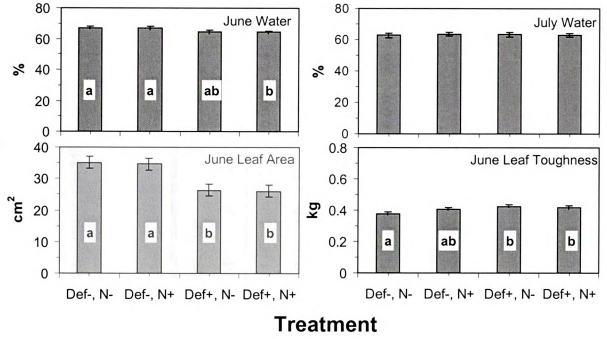


Figure 7. The effects of 1998 defoliation and fertilization treatments on measures (mean ± SE) of water content in June and July, and leaf area and leaf toughness in June. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 11. The effects of defoliation and fertilization treatments on water, leaf area, and leaf toughness in 1998. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

	Water - June			Water - July			Toughness - June			Leaf Area - June		
Source	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
Block	3	2.18	2.0	3	14.00	2.0	3	0.002	4.4 *	3	27.66	1.9
Defoliation	1	27.64	25.5 ***	1	0.04	0.0	1	0.003	7.7 *	1	302.54	20.7 **
Fertilization	1	0.11	0.1	1	0.15	0.0	1	0.000	1.1	1	0.34	0.0
Defol × Fert	1	0.02	0.0	1	1.21	0.2	1	0.001	3.6 †	1	0.05	0.0
Error	9	1.08		9	7.07		9	0.000		9	14.59	

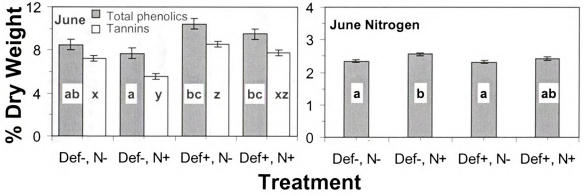


Figure 8. The effects of previous defoliation and fertilization treatments on foliar concentrations (mean ± SE) of total phenolics, condensed tannins, and nitrogen in June 1999. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 12. The effects of defoliation and fertilization treatments on major phenolics and nitrogen in 1999. Leaves were sampled in late June coinciding with presence of final instar gypsy moth larvae in the field. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		Total phenolics			Condensed tannins			Nitrogen		
Sample	Source	df	MS	*F*	df	MS	*F*	df	MS	*F*
June	Block	3	1.57	5.1 *	3	1.74	1.8	3	0.01	0.5
	Defoliation	1	12.39	40.4 ***	1	14.60	15.2 **	1	0.03	2.8
	Fertilization	1	6.13	20.0 **	1	3.44	3.6 †	1	0.11	11.0 **
	Defol x Fert	1	0.61	2.0	1	0.06	0.1	1	0.01	1.1
	Error	9	0.32		9	0.96		9	0.01	

there was no effect of previous fertilizer application (Figure 9, Table 13). In 1999, leaf area was not affected by previous defoliation or fertilization regimes (Figure 9, Table 13).

Effects of defoliation and fertilization on caterpillar performance

Gypsy moth lifelong bioassays - Neither defoliation nor fertilization had any effects on gypsy moth pupal mass in 1996 despite significant treatment effects on foliar biochemistry (Figure 10, Table 14). The development time of males was significantly increased in the defoliation plots, although the increase was small (less than one full day) and unlikely to have biological relevance. There was no impact of either treatment on female development time.

I increased the density of gypsy moth eggs in the defoliation plots by more than 40 fold in 1997 resulting in near complete defoliation by the end of the larval feeding period. Caterpillars in the plots switched from feeding only at night to feeding both diurnally and nocturnally, a typical density related response of gypsy moth larvae seen in natural outbreak populations (Lance et al. 1986). Surprisingly, only a marginal decrease in female pupal mass was detected, and there was no effect of defoliation on males (Figure 10, Table 14). There was no direct or interactive effect of fertilization on the pupal mass of either sex, nor were there any effects of defoliation or fertilizer on the development times. From the regression equation derived for female gypsy moth pupal mass and egg number (see methods), I estimated that fecundity was reduced from 717 to 632 eggs, a difference of 11.9% for caterpillars reared on foliage from the defoliated plots.

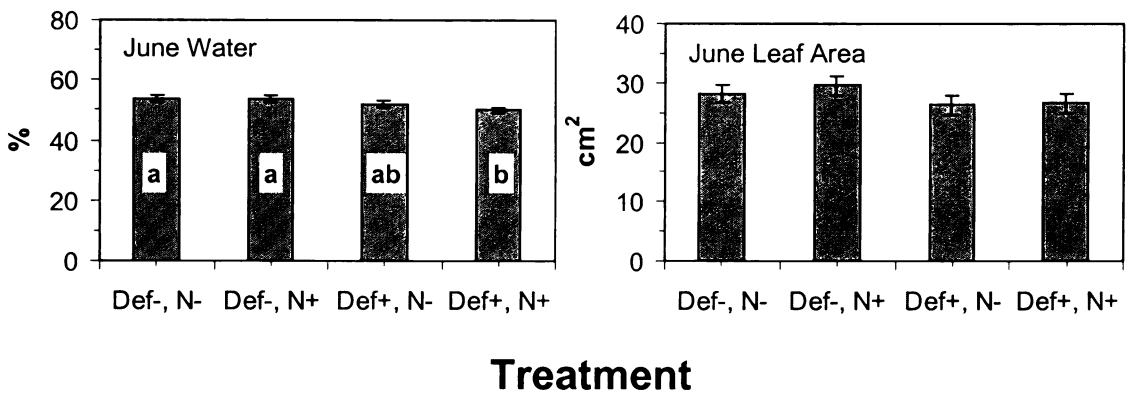


Figure 9. The effects of previous defoliation and fertilization treatments on water content (mean ± SE) and leaf area (mean ± SE) in June 1999. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 13. The effects of defoliation and fertilization treatments on water and leaf area in 1999. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

	Water - June				Leaf Area - June			
Source	df	MS	*F*		df	MS	*F*	
Block	3	3.29	0.8		3	4.44	0.5	
Defoliation	1	30.79	7.6	*	1	23.12	2.3	
Fertilization	1	4.79	1.2		1	2.97	0.3	
Defol × Fert	1	4.88	1.2		1	1.20	0.1	
Error	9	4.04			9	9.99		

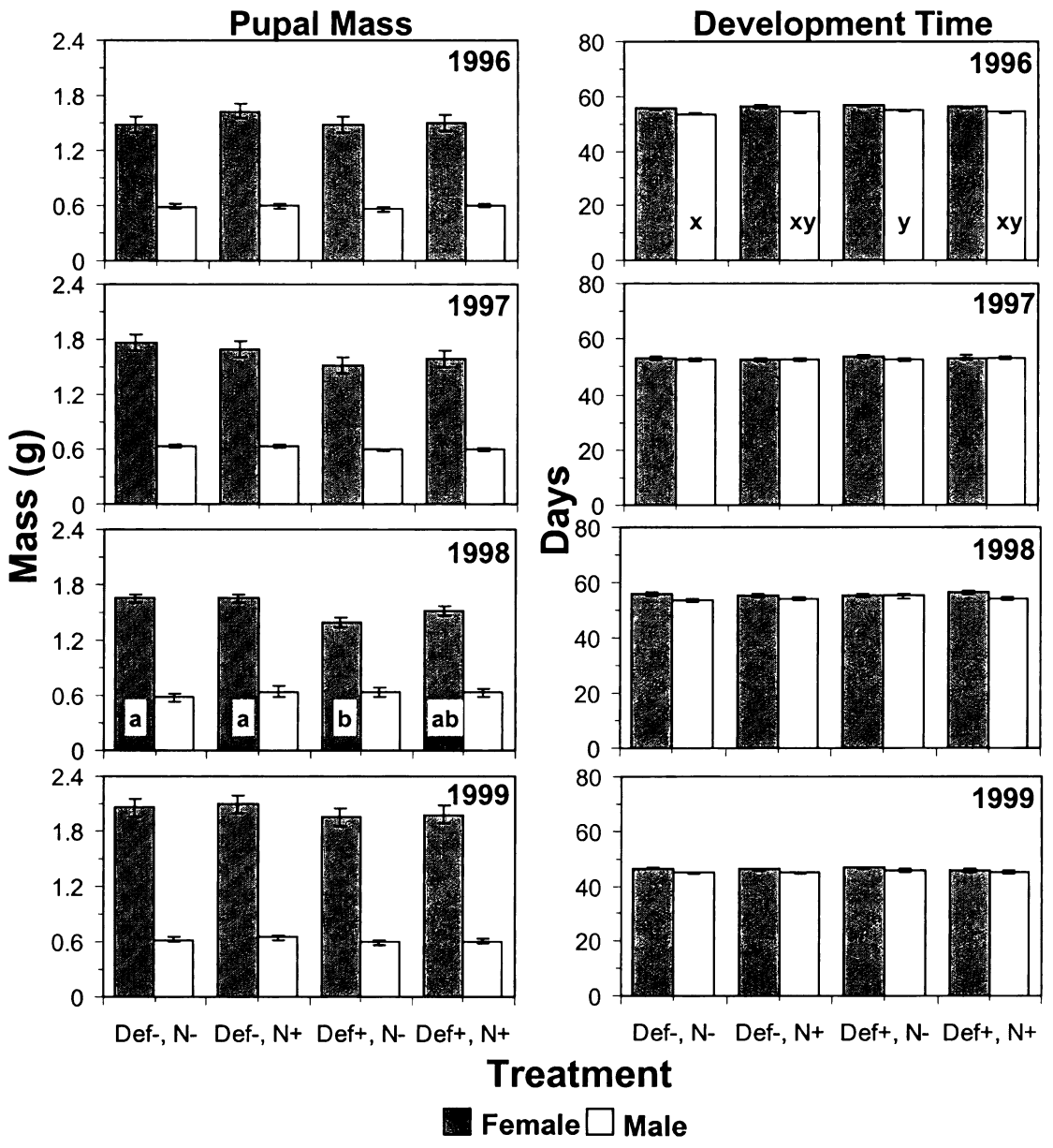


Figure 10. The effects of defoliation and fertilization treatments on male and female gypsy moth pupal mass and development time for 1996-1999. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 14. The effects of defoliation and fertilization on male and female gypsy moth pupal mass and development time in each year, 1996-1999. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. Note that in 1998, one block was dropped due to high mortality so analysis used only the remaining 3 blocks. No treatments were applied in 1999, therefore foliage consumed by caterpillars reflected 3 previous years treatments. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		PUPAL MASS						DEVELOPMENT TIME					
		Females			Males			Females			Males		
Year	Source	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
1996	Block	3	0.078	2.7	3	0.002	0.7	3	1.02	2.1	3	1.64	3.4 †
	Defoliation	1	0.023	0.8	1	0.000	0.1	1	1.29	2.6	1	3.20	6.6 *
	Fertilization	1	0.034	1.2	1	0.003	1.1	1	0.05	0.1	1	0.10	0.2
	Defol × Fert	1	0.012	0.4	1	0.001	0.4	1	1.70	3.4 †	1	2.12	4.4 †
	Error	9	0.003		9	0.029		9	0.50		9	0.48	
1997	Block	3	0.034	1.1	3	0.001	0.9	3	0.77	0.6	3	0.47	0.3
	Defol	1	0.128	4.1 †	1	0.003	3.0	1	1.52	1.3	1	0.43	0.3
	Fert	1	0.000	0.0	1	0.000	0.2	1	0.14	0.1	1	0.05	0.0
	Defol × Fert	1	0.016	0.5	1	0.000	0.2	1	0.00	0.0	1	1.06	0.7
	Error	9	0.001		9	0.032		9	1.22		9	1.48	
1998	Block	3	0.101	15.1 **	3	0.023	2.3	3	0.10	0.1	3	3.02	2.4
	Defol	1	0.119	17.8 **	1	0.001	0.1	1	0.00	0.0	1	1.54	1.2
	Fert	1	0.012	1.8	1	0.004	0.4	1	1.35	0.9	1	0.14	0.1
	Defol × Fert	1	0.011	1.7	1	0.007	0.8	1	1.03	0.7	1	1.92	1.5
	Error	6	0.007		6	0.010		6	1.55		6	1.27	
1999	Block	3	0.042	1.0	3	0.001	0.5	3	0.13	0.2	3	0.13	0.2
	Defol	1	0.041	1.0	1	0.007	2.1	1	0.00	0.0	1	0.85	1.1
	Fert	1	0.003	0.1	1	0.001	0.4	1	1.56	2.1	1	0.79	1.0
	Defol × Fert	1	0.000	0.0	1	0.000	0.1	1	0.13	0.2	1	0.53	0.7
	Error	9	0.040		9	0.003		9	0.75		9	0.79	

I relied on *in situ* egg masses and did not supplement gypsy moth densities in 1998. As a result, defoliation levels among plots were more variable than in the previous year, ranging from moderately to severely defoliated. In lifelong bioassays, gypsy moth females suffered a significant decline in pupal mass in defoliation treatments (-15.8% unfertilized plots, -8.3% fertilized plots) whereas males were not affected in 1998 (Figure 10, Table 14). There were no effects of defoliation or fertilization on development time of either males or females. Because of high mortality in my gypsy moth rearing stock (33% survival) due to an unidentified, presumably bacterial disease, data was available for only three of the four blocks (12 of 16 plots) and sample sizes within cells were reduced relative to previous years. Nonetheless, my data show that the combination of previous (1996 and 1997) and current year defoliation reduced fecundity by 14%, or 95 eggs per female, similar to the reduction seen in 1997.

In 1999, defoliation levels were very low and I did not apply fertilizer, thus any treatment effects reflect the history of the plots over the previous year or years. There was no significant effect of defoliation or fertilization on male or female gypsy moth pupal masses or male and female development time (Figure 10, Table 14). The lack of effects on gypsy moth following three consecutive years of defoliation indicate that induced-resistance in these poplars was driven primarily by within season changes in leaf quality, and not by DIR.

Gypsy moth short-term bioassays - In a short-term bioassay of 48 hours, relative growth rates (RGR) for 5^{th} instars did not vary among treatments in 1997 (Figure 11, Table 15). Relative consumption rate (RCR) increased by 9% in response to defoliation in fertilized plots and by 24% in unfertilized, defoliated plots. Defoliation and fertilization did not

Figure 11. The effects of previous defoliation and fertilization treatments on mean (± SE) relative growth rates (RGR), relative consumption rates (RCR), approximate digestibility of consumed foliage (AD), efficiency of conversion of ingested foliage (ECI), and efficiency of conversion of digested foliage (ECD), for fifth instar gypsy moth in 1997 and 1998. Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

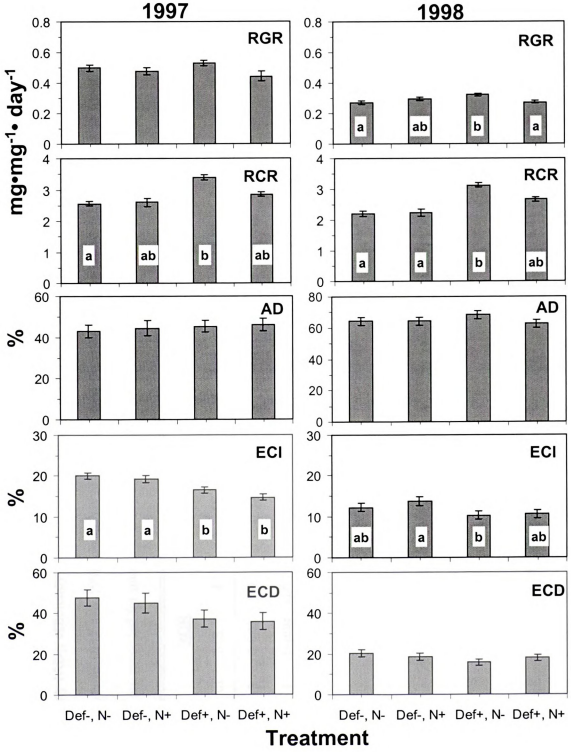

1997
1998
RGR
RCR
AD
ECI
ECD
mg•mg-1• day-1
%
a
ab
b
ab
a
a
b
ab
a
a
b
ab
ab
a
b
ab
Def-, N-
Def-, N+
Def+, N-
Def+, N+
Treatment

Table 15. Effects of defoliation and fertilization treatments on relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) for 5th instar gypsy moth in 1997 and 1998. Analysis of variance used the plot means with all factors treated as fixed. Consumption data was inadvertently not recorded for one plot in 1997. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

		RGR			RCR			AD			ECI			ECD	
Year	Source	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
1997	Block	3	0.003	1.0	3	0.515	3.8 †	3	37.10	1.0	3	47.12	23.2 ***	3	274.8	4.0 †
	Defoliation	1	0.000	0.0	1	0.804	6.0 *	1	12.89	0.4	1	34.14	16.8 **	1	257.0	3.8 †
	Fertilization	1	0.011	3.5 †	1	0.018	0.1	1	4.26	0.1	1	15.92	7.9 *	1	37.1	0.6
	Defol × Fert	1	0.004	1.3	1	0.250	1.9	1	3.97	0.1	1	0.14	0.1	1	16.2	0.2
	Error	9	0.003		8	0.134		8	36.62		8	2.03		8	68.0	
1998	Block	3	0.003	6.7 *	3	0.068	0.7	3	25.72	0.9	3	13.71	3.1 †	3	19.9	1.9
	Defoliation	1	0.001	1.6	1	1.820	19.9 **	1	0.54	0.2	1	25.88	5.9 *	1	26.5	2.5
	Fertilization	1	0.001	1.6	1	0.194	2.1	1	31.94	1.2	1	2.87	0.7	1	0.4	0.0
	Defol × Fert	1	0.005	11.3 **	1	0.228	2.5	1	30.43	1.1	1	1.41	0.3	1	12.6	1.2
	Error	9	0.000		9	0.091		9	27.69		9	4.37		9	10.7	

alter the approximate digestibility of foliage. However, defoliation significantly decreased the efficiency of caterpillars in converting ingested (ECI) and digested (ECD) foliage to biomass by 19% and 21%, respectively. Fertilizer did not affect either of these measures of feeding efficiency. The 1998 short-term bioassay yielded similar results to those seen in 1997 (Figure 11, Table 15). There was no treatment effect on RGR but RCR increased by 16% and 29% in caterpillars feeding on foliage from fertilized and unfertilized defoliation plots respectively. ECD was reduced by 23% and ECI by 22% for larvae fed foliage from defoliated plots. Fertilizer did not significantly affect measures of efficiency in the short-term bioassay.

I used Pearson correlation coefficients to assess the associations among phytochemical variables measured in this experiment, and pupal mass and development times of gypsy moth (Table 16). Nitrogen was positively correlated with the pupal mass of gypsy moth females in 1997 and 1998. In 1999, development time of both male and female gypsy moth was negatively correlated with nitrogen although for females the effect was only of marginal significance. Water content was not measured in 1997 but was positively correlated with female gypsy moth pupal mass in 1998. Of the secondary compounds determined, total phenolics were negatively correlated with pupal masses of both sexes of gypsy moth in 1997 and with female mass in 1998. Total phenolics were significantly correlated with longer development times for both males and females in 1996. Condensed tannins make up a large portion of the total phenolics in these poplars and thus had similar correlations with pupal mass and development times of gypsy moth. Male gypsy moth development time was marginally correlated with condensed tannins in 1996, 1997, and 1998. On the other hand condensed tannins were correlated with female

Table 16. Pearson correlations between male and female gypsy moth pupal mass and development time and concentrations of phytochemicals in leaves samples from the plots. Abbreviations are N = nitrogen, H20 = water, TOTPHN = total phenolics, CNDTAN = condensed tannins, PHENGLY = pooled phenolic glycosides, CINNAM = cinnamic acid, CATECH = catechins, LUTEOL = luteolin, QUERTN = quercetin glycosides, and MYRCTN = myricetin glycosides. Bold type p-values are $p < 0.05$, italicized p-values are $0.05 < p < 0.10$.

(a) Pupal Mass		N		H2O		TOTPHN		CNDTAN		PHENGLY		CINNAM		CATECH		LUTEOL		QUERTN		MYRCTN	
Year	Sex	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
1996	M	•	•	•	•	0.18	0.51	0.26	0.34	•	•	•	•	•	•	•	•	•	•	•	•
	F	•	•	•	•	0.03	0.92	-0.08	0.76	•	•	•	•	•	•	•	•	•	•	•	•
1997	M	0.35	0.19	•	•	-0.46	*0.07*	-0.43	*0.10*	0.00	0.98	0.12	0.67	0.12	0.66	0.10	0.71	0.31	0.24	0.00	0.97
	F	0.58	**0.02**	•	•	-0.52	**0.04**	-0.60	**0.02**	-0.44	*0.09*	-0.36	0.17	0.48	*0.06*	0.09	0.72	0.44	*0.09*	-0.12	0.67
1998	M	0.34	0.33	-0.09	0.77	-0.10	0.75	-0.29	0.36	0.77	**0.01**	0.44	0.15	0.47	0.13	0.50	0.11	0.62	**0.03**	0.58	**0.05**
	F	0.65	**0.02**	0.60	**0.04**	-0.68	**0.02**	-0.79	**0.01**	0.64	**0.03**	-0.38	0.23	0.37	0.24	-0.10	0.76	0.47	0.13	-0.04	0.90
1999	M	0.15	0.58	0.05	0.84	-0.19	0.48	-0.25	0.34	•	•	•	•	•	•	•	•	•	•	•	•
	F	-0.07	0.78	-0.17	0.58	-0.18	0.50	-0.22	0.40	•	•	•	•	•	•	•	•	•	•	•	•

(b) Development Time		N		H2O		TOTPHN		CNDTAN		PHENGLY		CINNAM		CATECH		LUTEOL		QUERTN		MYRCTN	
Year	Sex	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
1996	M	•	•	•	•	0.51	**0.05**	0.48	*0.08*	•	•	•	•	•	•	•	•	•	•	•	•
	F	•	•	•	•	0.54	**0.04**	0.52	**0.04**	•	•	•	•	•	•	•	•	•	•	•	•
1997	M	-0.12	0.67	•	•	0.42	0.11	0.46	*0.07*	0.02	0.96	0.10	0.72	-0.54	**0.04**	-0.07	0.79	-0.40	0.13	-0.03	0.92
	F	-0.36	0.18	•	•	0.28	0.29	0.35	0.18	0.26	0.34	0.32	0.23	-0.26	0.33	-0.18	0.52	-0.30	0.26	0.19	0.47
1998	M	-0.42	0.18	-0.53	*0.08*	0.47	0.12	0.50	*0.10*	-0.25	0.43	0.63	**0.03**	0.40	0.20	0.09	0.78	0.13	-0.78	-0.21	0.51
	F	0.00	0.99	-0.10	0.77	-0.13	0.70	0.14	0.66	0.22	0.48	0.11	0.74	0.23	0.48	-0.17	0.59	0.33	0.31	-0.05	0.87
1999	M	-0.50	**0.04**	-0.04	0.90	0.26	0.33	0.23	0.40	•	•	•	•	•	•	•	•	•	•	•	•
	F	-0.42	*0.10*	-0.03	0.90	0.00	0.99	0.14	0.60	•	•	•	•	•	•	•	•	•	•	•	•

gypsy moth development time in 1996 only. Phenolic glycosides had only a marginal negative association with female gypsy moth in 1997, and had a significant positive relationship to male and female pupal mass in 1998. Because concentrations were low (>1%) in this poplar clone, other intercorrelated phytochemical constituents may be determining the variable associations of phenolic glycosides with gypsy moth pupal mass. A similar process may explain why male pupal mass was positively correlated with myricetin- and quercetin-glycosides in 1998 but not in 1997 and had no effect in either year on females.

Long-term bioassays on other species of Lepidoptera - In addition to gypsy moth, I determined the effects of fertilization and gypsy moth defoliation on four other species of poplar-feeding lepidopterans in 1998 and three species in 1999. A large portion of the feeding period of forest tent caterpillar overlaps with gypsy moth (see Figure 2). In lifelong bioassays, pupal mass of female forest tent caterpillar was significantly reduced by defoliation whereas male pupal mass was unaffected in 1998 (Figure 12, Table 17). Using the regression equation for fecundity and pupal mass, I calculated that defoliation reduced fecundity by 9%, or 21 eggs/female. Pupal mass was not different among plots in 1999 suggesting that the reductions in 1998 were due to rapid-induced responses elicited by gypsy moth feeding damage. Fertilization did not affect forest tent caterpillar pupal mass in 1998 but significantly increased female mass in 1999 (Figure 12, 13, Table 17, 18). Development time of forest tent caterpillar was not affected by treatments in either year (Figure 12, 13, Table 17, 18).

The poplar tent maker initiates feeding in mid-season when gypsy moth defoliation is at its peak. Both female and male pupal masses were significantly reduced in the

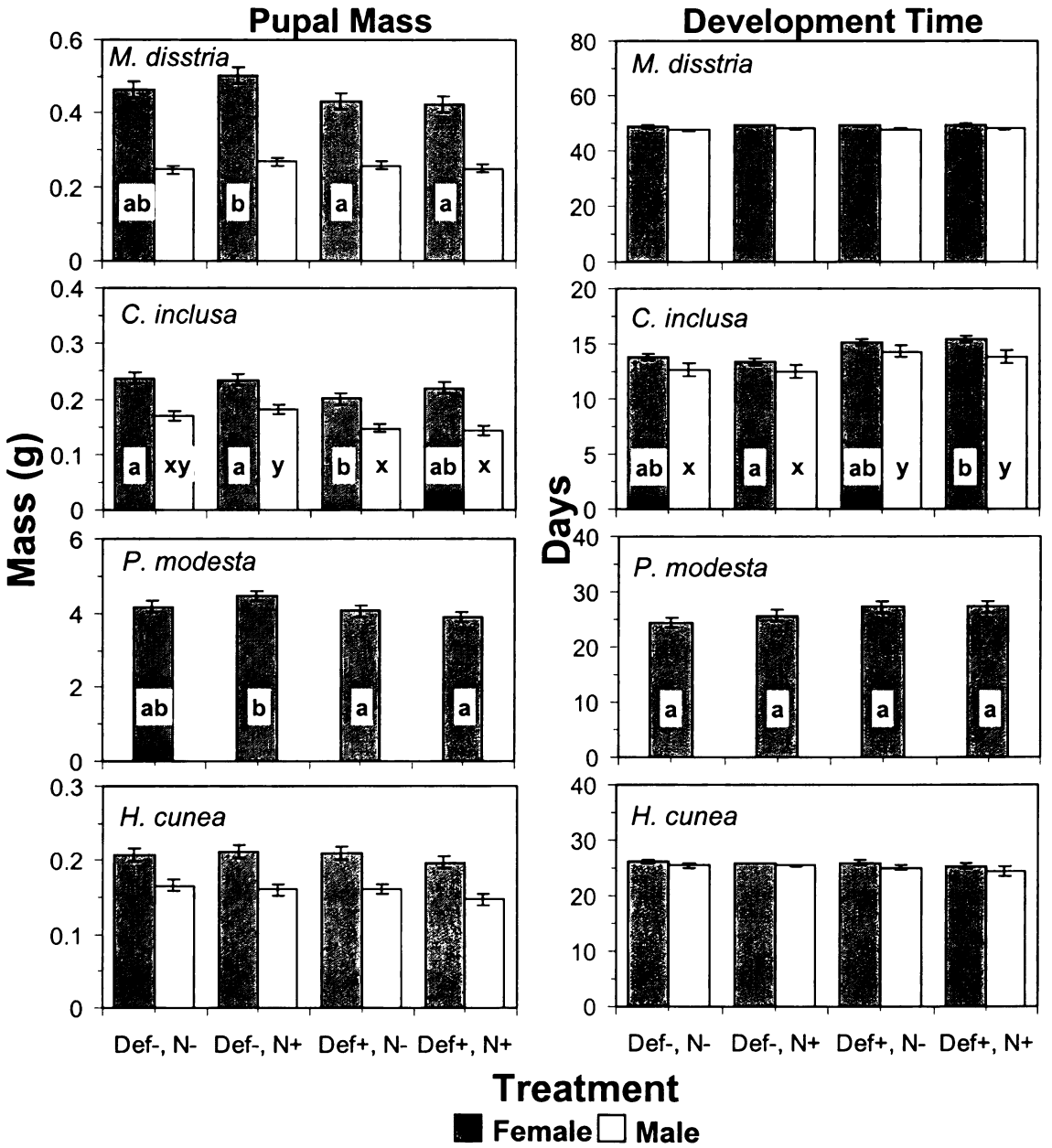


Figure 12. The effects of defoliation and fertilization treatments in 1998 on mean (± SE) pupal mass and development time of forest tent caterpillar (*M. disstria*), poplar tent maker (*C. inclusa*), big poplar sphinx (*P. modesta*), and fall webworm (*H. cunea*). Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 17. The effects of defoliation and fertilization on male and female pupal mass and development time for forest tent caterpillar (*M. disstria*), poplar tent maker (*P. inclusa*), big poplar sphinx (*P. modesta*), fall webworm (*H. cunea*), and white-marked tussock moth (*O. leucostigma*) in 1998. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. ‡Note that *P. modesta* were not sexually dimorphic so analysis was done on pooled males and females. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

			PUPAL MASS						DEVELOPMENT TIME					
			Females			Males			Females			Males		
Year	Species	Effect	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
1998	*M. disstria*	Block	3	0.019	9.6 **	3	0.003	6.0 *	3	6.72	10.7 **	3	6.48	12.1 **
		Defoliation	1	0.012	6.0 *	1	0.000	0.8	1	0.43	0.7	1	0.27	0.5
		Fertilization	1	0.001	0.6	1	0.000	0.0	1	0.56	0.9	1	1.05	2.0
		Defol x Fert	1	0.002	1.0	1	0.001	2.6	1	0.01	0.0	1	0.15	0.3
		Error	9	0.002		9	0.001		9	0.63		9	0.54	
	C. inclusa	Block	3	0.001	1.7	3	0.001	2.7	3	3.53	2.8	3	0.98	2.6
		Defol	1	0.003	5.9 *	1	0.004	12.0 **	1	10.83	8.4 *	1	9.00	23.8 **
		Fert	1	0.000	0.6	1	0.000	0.2	1	0.03	0.0	1	0.41	1.1
		Defol x Fert	1	0.001	1.2	1	0.000	1.1	1	0.39	0.3	1	0.15	0.4
		Error	9	0.001		9	0.000		9	1.29		9	0.38	
	P. modesta‡	Block	3	0.013	0.2	•	•	•	3	3.47	1.0	•	•	•
		Defol	1	0.556	7.1 *	•	•	•	1	19.29	5.4 *	•	•	•
		Fert	1	0.024	0.3	•	•	•	1	1.76	0.5	•	•	•
		Defol x Fert	1	0.112	1.4	•	•	•	1	1.76	0.5	•	•	•
		Error	9	0.079		•	•		9	3.59		•	•	
	H. cunea	Block	3	0.0001	0.2	3	0.0002	0.8	3	0.67	1.9	3	2.77	4.4 *
		Defol	1	0.0002	0.6	1	0.0003	1.3	1	0.36	1.0	1	2.34	3.7 †
		Fert	1	0.0002	0.2	1	0.0003	1.6	1	0.55	1.5	1	0.39	0.6
		Defol x Fert	1	0.0003	0.9	1	0.0001	0.3	1	0.14	0.4	1	0.63	1.0
		Error	9	0.0003		9	0.0002		9	0.35		9	0.63	

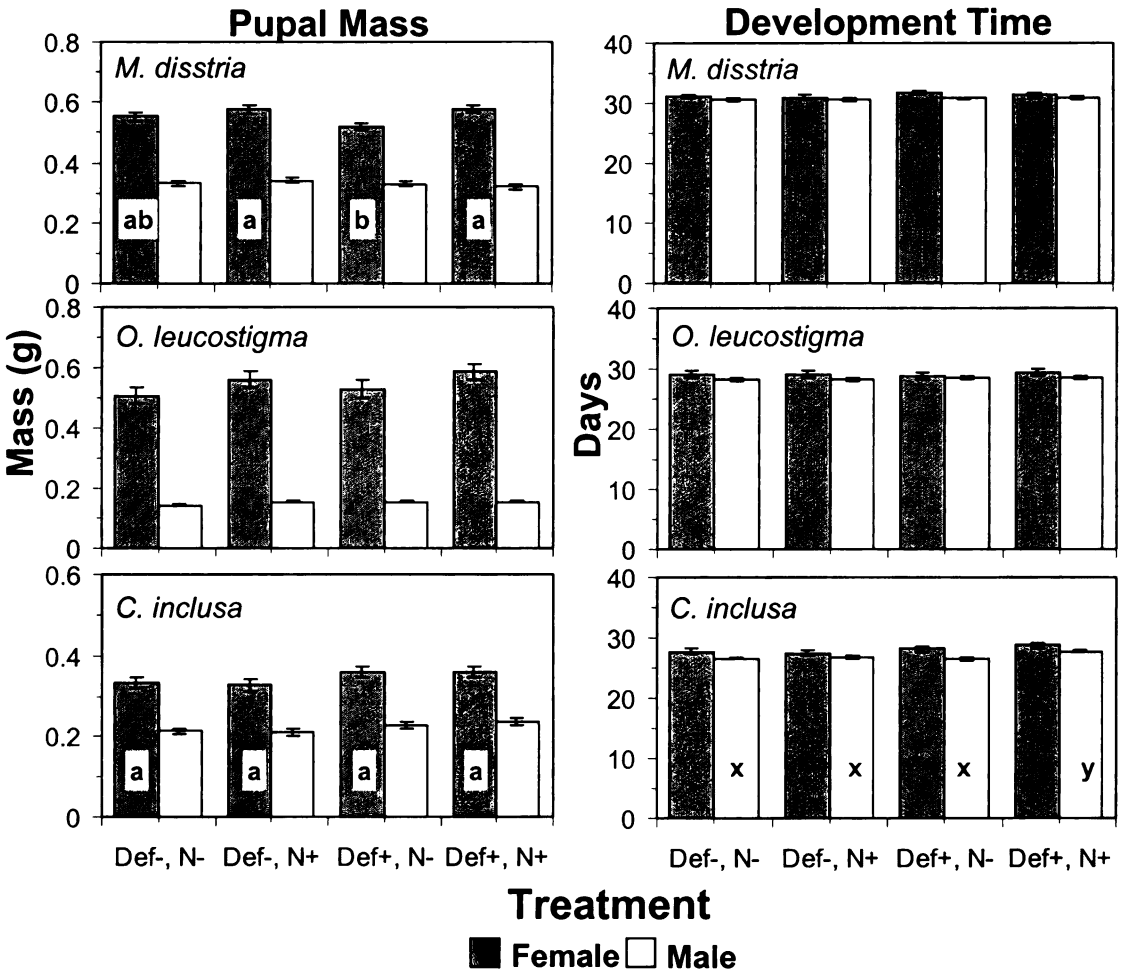


Figure 13. The effects of previous defoliation and fertilization treatments on 1999 pupal mass and development time of forest tent caterpillar (*M. disstria*), white-marked tussock moth (*O. leucostigma*) and poplar tent maker (*C. inclusa*). Means (± SE) were calculated from the four plots within each treatment category. Different letters indicate significant pairwise differences among means ($p < 0.05$) after significant main defoliation or fertilization effects in ANOVA. Treatment codes are undefoliated = Def -, defoliated = Def +, unfertilized = N-, and fertilized = N+.

Table 18. The effects of defoliation and fertilization on male and female pupal mass and development time for forest tent caterpillar (*M. disstria*), white-marked tussock moth (*O. leucostigma*), and poplar tent maker (*P. inclusa*) in 1999. Analysis of variance was done using the plot means with all factors treated as fixed and tested over MS Error. No treatments were applied in 1999, thus foliage consumed by caterpillars reflected treatments applied in the previous three years. Symbols are *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, † = < 0.10.

			PUPAL MASS						DEVELOPMENT TIME					
			Females			Males			Females			Males		
Year	Species	Effect	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
1999	*M. disstria*	Block	3	0.003	4.1 *	3	0.0011	3.6 †	3	0.14	0.2	3	0.30	1.5
		Defol	1	0.001	1.4	1	0.0006	2.0	1	0.91	1.6	1	0.21	1.1
		Fert	1	0.007	9.6 *	1	0.0000	0.0	1	0.25	0.5	1	0.02	0.1
		Defol x Fert	1	0.001	1.8	1	0.0003	0.9	1	0.03	0.1	1	0.00	0.0
		Error	9	0.001		9	0.0003		9	0.57		9	0.20	
	O. leucostigma	Block	3	0.006	1.9	3	0.0001	1.0	3	1.89	1.5	3	0.32	0.9
		Defol	1	0.002	0.7	1	0.0002	1.9	1	0.02	0.0	1	0.39	1.2
		Fert	1	0.012	3.7 †	1	0.0001	1.6	1	0.36	0.3	1	0.00	0.0
		Defol x Fert	1	0.000	0.0	1	0.0001	1.7	1	0.49	0.4	1	0.02	0.1
		Error	9	0.003		9	0.0001		9	1.30		9	0.34	
	C. inclusa	Block	3	0.000	0.2	3	0.0002	0.7	3	0.18	0.2	3	0.46	1.5
		Defol	1	0.004	5.5 *	1	0.0014	4.4 †	1	2.83	3.9 †	1	0.53	1.7
		Fert	1	0.000	0.0	1	0.0001	0.2	1	0.05	0.1	1	2.30	7.4 *
		Defol x Fert	1	0.000	0.2	1	0.0001	0.3	1	0.62	0.8	1	1.13	3.6 †
		Error	9	0.001		9	0.0003		9	0.73		8	0.31	

defoliated treatments in 1998 (Fig 12, Table 17). Using regression, I estimated that defoliation reduced fecundity by 9% (30 eggs/female). There were no main or interactive effects of fertilizer on pupal mass. Both male and female poplar tent maker took significantly longer to complete the larval period on trees which had been defoliated. The performance of poplar tent maker in 1999 was surprising because female pupal masses were significantly heavier and males marginally heavier on previously defoliated trees than on control trees (Figure 13, Table 18). Female development time was marginally faster on control trees. Observations suggested that this species may prefer vigorous shoots. The proliferation of rapidly growing epicormic shoots on surviving trees in previously defoliated plots may account for the apparent positive effects of the treatment history on this species. There was no effect of fertilizer on either sex in 1999.

Big poplar sphinx initiates feeding in July after previously defoliated trees have refoliated. In this study, big poplar sphinx did not exhibit sexual dimorphism in pupal mass. Males and females were pooled for analysis. I found that defoliation earlier in the season had significant, negative effects on big poplar sphinx pupal mass in 1998 (Figure 12, Table 17). Although not estimated in this study, the declines in pupal mass would likely translate to significant reductions in fecundity because the adults do not feed. In addition, development time of big poplar sphinx was lengthened significantly on trees defoliated earlier in the season (Figure 12, Table 17. I was unable to obtain sufficient numbers of big poplar sphinx to use in bioassays in 1999.

Fall webworm had the latest feeding phenology of any species used in my bioassays, beginning near the end of July. I found no effect of either defoliation or fertilizer treatment on male or female pupal mass for this species nor was there an effect on female

development time (Figure 12, Table 17). Male development time was marginally faster on previously defoliated trees although the difference was less than one day. Fall webworm was not assayed in 1999.

White-marked tussock moth was used in bioassays for the first time in 1999. In Michigan this species is bivoltine having a spring and summer generation. I used first generation larvae only and found that previous defoliation did not significantly affect pupal masses of either males or females (Figure 13, Table 18). Female pupal mass was marginally heavier on trees that had received fertilizer in previous years. There were no effects of defoliation and fertilizer on development time of white-marked tussock moth.

As with gypsy moth above, correlation analysis was used to assess the association of individual phytochemical components with pupal mass and development time of the insects (Table 19). Pupal mass of female forest tent caterpillars was positively correlated with foliar nitrogen in 1998 and 1999. Male forest tent caterpillar pupal mass was marginally correlated with nitrogen in both years. Pupal masses of male and female poplar tent makers as well as big poplar sphinx were correlated with nitrogen levels in 1998 although the significance was only marginal. Pupal mass of fall webworm was not correlated with foliar nitrogen levels. In 1998, development times were longer when nitrogen was low for both sexes of forest tent caterpillar and for female poplar tent makers. Nitrogen was also negatively correlated with development time of male poplar tent makers although the effects were only marginal. There was no correlation between development time of any other species and foliar nitrogen in 1999.

Water content was correlated with pupal masses of female forest tent caterpillar, and male poplar tent makers in 1998 (Table 19). Water was also correlated with male poplar

Table 19. Pearson correlations between male and female pupal mass and development time for forest tent caterpillar (*M. disstria*), poplar tent maker (*C. inclusa*), big poplar sphinx (*P. modesta*), fall webworm (*H. cunea*) and white-marked tussock moth (O. leucostigma) and concentrations of phytochemicals in leaves sampled from the plots. Correlations were done between the leaf sample that was collected at the most phenologically relevant time for the insect species involved. Abbreviations are N = nitrogen, H20 = water, TOTPHN = total phenolics, CNDTAN = condensed tannins, PHENGLY = pooled phenolic glycosides, CINNAM = cinnamic acid, CATECH = catechins, LUTEOL = luteolin, QUERTN = quercetin glycosides, and MYRCTN = myricetin glycosides. Bold type *p*-values are $p < 0.05$, italicized *p*-values are $0.05 < p < 0.10$.

(a) Pupal Mass			N		H2O		TOTPHN		CNDTAN		PHENGLY		CINNAM		CATECH		LUTEOL		QUERTN		MYRCTN	
Year	Species	Sex	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
1998	*M. disstria*	M	0.43	*0.10*	0.35	0.18	-0.33	0.21	-0.62	**0.01**	0.27	0.35	-0.18	0.53	-0.04	0.88	0.14	0.63	0.42	0.14	0.08	0.78
		F	0.71	**0.01**	0.53	**0.04**	-0.42	0.11	-0.78	**0.01**	0.36	0.20	-0.36	0.20	0.05	0.86	-0.02	0.96	0.30	0.30	-0.15	0.65
	C. inclusa	M	0.46	*0.08*	0.68	**0.01**	-0.50	**0.05**	-0.80	**0.01**	0.03	0.92	-0.45	0.11	-0.32	0.27	-0.17	0.55	0.07	0.81	-0.53	**0.05**
		F	0.47	*0.07*	0.40	0.13	-0.38	0.15	-0.49	**0.05**	0.08	0.77	-0.30	0.29	0.02	0.94	-0.17	0.55	0.01	0.98	-0.05	0.87
	P. modesta	M/F	0.47	*0.07*	0.42	0.11	-0.44	*0.09*	-0.63	**0.01**	-0.23	0.39	-0.46	*0.07*	-0.62	**0.01**	-0.53	**0.03**	-0.51	**0.05**	-0.48	*0.06*
	H. cunea	M	0.11	0.69	0.11	0.67	-0.27	0.31	-0.32	0.22	-0.02	0.95	-0.10	0.72	-0.29	0.39	-0.11	0.66	0.02	0.94	-0.62	**0.01**
		F	0.35	0.19	0.19	0.48	-0.26	0.32	-0.39	0.14	0.10	0.70	0.08	0.76	-0.19	0.49	0.02	0.93	0.28	0.29	-0.51	**0.04**
1999	*M. disstria*	M	0.47	*0.07*	0.39	0.13	-0.03	0.72	-0.22	0.39	•	•	•	•	•	•	•	•	•	•	•	•
		F	0.43	*0.10*	-0.23	0.38	-0.39	0.13	-0.37	0.15	•	•	•	•	•	•	•	•	•	•	•	•
	O. leucostigma	M	0.03	0.91	0.14	0.59	-0.08	0.76	-0.06	0.80	•	•	•	•	•	•	•	•	•	•	•	•
		F	0.16	0.56	-0.21	0.43	-0.12	0.64	-0.23	0.39	•	•	•	•	•	•	•	•	•	•	•	•
	C. inclusa	M	-0.14	0.60	-0.58	**0.02**	0.28	0.28	0.37	0.17	•	•	•	•	•	•	•	•	•	•	•	•
		F	0.02	0.92	-0.34	0.19	0.22	0.41	0.36	0.16	•	•	•	•	•	•	•	•	•	•	•	•

(b) Development Time			N		H2O		TOTPHN		CNDTAN		PHENGLY		CINNAM		CATECH		LUTEOL		QUERTN		MYRCTN	
Year	Species	Sex	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
1998	*M. disstria*	M	-0.58	**0.02**	-0.17	0.52	0.16	0.56	0.40	0.13	-0.37	0.17	0.45	*0.08*	-0.68	0.80	-0.33	0.21	-0.47	*0.07*	-0.47	*0.07*
		F	-0.61	**0.02**	-0.20	0.46	0.26	0.34	0.48	**0.06**	-0.66	**0.01**	0.14	0.60	-0.34	0.21	-0.41	0.12	-0.58	**0.02**	-0.67	**0.01**
	C. inclusa	M	-0.46	*0.08*	-0.75	**0.01**	0.73	**0.01**	0.84	**0.01**	0.02	0.95	0.66	**0.01**	0.52	**0.04**	0.40	0.13	0.21	0.43	0.12	0.67
		F	-0.51	**0.05**	-0.69	**0.01**	0.62	**0.01**	0.87	**0.01**	-0.10	0.72	0.54	**0.03**	0.42	0.11	0.10	0.71	0.14	0.61	-0.16	0.55
	P. modesta	M/F	-0.23	0.40	0.64	**0.01**	-0.11	0.69	0.12	0.66	0.25	0.36	0.12	0.67	0.08	0.78	0.13	0.63	0.25	0.36	0.31	0.24
	H. cunea	M	0.15	0.56	-0.42	0.11	-0.26	0.32	-0.35	0.19	-0.24	0.36	-0.41	0.12	-0.35	0.18	-0.34	0.20	-0.33	0.21	-0.58	**0.02**
		F	0.13	0.62	0.00	0.98	0.11	-0.68	0.03	0.90	0.32	0.22	0.08	0.78	-0.28	0.29	-0.42	0.11	-0.43	*0.10*	-0.59	**0.02**
1999	*M. disstria*	M	-0.02	0.96	-0.04	0.88	-0.10	0.70	-0.04	0.86	•	•	•	•	•	•	•	•	•	•	•	•
		F	0.04	0.70	0.21	0.42	0.23	0.42	0.30	0.27	•	•	•	•	•	•	•	•	•	•	•	•
	O. leucostigma	M	0.03	0.91	0.02	0.97	0.20	0.47	0.19	0.49	•	•	•	•	•	•	•	•	•	•	•	•
		F	0.16	0.57	0.05	0.83	0.04	0.86	0.01	0.96	•	•	•	•	•	•	•	•	•	•	•	•
	C. inclusa	M	0.07	0.80	-0.52	**0.05**	-0.07	0.80	0.05	0.96	•	•	•	•	•	•	•	•	•	•	•	•
		F	-0.20	0.44	-0.36	0.18	0.20	0.46	0.28	0.30	•	•	•	•	•	•	•	•	•	•	•	•

tent maker pupal mass in 1999. Pupal masses of the late-feeding species, big poplar sphinx and fall webworm, were not affected by water content. Correlation analysis suggested that development time of big poplar sphinx was faster when feeding on leaves with higher water content, as was development time of both sexes of poplar tent maker in 1998. Only the development of male poplar tent makers varied with water content in 1999.

Male poplar tent maker and big poplar sphinx pupal mass were negatively correlated with total phenolics in 1998, although only marginally in the case of big poplar sphinx (Table 19). In fact, the relationship between total phenolics and pupal mass had a negative sign for all species in both years except for poplar tent maker in 1999. The development times of both male and female poplar tent maker increased with total phenolic concentration in 1998. The pupal masses of female forest tent caterpillar and big poplar sphinx were negatively correlated with condensed tannins in 1998. There was a marginal association between female forest tent caterpillar development time and condensed tannins and a significant relationship for condensed tannins and development time for both sexes of poplar tent maker in 1998. However, there was no correlation between condensed tannins and pupal masses of fall webworm in 1998 or for any species in 1999.

Other phenolic constituents in poplar leaves had variable and species specific effects on pupal mass and development time. Of the species assayed, only pupal mass of big poplar sphinx was consistently associated with concentrations of these compounds (Table 19). Cinnamic acid, catechins, luteolin, quercetin, and myricetin were all negatively correlated with pupal mass of this herbivore, although the relationship was of only

marginal significance in some cases. Of the minor phenolics, myricetin showed the strongest correlation with herbivore performance, having a negative association with pupal mass of male poplar tent maker, big poplar sphinx, and both sexes of fall webworm. This was the only compound I measured that correlated either positively or negatively with pupal mass of fall webworm. Forest tent caterpillar development time was sensitive to phenolic glycosides, myricetin-glycosides, and quercetin-glycosides, which all negatively correlated with female development time. Male development time was also negatively correlated with myricetin and quercetin although only marginally whereas cinnamic acid exhibiting a positive correlation. Decreases in the development time of male and female fall webworm were associated with increasing myricetin concentrations.

DISCUSSION

Effects of defoliation-induced changes in phytochemistry on insect herbivores

I conducted laboratory bioassays using gypsy moth in each of the four years of the study, and in addition to gypsy moth, used five other species over the last two years of the study. In 1996 gypsy moth pupal mass was unchanged among treatments despite defoliation-induced increases in total phenolics and condensed tannins. In contrast, gypsy moth fecundity was reduced by 12% by defoliation in 1997 and by 14% in 1998. The fecundity of forest tent caterpillar, poplar tent maker, and big poplar sphinx was also reduced in 1998 in the defoliation plots by 9%, 9%, and 10%, respectively. The performance of gypsy moth and three other species assayed in 1999 were unaffected by three consecutive years of previous defoliation.

The complete lack of significant negative effects on herbivores in 1999 suggests that RIR rather than DIR was determining foliar quality in these poplars in 1997 and 1998. However there was considerable variation in the strength of RIR on individual herbivore species both within a season and among years. In the first year of the study, large increases in total phenolics and condensed tannins had no effect on gypsy moth fecundity or female development time and only minimal effects on male development time. In contrast, partial defoliation has had significant effects on gypsy moth in other experiments using poplars. Havill and Raffa (1999) compared RIR induced by gypsy moth feeding, the elicitor jasmonic acid, and mechanical damage to 12 poplar cultivars. Not only did the type of damage influence induction, the strength of the induced resistance on gypsy moth varied by as much as 72% among different cultivars. Similarly, growth rates of early instar forest tent caterpillar were three times higher on undefoliated poplars than on trees partially defoliated (Robison and Raffa 1997). Studies using other tree species have also shown significant RIR effects on gypsy moth. On red oak with current year defoliation of 10-58%, Rossiter et al. (1988) found that female gypsy moth pupal masses were reduced by as much as 20%. Furthermore, the largest portion of the variance in gypsy moth fecundity was explained by the concentration of phenolics which in turn, increased with defoliation. Data from natural populations of gypsy moth also suggest that RIR can reduce the pupal mass and fecundity of gypsy moths while lengthening development times. Relative to an undefoliated site, pupal mass was reduced by 22% for caterpillars fed foliage from a site with a single year of defoliation and 32% from a site with one year of previous and one year of current defoliation (Lance et al. 1991).

There are a number of possibilities why I did not see any effect of elevated levels of phenolics on gypsy moth in 1996. Densities of gypsy moth were relatively low in 1996 compared to 1997 and 1998. It is possible that the negligible feeding damage by early instars may have been insufficient to induce any changes. Subsequent feeding by large larvae may have triggered the induced response, but too late to have any significant impact on growth. The data of Schultz and Baldwin (1982) indicates that tannin levels in oaks reach a peak after gypsy moth has finished feeding. Similarly, D'Amico et al. 1998 found no difference in tannin levels between defoliated and undefoliated oaks in May and June when gypsy moth are actively feeding, although significant differences were detected later in the season. My short-term bioassays in 1997 and 1998 indicate that growth rates of late instars were not affected by the defoliation treatments because they were able to compensate for reduced leaf quality by increasing consumption rates. Havill and Raffa (1999) showed that feeding damage to *P. × euramericana* "Eugeneii", the same cultivar used in my experiments, reduced growth rates of laboratory reared second instar gypsy moth by 44%, suggesting that larval ontogeny may influence susceptibility to secondary compounds.

Similar variability in RIR response to defoliation is apparent in other *Populus* species. On aspen, Lindroth and Kinney, (1998) showed that herbivory by forest tent caterpillar elicited a significant increase in condensed tannins, but that most of the increase came late in the larval period when caterpillars had nearly completed their development. As a result, there was no significant effect on the growth of the caterpillars. In contrast, Parry et al. 2000, found that current year defoliation of aspen significantly reduced growth, pupal mass of both sexes, and fecundity of forest tent caterpillar. They used much higher

defoliation levels (ca. 80% vs. 30%) than did Lindroth and Kinney (1998) suggesting that induction of RIR may be density dependent and that the timing of induction mediates the outcome of experiments. If the timing of induction also determines the effects on gypsy moth, then the significant reductions in gypsy moth fecundity in 1997 and 1998 may be due to increased levels of defoliation achieved in those years relative to 1996. Because densities of larvae were much higher and significant damage occurred earlier, induction could have occurred at an earlier point in the season thus affecting the more sensitive early instars.

A more parsimonious explanation for the lack of effects on gypsy moth in 1996 is that by themselves, total phenolics and condensed tannins play little role in determining gypsy moth performance. This explanation seems contradictory given the strong negative correlations between gypsy moth pupal mass and the concentrations of total phenolics and condensed tannins in 1997 and 1998. In those two years however, nitrogen levels were significantly lower in the leaves of defoliated trees. Thus, the performance of gypsy moth in 1997 and gypsy moth and the other species assayed in 1998 may reflect a response to foliar nitrogen concentration. This suggests that the negative correlations between caterpillar performance and total phenolics or condensed tannins are spurious. A number of studies have shown that nitrogen and condensed tannins are themselves negatively correlated (see Tuomi et al. 1990). In 1999, levels of foliar nitrogen in the previously defoliated plots were not different from controls, and there were no negative effects of treatments on the four species assayed. The lack of any effects on larval performance in 1999 occurred despite total phenolic and condensed tannin levels in trees from previously defoliated plots that were as high as the concentrations in 1997 and

1998. This strongly suggests that induced secondary phenolics were not substantially influencing the performance of the caterpillars in this experiment.

The lack of a direct effect of condensed tannins on herbivore performance does not necessarily mean that they had no biological activity. Negative correlations between pupal mass and condensed tannins were higher than were positive correlations between foliar nitrogen and pupal mass for all species assayed in 1998. This suggests that when nitrogen levels vary as in 1997 and 1998, condensed tannins have greater negative effects on herbivore performance than when nitrogen levels were more uniform among treatments, as in 1999. Bryant et al. (1993) describe a similar relationship between nitrogen and condensed tannins on the performance of the lepidopteran *Rheumaptera hastata* feeding on paper birch. Condensed tannins were highly negatively correlated with pupal masses of *R. hastata* whereas nitrogen had the opposite effect. They concluded that condensed tannins were the most important determinant of the induced-resistance they found in birch because in laboratory bioassays, artificially increasing tannin levels reduced performance of the larvae. However, they did not vary the nitrogen content simultaneously, which may have better elucidated the relationship between condensed tannins, nitrogen, and insect growth. Bryant et al. (1987) varied the concentration of condensed tannins and nitrogen in artificial diets fed to large aspen tortrix. The magnitude of the tannin effects was greater on the low nitrogen diet suggesting that tannins may interact with dietary nitrogen. Low nitrogen levels have been found to exacerbate the activity of other phenolic compounds (Lindroth and Bloomer 1990). In the study, phenolic glycosides were more detrimental to forest tent caterpillars when they

were reared on diets low in nitrogen. Interactions between secondary phenolics and foliar nitrogen may be a fruitful area of future research.

My study does suggest that the principal induced-response in poplars to herbivory has little direct effect on several of its herbivores. This raises interesting evolutionary questions about the actual function of condensed tannins. In other *Populus* spp. such as aspen, condensed tannins have also been shown to have little or no effect on herbivorous insects. Constitutive levels of condensed tannins do not affect forest tent caterpillar and gypsy moth growth (Hemming and Lindroth 1995, Hwang and Lindroth 1997). Condensed tannins exhibit major differences in structure among tree species and also vary considerably in their antiherbivore activity (Ayres et al. 1997). An assay of six insect herbivores against condensed tannins from 16 woody plants found that condensed tannins had significant effects in only eight of the 45 insect-tannin combinations that were run (Ayres et al. 1997), suggesting that as a resistance mechanism, tannins have limited utility.

Not all studies have shown effects of DIR following defoliation. The performance of winter moth feeding on oak and apple was found to decrease with increasing levels of current year defoliation. However, performance on oak was positively correlated with previous years defoliation, at least across a range of 10-50% (Roland and Myers 1987). In the same study, higher defoliation of apple in the previous year significantly decreased the pupal mass of winter moth. In natural gypsy moth populations, Lance et al. (1991) found that 70% defoliation of red oak had little effect on gypsy moth pupal mass or development time in the following year. Current year defoliation was much more important in reducing pupal mass, although the site with the lowest pupal masses had

experienced both current and previous defoliation. On red alder, severe (>70%) defoliation by western tent caterpillar had no effect on larval performance in the following year. Similarly, Harrison (1995) found that pupal mass or fecundity of western tussock moths was not reduced on bush lupines that had been completely defoliated in the previous year.

Most experimental tests of DIR have consisted of a single year's defoliation followed by assays in the following year or years. However, it has been suggested that the cumulative effects of successive defoliation may have larger effects than a single event (Haukioja et al. 1988). Many natural insect outbreaks partially or completely defoliate trees for at least two years in succession, indicating that longer studies may be more realistic. Some studies have shown that there may indeed be cumulative effects of sustained defoliation on herbivores. Werner (1979) found that additional years of defoliation had additive effects on mortality of spear-marked black moth. Similarly defoliation of black oak for three consecutive years reduced gypsy moth pupal masses by increasing amounts in each successive year presumably because of long term depletions in foliar nitrogen (Walton and Wallner 1979, Valentine et al. 1983).

A few other studies have also experimentally determined the effects of cumulative defoliation on insect performance. The results of Clausen et al. (1991) are difficult to interpret. Treatments of one, two, or three consecutive years of defoliation were applied to aspen, and the performance of large aspen tortrix fed foliage from these trees compared with insects fed foliage from undefoliated controls. Unfortunately, the researchers reared tortix larvae on the refoliated leaves of the aspen, which is not phenologically relevant because the caterpillars normally initiate feeding at bud break

and complete their development on the spring leaves (Parry et al. 1997). Thus, the instars (2-5) used in their bioassay would never encounter reflush foliage in nature. The large negative effects on large aspen tortix pupal mass are interesting from a physiological standpoint but do not reveal anything about the effects of defoliation on populations of this insect. Research with mountain birch has shown that the effects of two consecutive years of defoliation were not significantly greater than a single defoliation (Kaitaniemi et al. 1999b). They found no difference in the fecundity of two defoliators, autumnal moth and winter moth, *Opherophtera brumata*, reared on foliage from plots suffering 25% and 75% defoliation, or two years of 75% defoliation. Similarly, four consecutive years of high defoliation (>70%) did not reduce forest tent caterpillar fecundity more than a single year of high defoliation in two clones of trembling aspen (Parry et al. 2000), although there was a trend towards lower performance on trees with the highest cumulative defoliation. My study also showed that the effects of repeated defoliation events did not elicit any additional response in the poplars beyond that occurring in a single season.

Effects of defoliation on tree growth and phytochemistry

The CNB hypothesis predicts that under conditions where growth but not photosynthesis is limited by nutrient deficiencies, excess carbon will be allocated to carbon-based secondary compounds such as phenolics (e.g., Bryant et al. 1983, Tuomi et al. 1984, 1990). The ability of trees to recover nitrogen after defoliation is contingent upon both species-specific compensatory responses and on environmental constraints such as resource availability (Tuomi 1990). Carbon-based secondary compounds are predicted to remain elevated until a tree's nitrogen pool has recovered to predefoliation levels, a process that may take several years in nutritionally poor environments (Tuomi et al.

1984, 1990, 1991, Bryant et al. 1991). Under these conditions, the application of nitrogen fertilizer to trees suffering defoliation should mitigate the production of secondary phenolics because carbon will be preferentially allocated to growth.

The effects of defoliation on foliar nitrogen within a growing season have received less attention than processes occurring in the subsequent year. I found lower nitrogen levels in poplar leaves from defoliated plots in both mid and late season samples in 1997 and 1998. Since early season nitrogen levels did not differ among treatments, current year herbivory was likely responsible for the change. Declines in nitrogen concurrent with defoliation, or later within the same growing season, have been shown in several other tree species (e.g., Faeth 1986, Robison and Raffa 1997, Lindroth and Kinney 1998, Roth et al. 1998). The mechanism behind nitrogen decreases in damaged leaves is not well understood. Some nitrogen may be leached from the leaves, or the tree may withdraw nitrogen from the damaged leaf as has been shown for other nutrients (Chapin 1980, Nef 1988). Reasons for nitrogen reductions in refoliated leaves are more obvious. When trees produce a second set of leaves from dormant buds, they must do so without the stored nitrogen pools available for leaf expansion during the spring, leading to lower levels of foliar nitrogen. The poplars in my study exhibited this pattern in 1997. However, in 1998, many of the trees were not defoliated to the extent that extensive reflush occurred and instead, retained damaged leaves for the duration of the season. Foliar nitrogen was significantly lower in these damaged leaves than in control foliage. This suggests that feeding damage and the physiological constraints of producing new leaves may both account for within season declines in nitrogen following defoliation.

Increases in secondary phenolic compounds are often correlated with decreases in foliar nitrogen (Tuomi 1990, Bryant et al. 1993). The two main secondary phenolic classes found in poplars are condensed tannins and phenolic glycosides, both products of the shikimic acid pathway (Clausen et al. 1991, Lindroth and Hwang 1996). I found that gypsy moth feeding induced significant within season changes in total phenolics and condensed tannins in each of the three years that defoliation treatments were applied. In 1996, the first year of the study, I recorded significant increases in total phenolics and condensed tannins. Within season increases in condensed tannins in response to defoliation have been shown repeatedly (e.g., Schultz and Baldwin 1982, Rossiter et al. 1988, Faeth 1988, Hunter and Schultz 1995, Roth et al. 1998). In my study, foliage samples collected in 1999, one year after the cessation of defoliation also had elevated levels of secondary phenolics in previously defoliated plots showing that induced effects on condensed tannins occur both within and across years. Given these results, the elevated levels of condensed tannins in 1997 and 1998 likely reflect defoliation effects from both current and past years, a result that has not been documented previously.

The addition of N-fertilizer reduced levels of both total phenolics and condensed tannins in defoliated plots in 1996 and 1998 as predicted by the CNB. In 1997, the year with most severe defoliation, there was no effect of fertilizer on condensed tannins in the defoliated plots. Why fertilizer did not decrease phenolic concentrations in 1997 as it did in other years is not clear. One possibility is that very high levels of defoliation and/or input of large amounts of nitrogen-rich frass in 1997 may have obscured any effects of fertilization. Alternatively, the near complete loss of leaf tissue could have reduced photosynthesis so much that carbon also became a limiting factor. There was no

difference in tree growth between unfertilized and fertilized trees in the defoliated plots in 1997 suggesting that nitrogen was not limiting growth in contrast to the growth differences in 1998 when defoliation was less severe.

In 1997, levels of condensed tannins in the refoliated leaves were lower than foliage from the control plots. During refoliation, defoliated trees must allocate substantial amounts of carbon to producing a new set of leaves, which are major carbon sinks. Furthermore, trees must also replenish the depleted carbohydrate stores prior to autumn leaf fall. Both of these processes are likely to take precedence over the production of carbon-demanding phenolics leading to lower concentrations of carbon-based secondary compounds such as condensed tannins. In marked contrast, total phenolics and condensed tannins were significantly higher in late season leaf samples taken from plots defoliated earlier in the season in 1998. This pattern may be due to lower defoliation levels and the retention of damaged leaves rather than a complete reflush as was seen in 1997. Thus the levels of condensed tannins in the late season samples in 1998 were similar to those taken at mid-season. Schultz and Baldwin (1982) and Faeth (1988) also found lower levels of condensed tannins in reflush leaves following complete defoliation of two different species of oak. As in my study, partially damaged oak leaves had higher levels of condensed tannins than either undamaged controls or refoliated leaves in late season samples.

The six minor phenolic compounds measured in my study had variable responses to defoliation and fertilization. In 1997, defoliation resulted in a significant increase in phenolic glycoside concentrations but this did not occur in 1998. In aspen, phenolic glycoside response to defoliation has also been less predictable than condensed tannins

with some studies showing short-term increases in phenolic glycosides while others did not (Clausen et al. 1991, Roth et al. 1998, Lindroth and Kinney 1998). Phenolic glycosides play a role in very rapid responses and may rapidly turnover (Clausen et al. 1989, 1991), making detection of changes highly dependent on the timing of sampling. Low levels of damage in hybrid poplar has been shown to induce rapid increases in total phenolics but the effects disappeared within five days (Baldwin and Schultz 1983), indicating that unless there is subsequent damage to reinforce the response, the induction is ephemeral. This was particularly evident in one control plot in 1997 where significant numbers of gypsy moth circumvented the sticky trap barriers. Despite relatively low herbivory levels, at least compared to the defoliation plots, total phenolics increased dramatically in foliage from this plot and were as high or higher than in the defoliation plots. Yet after the removal of the caterpillars, the levels of total phenolics had returned to control levels in samples taken one month later. At least with respect to fertilizer and light availability, phenolic glycosides do not respond as predicted by the CNB (Hemming and Lindroth 1999). In my study, fertilization resulted in increased phenolic glycoside levels in July leaves, opposing predictions of the CNB. On the other hand, I found that fertilization reduced levels of quercetin and myricetin in June 1997, as predicted by CNB although concentrations of the other minor phenolic compounds did not vary among treatments.

Processes driving DIR have received much more attention in trees than those responsible for RIR. Reduced nitrogen levels in the year or years following defoliation are thought to underlie DIR in deciduous trees (Tuomi et al. 1984, 1990). In forests growing in nutrient poor soil, the recovery of foliar nitrogen to predefoliation levels may

take several years as shown for mountain birch (Tuomi et al. 1984). Shorter recovery times have been recorded for defoliated birches in Alaska (Bryant et al. 1991) and willows in Finland (Tuomi et al. 1991), although two years were still required for nitrogen to return to control levels. Reductions in nitrogen in the year following defoliation have also been shown in black oak (*Quercus velutina* Lambert), gray birch (*Betula populifolia* Marshall), and red alder (*Alnus rubra* Bong) (Valentine et al 1983, Myers and Williams 1987). In contrast, I found that foliar nitrogen in previously defoliated poplars, while slightly depressed was not statistically different from levels in control leaves. This pattern was evident in May 1998 and June 1999 even though late season leaf samples from the previous year had markedly reduced nitrogen levels relative to control foliage. These data indicate that the poplars were able to rapidly replenish nitrogen pools prior to the start of the following growing season, which has not previously been shown for any tree species.

The rapid recovery of foliar nitrogen levels in the defoliated poplars is somewhat puzzling given that their rate of ammonium and nitrate uptake was only 33% of that in control trees even though there was little evidence of fine root mortality (Kosola et al. 2000). The management practices in this poplar plantation may provide a possible explanation for this pattern. Application of herbicide in the spring and summer to control understory vegetation resulted in essentially bare soil beneath the trees, in marked contrast to many natural forest systems that have extensive woody and herbaceous undergrowth. Thus, following decomposition, nitrogen in frass, greenfall, insect exuviae, and cadavers is available for uptake by trees free of competition from other vegetation. In contrast, nitrogen lost through defoliation may not be recovered by trees in natural

forests because competing herbaceous and woody plants in the understory intercept and utilize it. Furthermore, the poplars were growing in fields formerly used in a corn, alfalfa, and soybean rotation, and were undoubtedly richer in nitrogen than soils in boreal forests and subarctic soils where the strongest DIR responses have been observed. The tree growth data mirror the changes in foliar nitrogen levels. Despite reductions in diameter growth of ca. 11%, 50%, and 50% in 1996, 1997, and 1998 respectively, there was no difference in growth among previously defoliated and control trees in 1999. My results are congruent with the prediction of Tuomi et al. (1990) that recovery of foliar nitrogen to predefoliation levels would be most rapid for trees growing in nutritionally rich environments.

In contrast to the ephemeral effects on foliar nitrogen, strong across-year effects of defoliation on levels of total phenolics and condensed tannin levels were recorded. This was most pronounced in 1999, where condensed tannins remained 16% and 28% higher than control leaves in the previously unfertilized and fertilized defoliated trees, respectively. Similar differences in condensed tannin levels (10% and 22%) were evident in foliage sampled from the defoliated plots in May 1998. Since herbivory was minimal at the time of sampling, these differences reflect effects of treatments from the previous year. These results are not unexpected given that the return of phenolic concentration to levels found in control trees generally lags behind nitrogen by at least a year (Tuomi et al. 1991).

Variability in the induced responses of phenolic compounds to defoliation and fertilization both within and between years suggests that more than one mechanism may be governing the response. While condensed tannins responded as predicted to

defoliation and fertilization, other phenolic compounds did not. Outside of the phenolic glycosides, little is known about the majority of the other minor phenolic compounds identified from poplar foliage. It is possible that some of these biochemicals are involved in wound-specific responses and thus are unaffected by alterations in the nutritional environment of the tree. Tuomi et al. (1991) suggested that non-specific chemical changes caused by alterations of the carbon/nutrient balance and specific wound-induced resistance mechanisms may both be components of plant resistance to herbivory. Characterization of wound-specific responses in trees lags behind analogous research with herbaceous plants. A full understanding of induced-responses in trees will not be possible until the relationship between both nutritionally driven and wound specific responses has been identified.

In a seeming paradox, I view the results of my experiment as complimentary rather than contradictory to other studies on DIR. My study suggests that rather than a general response, the outcome of experiments is system specific and highly contingent upon environment. Tuomi et al. (1984, 1990) suggested that expression of DIR would be greatest in marginal habitats deficient in nitrogen. Because my experimental system is at the other end of a fertility continuum, a significant DIR response should not be expected. I note that in two well-designed studies where DIR responses were not detected (Harrison 1995, Rothman 1997), both were conducted on nitrogen fixing species. In these trees, nitrogen depletion is unlikely to occur unless defoliation occurred repeatedly for many years; thus any expression of DIR should be negligible.

Indirect effects of induced resistance on herbivores - Indirect interactions between induced resistance and the natural enemies of caterpillars are increasingly recognized as

important. For example, the induction of secondary compounds such as phenolics may interact with pathogens to either enhance or reduce mortality of the insect host. In a laboratory study, gypsy moth mortality from nuclear polyhedrosis virus (NPV) was negatively correlated with hydrolyzable tannin concentrations in oak leaves induced by defoliation (Hunter and Schultz 1993), presumably because tannins precipitate viral proteins, interfering with the infection process (Keating et al. 1990). Hunter and Schultz (1993) suggested that the interaction between secondary phenolics and pathogens could prolong gypsy moth outbreaks in oak-dominated forests. In field studies however, D'Amico et al. (1998) found no evidence that mortality of gypsy moth from NPV was reduced by defoliation on two species of oak. They attributed their findings to the lack of induction of tannins during the critical time period early in the season when most viral transmission between larvae occurs.

An aspect of the relationship between pathogens and tannins that has been hypothesized (e.g., Herms and Mattson 1992), but not explored in detail, is the effect of increased consumption rate on susceptibility to pathogen infection. Elevated consumption rates by gypsy moth and other species such as forest tent caterpillar are likely a generalized response to low nutritive levels in leaves (e.g., Slansky and Feeny 1977, Stockhoff 1992, Lindroth et al. 1997, Williams et al. 1998, Parry et al. 2000). If tannins interact negatively with NPV, decreases in susceptibility may be mitigated if the caterpillars simultaneously increase consumption rates on foliage with elevated phenolics, thus ingesting greater numbers of viral propagules and resulting in no net change in mortality. Such effects are unlikely in poplars as their condensed tannins only weakly reduce NPV mortality (Lindroth et al. 1999). The effects of DIR on pathogens has

received little attention despite Wallner's (1983) study showing increased gypsy moth susceptibility to NPV when reared on foliage from previously defoliated oak trees. Disparate effects of tannins on insect pathogens documented in previous studies suggest that the relationship between primary nutrients, inducible secondary allelochemicals, and pathogens is not yet understood and should be explored further.

Until recently, it was generally thought that herbivorous insect communities were not structured by competition (e.g., Hairston et al. 1960, Schoener 1983, Strong et al. 1984). Evidence has now accumulated suggesting that these earlier views were not entirely accurate. A comprehensive review found that interspecific competition between herbivorous insects occurred in more than 75% of the cases examined (Denno et al. 1995). Among chewing folivores, plant-mediated competitive interactions such as those driven by induced-resistance accounted for more than half of reported negative interactions between species. Early season defoliation may impact folivorous insect communities in two ways. First, the outbreak species competes directly with other species for foliage. Secondly, the feeding activity of an outbreak species may trigger induced-resistance that reduces the quality of remaining foliage for co-occurring species. Changes in host plant quality due to defoliation may persist thus affecting herbivores feeding later in the season. For example, defoliation of paper birch, sugar maple, and aspen by forest tent caterpillars induced both RIR and DIR, reducing the performance of gypsy moth larvae early in the season as well as the tiger swallowtail and second generation white-marked tussock moth feeding later in the year (Dankert et al. 1997). Similarly, Harrison and Karban (1986) found that early season feeding by an arctiid caterpillar on bush lupine reduced the quality of foliage for a tussock moth that initiated feeding later in the

growing season. Early season damage to oak leaves reduces the probability of herbivory later in the season (Hunter and Schultz 1995, Wold and Marquis 1997) although Faeth (1988) found that the effects on late season species depended on both the severity of the previous herbivory and the species examined.

Effects of induction may be perceived differently among different species of herbivores. There are two sides to the specificity of induction. Plants may perceive and react to herbivory caused by different species differently, and induced responses elicited by a single species may have variable effects on different species of herbivore (Agrawal 1999, 2000). Dietary specialization can mediate the outcome of the effects of induced responses on herbivores (Hartley and Lawton 1987, Bowers and Stamp 1993, Agrawal and Karban 1999). I predicted that the polyphagous and outbreak species in the study would be buffered to changes in poplar quality after defoliation, because substantive variation in the nutritional quality and biochemical profile of host plants is incumbent to these life-history strategies. Contrary to expectation, the pupal mass and/or fecundity of gypsy moth, forest tent caterpillar, poplar tent maker, and big poplar sphinx were reduced by similar magnitudes in 1998. Thus, uniform responses to changes in foliage occurred despite the disparate life histories represented among these species. One explanation is that all species were responding to changes in nitrogen rather than an induced secondary compound. Similar interspecific responses to a primary nutrient such as nitrogen is more probable than uniform responses to secondary compounds that may require the evolution of specific detoxification mechanisms. In contrast to my study, Agrawal (2000) found considerable variation in the effects of induced physical and biochemical resistance in wild radish (*Raphanus sativa*) among a guild of foliage-feeding caterpillars. The

differences in performance among species could not predicted by the degree of dietary specialization.

The only species not affected by my defoliation treatments in 1998 was fall webworm. This species is extremely polyphagous suggesting that it has evolved mechanisms to process leaves with wide variations in secondary compounds, toughness, water, and nutrient concentrations. Williams and Myers (1984) concluded that fall webworm benefits from early season defoliation, although they found no differences in pupal mass of larvae reared on undefoliated and previously defoliated trees at one site and only a 12 mg increase at a second site. Their interpretation is even more problematic because sexes in this dimorphic species were not analyzed separately and the insects rather than the experimenters selected the trees. Rather than improving foliar quality, I suggest that their results concur with mine: early season defoliation has no biologically meaningful effect on fall webworm performance.

I measured only the indirect effects of gypsy moth defoliation on other lepidopterans in the poplar-feeding community. In natural outbreaks, direct competition for foliage undoubtedly compounds the indirect effects on the folivorous community. Displacement from preferred hosts and habitats by defoliation may be associated with higher mortality, especially if the species relies on crypsis to avoid predation or parasitism (Heinrich 1993). The protective structures of leaf rolling and leaf mining species can be compromised by the feeding activities of defoliators. Hunter and Willmer (1989) showed that the free-feeding winter moth had a competitive advantage over *Tortrix viridana* when they co-occurred on oak because winter moth damaged the leaf-rolls essential for osmoregulation of tortrix. In several studies, leafminer survival was negatively correlated

with free-feeding caterpillar damage (Faeth 1988, West 1985). Gypsy moth defoliation has been shown to reduce abundance and/or species diversity of other lepidopterans in oak forests (Sample et al. 1996, Work and McCullough 2000), although the effects are relatively minor. It is unclear if indirect or direct competitive interactions, or some other process drive these changes.

Simulation models have suggested that induced resistance can regulate herbivore populations and drive cyclic dynamics without the influence of other density-dependent factors (Edelstein-Keshet and Rausher 1989, Underwood 1999). In Underwood's (1999) model, whether populations were regulated or cycled depended on both the decay rate of the resistance and the lag time to maximum strength of the induced-response. In forest systems at least, induced-responses appear unlikely to be capable of regulating insect populations by themselves. Only one study has looked at the effects of induced-resistance on the population growth rate of a defoliator. In the absence of natural enemies, Haukioja et al. (1988) calculated the per capita growth rate, r, for autumnal moth feeding on undefoliated trees to be 2.9 compared with 1.1 on trees defoliated the previous year. Although DIR decreased population growth rate significantly, by itself, it was not capable of preventing this population from growing. In natural systems, DIR interacts with other biotic and abiotic factors and its effects may be sufficient to slow population growth to the point where other time-lagged density-dependent agents such as pathogens or parasitoids can drive populations down.

Considerable knowledge has accumulated on the effects of DIR on herbivorous insects, albeit in relatively few systems. Lacking, however, are synthetic studies where the effects of DIR are measured relative to the contributions of other top-down and

bottom up regulating factors. Bylund (1995) attempted such a study using primarily historical data and demonstrated that delayed density-dependent parasitism was the most important factor correlated with fluctuations of autumnal moth populations in Sweden. Other factors including depletion of food, low winter temperatures, and induced-resistance contributed to population regulation. Over a three-year period, Virtanen and Neuvonen (1999) estimated the relative contribution of altitude, climate, host quality, and parasitoids on autumnal moth in Finland, and like Bylund (1995), concluded that parasitoids were more important than host plant quality in determining population dynamics. Although not looking explicitly at induced-resistance, Hunter et al. (1997) used time series analysis and data from supplementary experiments to determine the relative contributions of top-down and bottom-up forces to the population dynamics of two herbivores on English oak. This approach could be adapted for use with long-term data sets from other forest insects to look at the relative contribution of induced-responses and natural enemies to population dynamics. Unfortunately, in many of the systems where induced-resistance has been intensively studied, there is a dearth of information on other potential regulating factors. For example, the paper birch-black-marked spear moth system and the large aspen tortrix-trembling aspen system have been subjected to detailed research on induced-resistance mechanisms in Alaska, yet lacking entirely from these systems is any quantitative estimates of the impact of natural enemies. Given the intractability of studying outbreak species over the decades required to collect data on natural cycles, manipulative studies such as mine may be a useful tool to address these questions, especially if interactions with the third trophic level are incorporated into the experimental design.

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LITERATURE CITED

Agrawal, A.A. 2000. Specificity of induced-resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos* **89**: 493-500.

Agrawal, A.A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology* **80**: 1713-1723.

Agrawal, A.A. and R. Karban. 1999. Why induced defenses may be favored over constitutive strategies in plants. Pp. 45-61 in R. Tollrian and C.D. Harvell, eds, The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, New Jersey.

Ayres, M.P., T.P. Clausen, S.F. MacLean, Jr., A.M. Redman and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78**: 1696-1712.

Baldwin, I.T. and J.C. Schultz. 1983. Rapid-changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science* **221**: 277-279.

Baltensweiler, W. and A. Fischlin. 1988. The larch budmoth in the Alps. Pp. 331-351 in A.A. Berryman, ed, Dynamics of Forest Insect Populations. Patterns, Causes, and Implications. Plenum Press, New York.

Bate-Smith, E.C. and V. Rašper. 1969. Tannins of grain sorghum: luteforol (leucoluteolinidin) 1',4,4'5,7-pentahydroxyflavan. *Journal of Food Science* **34**: 203-209.

Bae, H.-E., T.A. McAllister, A.D. Muir, L.J. Yanke, K.A. Bassendowski and K.J. Cheng.1993. Selection of a method of condensed tannin analysis for studies with rumen bacteria. *Journal of Agricultural and Food Chemistry*. **41**: 1256-1260.

Bell, R.A., C.D. Owens, M. Shapiro and J.R. Tardif. 1981. Development of mass-rearing technology. Pp. 599-633 in C.C. Doane and M.L. McManus, eds, The Gypsy

Moth: Research Toward Integrated Pest Management. United States Department of Agriculture Technical Bulletin 1584.

Benz, G. 1974. Negative feedback by competition for food and space, and by cyclic induced changes in the nutritional base as regulatory principles in the population dynamics of the larch budmoth, *Zieraphera dineana* (Guenee) (Lepidoptera: Tortricidae). *Zietschrift fur Angewandte Entomologie* **76**: 196-228.

Bergey, D.R., G.A. Hoi and C.A. Ryan. 1996. Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. *Proceedings of the National Academy of Science* **93**: 12053-12058

Berryman, A.A., N.C. Stenseth and A.S. Isaev. 1987. Natural regulation of herbivorous forest insect populations. *Oecologia* **71**: 174-184.

Berryman, A.A. 1996. What causes population cycles of forest Lepidoptera? *Trends in Ecology and Evolution* **11**: 28-32.

Bowers, M.D. and N.E. Stamp. 1993. Effects of plant age, genotype, and herbivory on Plantago performance and chemistry. *Ecology* **74**: 1778-1791.

Bryant, J.P., F.S. Chapin, D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in realtion to vertebrate herbivory. *Oikos* **40**: 357-368.

Bryant, J.P. J. Tuomi and P. Niemela. 1988. Environmental constraints of constitutive and long-term inducible defenses in woody plants. Pp. 367-389 in K.G. Spencer, ed, Chemical Mediation of Coevolution. Academic Press, San Diego, CA.

Bryant, J.P., K. Danell, F. Provenza, P.B. Riechardt, T.A. Clausen and R.A. Werner. 1991. Effects of mammal browsing on the chemistry of deciduous woody plants.

Pp. 135-154 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Bryant, J.P., P.B. Reichardt, T.P. Clausen and R.A.Werner. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. *Ecology* **74**: 2072-2084.

Bryant J.P., T.P. Clausen, P.B. Reichardt, M.C. McCarthy and R.A.Werner 1987. Effects of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortrix (*Choristoneura conflictana* (Walker)). *Oecologia* **73**: 513-517.

Bylund, H. 1995. Long-term interactions between the autumnal moth and mountain birch: the role of resources, competitors, natural enemies, and weather. PhD Dissertation. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Chapin, F.S. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**: 233-260.

Chapin, FS and R.A. Kedrowski. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology* **64**: 376-391.

Clausen, T.P., P.B. Reichardt, J.P. Bryant and R.A. Werner. 1991. Long-term and short-term induction in quaking aspen: related phenomena? Pp. 71-83 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Constabel, P. 1999. A survey of herbivore inducible proteins and phytochemicals. Pp. 137-166 in A.A. Agrawal, S. Tuzin and E. Bent, eds, Induced plant defenses against Pathogens and herbivores. APS Press, St. Paul, Minnesota.

D'Amico, V., J.S. Elkinton, G. Dwyer, R.B. Willis and M.E. Montgomery. 1998. Foliage damage does not affect within-season transmission of an insect virus. *Ecology* **79**: 1104-1110.

Dankert, B.A., D.A. Herms, D. Parry, J.M. Scriber and L.A. Haas. 1997. Mediation of interspecific competition between folivores through defoliation induced changes in host quality. Pp. 71-88 in A. Raman, ed, Ecology and Evolution of Plant Feeding Insects in Natural and Man-Made Environments. International Scientific Publications. New Delhi, India

Denno, R.F., M.S. McClure and J.R. Ott. 1995. Interspecific interactions in phytophagous insects: competition revisited and resurrected. *Annual Review of Entomology* **40**: 297-331.

Diss A.L, J.G. Kunkel, M.E. Montgomery and D.E. Leonard. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. *Oecologia* **106**: 470-477.

Edelstein-Keshet, L. and M.D. Rausher. 1989. The effects of inducible plant defenses on herbivore populations. I. Mobile herbivores in continuous time. *The American Naturalist* **133**: 787-810.

Faeth, S.H. 1986. Indirect interactions between temporally-separated herbivores mediated by the host plant. *Ecology* **67**:479-494.

Faeth S.H. (1988). Plant mediated interactions between seasonal herbivores: Enough for evolution or coevolution. Pp. 391-414 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Feeny, P.P. 1970. Changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**: 565-581.

Ginzburg, L.R. and D.E. Taneyhill. 1994. Population-cycles of forest Lepidoptera – a maternal effects hypothesis. *Journal of Animal Ecology* **63**: 79-92.

Gordon, H.T. 1968. Quantitative aspects of insect nutrition. Zoology 8: 131-138.

Hairston, N.G., F.E. Smith and L.B. Slobodkin. 1960. Community structure, population control and competition. *The American Naturalist* **115**: 354-366.

Harper, J.L. 1977. Population Biology of Plants. Academic Press, San Diego.

Harrison, S. 1995. Lack of strong induced or maternal effects in tussock moths *(Orgyia vetusta)* on bush lupine (*Lupinus arboreus*). *Oecologia* **103**: 343-348.

Harrison, S. and R. Karban. 1986. Effects of an early season folivorous moth on the success of a later-season species mediated by a change in the quality of a shared host, *Lupinus arboreus*. *Oecologia* **69**: 354-359.

Hartley, S.E. and J.H. Lawton. 1987. Effects of different types of damage on the chemistry of birch foliage and the response of birch feeding insects. *Oecologia* **74**: 423-437.

Haukioja, E. 1980. On the role of plant defenses in the fluctuation of herbivore populations. *Oikos* **35**: 202-213.

Haukioja, E. 1982. Inducible defenses of white birch to a geometrid defoliator, *Epirrita autumnata*. Proceedings of the 5th International Symposium on Insect-Plant Relationships, Wageningen.

Haukioja, E. and P. Niemela. 1977. Retarded growth of a geometrid larvae after mechanical damage of leaves of its host tree. *Annales Zoologici Fennici* **14**: 48-52.

Haukioja, E. and S. Neuvonen. 1985. Induced-long term resistance of birch foliage against defoliators: defensive or incidental. *Ecology* **66**: 1303-1308.

Haukioja, E. and S. Neuvonen. 1987. Insect population dynamics and the induction of plant resistance: the testing of hypotheses. Pp. 411-432 in P. Barbosa and J.C. Schultz, eds, Insect Outbreaks. Academic Press, San Diego.

Haukioja, H., P. Niemela and S. Sirena. 1985. Foliage phenols and nitrogen in relation to growth, insect damage, and the ability to recover after defoliation, in the mountain birch *Betula pubescens ssp. tortuosa*. *Oecologia* **65**:214-222.

Haukioja, E., S. Neuvonen, S. Hanhimaki and P. Niemela. 1988. The autumnal moth in Fennoscandia. Pp. 163-178 in A.A. Berryman, ed, Dynamics of Forest Insect Populations. Patterns, Causes, and Implications. Plenum, New York.

Havill, N.P. and K.F. Raffa. 1999. Effects of elicitation treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior. *Oecologia* **120**: 295-305.

Heinrich, B. 1993. How avian predators constrain caterpillar foraging. Pp. 224-247 in N. E. Stamp and T. M. Casey, eds, Caterpillars: Ecological and Evolutionary Constraints on Foraging. Chapman and Hall, New York.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants – to grow or defend. *Quarterly Review of Biology* **67**: 283-335.

Hemming J.D.C., R.L. Lindroth. 1995. Intraspecific variation in aspen phytochemistry – effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* **103**: 79-88.

Hunter, A.F. 1995. Ecology, life-history, and phylogeny of outbreak and non-outbreak species. Pp. 41-64 in N. Cappuccino and P.W. Price, eds, Population Dynamics: New Approaches and Synthesis. Academic Press, New York.

Hunter, M.D., and P.G. Willmer. 1989. The potential for interspecific competition between 2 abundant defoliators on oak – leaf damage and habitat quality. *Ecological Entomology* **14**: 267-277.

Hunter, M.D. and J.C. Schultz. 1993. Induced plant defenses breached? Phytochemical induction protects an herbivore from disease. *Oecologia* **94**: 195-203.

Hunter, M.D. and J.C. Schultz. 1995. Fertilization mitigates chemical induction and herbivore responses within damaged oak trees. *Ecology* **76**: 1226-1232.

Hunter MD, G.C. Varley and G.R. Gradwell. 1997. Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: A classic study revisited. *Proceedings of the National Academy of Science* **94**: 9176-9181.

Hwang, S.Y. and R.L. Lindroth. 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* **111**: 99-108.

Julkunen-Tiitto, R, M. Rousi, J.P. Bryant, S. Sorsa, M. Keinanen and H. Sikanen. 1996. Chemical diversity of several Betulaceae species: Comparison of phenolics and terpenoids in northern birch stems. *Trees-Structure and Function* **11**:16-22.

Kaitaniemi, P. K. Ruohomaki, V. Ossipov, E. Haukioja and K. Pihlaja. 1998. Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. *Oecologia* **116**: 182-190.

Kaitaniemi, P., K. Ruohomaki, T. Tammaru and E. Haukioja. 1999a. Induced resistance of host tree foliage during and after natural insect outbreak. Journal of Animal *Ecology* **68**: 382-389.

Kaitaniemi, P., S. Neuvonen and T. Nyyssonen.1999b. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. *Ecology* **80**: 524-532.

Karban, R. and I.T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago, Illinois.

Kause A, V. Ossipov, E. Haukioja, K. Lempa, S. Hanhimaki and S. Ossipova. 1999. Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia* **120**:102-112.

Keating, S.T., M.D. Hunter and J.C. Schultz. 1990. Leaf phenolic inhibition of gypsy moth nuclear polyhedrosis virus: role of polyhedral inclusion body aggregation. *Journal of Chemical Ecology* **16**: 1445-1457.

Koricheva J, S. Larsson, E. Haukioja and M. Keinanen. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* **83**: 212-226.

Kosola, K.R., D.I. Dickmann, E.A. Paul and D. Parry, 2000. Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates and root demography of poplars. *Oecologia*. **In Press**.

Krause, S.C. and K.F. Raffa. 1992. Comparison of insect, fungal, and mechanically induced defoliation of larch – effects on plant productivity and subsequent host susceptibility. *Oecologia* **90**: 411-416.

Lance, D.R., J.S. Elkinton and C.P. Schwalbe. 1986. Feeding rhythms of gypsy moth larvae: effect of food quality during outbreaks. *Ecology* **67**: 1650-1654.

Lance, D.R., J.S. Elkinton and C.P. Schwalbe. 1991. Responses of gypsy moth larvae (Lepidoptera: Lymantriidae) to foliage of oaks from naturally infested sites on Cape Cod, Massachusetts. *Journal of Entomological Science* **26**: 214-222.

Liebhold, A.M., K.W. Gottschalk. R.-M. Muzika, M.E. Montgomery, R. Young, K. O'Day and B. Kelley. 1995. Suitability of North American tree species to the gypsy moth: A summary of field and laboratory tests. United States Department of Agriculture, Forest Service. Northeast Forest Experimental Station General Technical Report NE-211.

Lindroth, R.L. and M.S. Bloomer. 1991. The chemical ecology of the forest tent caterpillar: responses to dietary protein and phenolic glycosides. *Oecologia* **86**: 408-413.

Lindroth, R.L. and S.-Y. Hwang. 1996. Diversity, redundancy, and multiplicity in chemical defense systems of aspen.), Pp. 25-56 J.T. Romeo, J.A. Saunders and P. Barbosa, eds, Phytochemical Diversity and Redundancy in Ecological Interactions. Plenum Press, New York.

Lindroth, R.L. and P.A. Koss. 1996. Preservation of Salicaceae leaves for phytochemical analyses: Further assessment. *Journal of Chemical Ecology* **22**:765-772.

Lindroth, R.L. and M.S. Pajutee. 1987. Chemical analysis of phenolic glycosides: art, facts, and artifacts. *Oecologia* **74**: 144-148.

Lindroth, R.L and K.K. Kinney. 1998. Consequences of enriched atmospheric CO2 and defoliation for foliar chemistry and gypsy moth performance. *Journal of Chemical Ecology* **24**:1677-1695.

Lindroth, R.L., K.A. Klein, J.D.C. Hemming and A.M. Feuker. 1997. Variation in temperature and dietary nitrogen affect performance of gypsy *moth (Lymantria dispar* L.). *Physiological Entomology* **22**: 55-64.

Lindroth, R.L., S. Hwang and T.L. Osier. 1999. Phytochemical variation in quaking aspen: effects on gypsy moth susceptibility to nuclear polyhedrosis virus. *Journal of Chemical Ecology* **25**: 1331-1341.

Marino, P.C. and K.L. Gross. 1988. Competitive effects of conspecific and herbaceous plants (weeds) on growth and branch architecture of *Populus* × *euramericana* cv. Eugenei. *Canadian Journal of Forest Research* **28**: 359-367.

Mason, C.J. and M.L. McManus, 1981. Larval dispersal of the gypsy moth. Pp. 161-202 in C.C. Doane and M.L. McManus, eds, The Gypsy Moth: Research Toward Integrated Pest Management. United States Department of Agriculture Technical Bulletin 1584.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen-content. *Annual Review of Ecology and Systematics* **11**: 119-161.

Mattson, W.J. and N.D. Addy. 1975. Phytophagous insects as regulators of forest primary production. *Science* **190**: 515-522.

Mattson, W.J., D.A. Herms, J.A. Witter and D.C. Allen. 1991. Woody plant grazing systems: North American outbreak folivores and their host plants. Pp. 53-84 in Y.N. Baranchikov, W.J. Mattson, F.P. Hain and T.L. Payne, eds, Forest Insect Guilds: Patterns of Interactions with Host Trees. United States Department of Agriculture, Forest Service. General Technical Report NE-153.

May, R.M. 1973. Stability and Complexity in Model Ecosystems. Princeton University Press, Princeton, New Jersey.

Meyer, G.A. and M.E. Montgomery (1987). Relationships between leaf age and the food quality of cottonwood foliage for the gypsy moth, *Lymantria dispar*. *Oecologia* **72**: 527-532.

Myers, J.H. and K.S. Williams. 1987. Lack of short or long term inducible defenses in the red alder- western tent caterpillar system. *Oecologia* **48**: 73-78.

Nef, L. 1988. Interactions between the leaf miner, *Phyllocnitis suffusella*, and poplars. Pp. 239-251 in W.J. Mattson, J. Levieux and C. Bernard-Dagan, eds, Mechanisms of Woody Plant Defenses against Insects. Springer, New York.

Neuvonen, S. and E. Haukioja. 1991. The effects of inducible resistance in host foliage on birch-feeding herbivores. Pp. 277-291 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Nitao, J.K, B.A. Birr, M.G. Nair, D.A. Herms, and W.J. Mattson. 2000. Rapid quantification of condensed tannins with a continuous flow analyzer. *Journal of Agricultural and Food Chemistry*. **In review**.

Parry, D. W.J.A. Volney and C.R. Currie. 1997. The relationship between trembling aspen phenology and larval development of the large aspen tortrix. Natural

Resources Canada, Canadian Forest Service, Northwest Region, Northern Forestry Centre NOR-X-350. 14 pp.

Parry, D., D.A. Herms and W.J. Mattson. 2000. Induced-responses of aspen to multiple, consecutive defoliations: their effects on an outbreak folivore and its natural enemies. *Journal of Animal Ecology*. **Submitted**.

Reynolds, B.C. and D.A. Crossley. 1997. Spatial variation in herbivory by forest canopy arthropods along an elevation gradient. *Environmental Entomology* **26**: 1232-1239.

Rhoades, DF. 1983. Herbivore population dynamics and plant chemistry. Pp. 155-220 in . R.F. Denno and M.S. McClure, eds, Variable plants and Herbivores in Natural and Managed Systems. Academic Press, San Diego.

Robison, D.J. and K.F. Raffa.1997. Effects of constitutive and inducible plant traits of hybrid poplars on forest tent caterpillar feeding and population ecology. *Forest Science* **43**: 252-267.

Roland, J. and J.H. Myers 1987. Improved insect performance from host-plant defoliation – winter moth on oak and apple. *Ecological Entomology* **12**: 409-414.

Rossiter, M.-C., J.C. Schultz, I.T. Baldwin. 1988. Relationships among defoliation, red oak phenolics and gypsy moth growth and reproduction. *Ecology* **69**: 267-277.

Roth, S., R.L. Lindroth, J.C. Volin and E.L. Kruger. 1998. Enriched atmospheric CO2 and defoliation: effects on tree chemistry and insect performance. *Global Change Biology* **4**: 419-430.

Rothman, L.D. 1997. Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. *Ecology* **78**: 1481-1493.

Ruohomaki, K., S. Hanhimaki, E. Haukioja, L. Iso-Iivari, S. Neuvonen, P. Niemela and S. Suomela. 1992. Variability in the efficacy of delayed-inducible resistance in mountain birch. *Entomologia Experimentalis et Applicata* **62**: 107-115.

Sample BE, L. Butler, C. Zivkovich, R.C. Whitmore and R. Reardon. 1996. Effects of *Bacillus thuringiensis* Berliner var Kurstaki and defoliation by the gypsy moth [*Lymantria dispar* (L)(Lepidoptera: Lymantriidae)] on native arthropods in West Virginia. *The Canadian Entomologist* **128**: 573-592.

Schoener, T.W. 1983. Field experiments on interspecific competition. *The American Naturalist 122*: 240-285.

Schultz, J.C. 1988. Plant responses induced by herbivores. *Trends in Ecology and Evolution* **3**: 45-49.

Schultz, J.C. and I.T. Baldwin. 1982. Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science* **217**: 149-151.

Scriber, J.M. and F. Slansky. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology* **26**: 183-211.

Slansky, F. and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by the larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs* **47**: 209-228.

Stockhoff, B. A. 1992. Diet-switching by gypsy moth: effects of diet nitrogen history vs. switching on growth, consumption and food utilization. *Entomologia Experimentalis et Applicata* **64**: 225-238.

Strong, D.R., J.H. Lawton and T.R.E. Southwood. 1984. Insects on Plants: Community Patterns and Mechanisms. Harvard University Press, Cambridge.

Swain, T. and W.E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic compounds. *Journal of the Science of Food Agriculture* **10**: 63-68.

Tammaru, T. & Haukioja, E. (1996) Capitol breeders and income breeders among Lepidoptera – consequences to population dynamics. *Oikos* **77**: 561-564.

Tuomi, J., P. Niemela, E. Haukioja, S. Siren and S. Neuvonen. 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia* **61**: 208-210.

Tuomi, J., P. Niemela and S. Siren.1990. The Panglossian paradigm and delayed-inducible accumulation of foliar phenolics in mountain birch. *Oikos* **59**:399-410.

Tuomi, J., T. Fagerstrom and P. Niemela. 1991. Carbon allocation, phenotypic plasticity, and induced defenses. Pp. 85-104 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Turchin, P. 1990. Rarity of density-dependence or population regulation with lags? *Nature* **344**: 660-663.

Turchin, P. 1995. Population regulation: Old arguments and a new synthesis. Pp. 19-40 in N. Cappuccino and P.W. Price, eds, Population Dynamics: New Approaches and Synthesis. Academic Press, New York.

Underwood, N. 1999. The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist* **153**: 283-294.

Valentine, H.T., W.E. Wallner and P.M. Wargo. Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia* **57**: 298-302.

Virtanen, T. and S. Neuvonen (1999). Performance of moth larvae on birch in relation to altitude, climate, host quality, and parasitoids. *Oecologia*. **120**: 92-101.

Waldbauer, G.P. 1968. The consumption and utilization of food by insects. *Advances in Insect Physiology* **5**: 229-289.

Wallner, W.E. 1983. Gypsy moth host interactions: a concept of room and board. Pp. 5-8 in R.L. Talerco and M. Montgomery, eds, Proceedings: Forest Defoliator –Host Interactions: a Comparison between Gypsy Moth and Spruce Budworms. USDA Forest Service, Northeastern Station General Technical Report NE-85.

Wallner, W.E. and G.S. Walton. 1979. Host defoliation: a possible determinant of gypsy moth population quality. *Annals of the Entomological Society of America* **72**: 62-67.

Werner, R.A. 1979. Influence of host foliage on development, survival, fecundity and oviposition of the spear-marked black moth, *Rheumaptera hastata* (Lepidoptera: Geometridae). *The Canadian Entomologist* **111**: 317-322.

West, C. 1985. Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. *Ecological Entomology* **10**: 111-120.

Williams, K.S. and J.H. Myers.1984. Previous attack of red alder may improve food quality for fall webworm larvae. *Oecologia* **62**: 74-79.

Williams, R.S., D. E. Lincoln and R.J. Norby. 1998. Leaf age effects of elevated CO2-grown white oak leaves on spring-feeding lepidopterans. *Global Change Biology* **4**: 235-246.

Wold, E.N. and R.J. Marquis. 1997. Induced defense in white oak: effects on herbivores and consequences for the plant. *Ecology* **78**: 1356-1369.

Woods, S.A. and J.S. Elkinton. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. *Journal of Invertebrate Pathology* **50**: 151-157.

Work, T.T. and D.G. McCullough. 2000. Lepidopteran communities in two forest ecosystems during the first gypsy moth outbreaks in northern Michigan. *Environmental Entomology* **29**: 884-900.

CHAPTER 3:

INDUCED-RESPONSES OF ASPEN TO SUCCESSIVE YEARS OF DEFOLIATION: THE EFFECTS ON AN OUTBREAK FOLIVORE AND ITS NATURAL ENEMIES

INTRODUCTION

Decline in foliage quality following defoliation of trees is one of several mechanisms potentially responsible for driving the delayed-density dependent feedback necessary to produce the cyclic population dynamics characteristic of some outbreak forest Lepidoptera (Berryman et al. 1987). In response to defoliation, two types of induced resistance have been identified, rapid-induced resistance (RIR) and delayed-induced resistance (DIR) (Neuvonen and Haukioja 1991). Induction of RIR occurs within hours or days of herbivory, affecting the herbivore generation responsible for the damage, and is thereby expected to exert a stabilizing influence on population density. In contrast, DIR responses are not manifested until the following growing season, and therefore affect future generations of herbivores (Haukioja 1982). A number of authors have speculated that long-term reductions in host plant quality and the time-lag in the recovery of trees after defoliation, act to destabilize populations thus contributing to population cycles (e.g., Benz 1974, Haukioja 1980, Rhoades 1983). A recent model has verified that DIR can produce population cycles if there is a sufficient time-lag before maximum induction, or if following induction, the relaxation of DIR is sufficiently protracted (Underwood 1999).

Research on mountain birch, *Betula pubescens* ssp. *czerepanovii* (Orlova) Hamet-Ahti, and its primary herbivore, the autumnal moth, *Epirrita autumanta* (Lepidoptera:

Geometridae) in Fennoscandia, has generated substantive contributions to the understanding of the role of induced-resistance in insect population dynamics. Defoliation of mountain birch induces phytochemical changes in the leaves characterized by elevated levels of secondary compounds such as hydrolyzable and condensed tannins, and reductions in primary nutrients including nitrogen and water (e.g., Tuomi et al. 1984, 1990, Kaitaniemi et al. 1998). These changes in host quality persist for at least the subsequent growing season, and in some cases do not return to predefoliation levels for several years (Tuomi 1984). Caterpillars feeding on foliage from previously defoliated mountain birch suffer significantly reduced growth and fecundity relative to undefoliated control trees. In some studies, reductions in fecundity as high as 60-80% were found, although lesser effects are more common (Haukioja 1982, Haukioja and Neuvonen 1987, Ruohomaki et al. 1992, Kaitaniemi et al. 1999a, 1999b). In other systems, artificial defoliation of paper birch, *Betula papyrifera* Marshall, and trembling aspen, *Populus tremuloides* Michaux, simulating outbreaks of the black-marked spear moth, *Rheumaptera hastata* (Lepidoptera: Geometridae), and large aspen tortrix, *Choristoneura conflictana* (Lepidoptera: Tortricidae), respectively, induced phytochemical changes and reductions in herbivore performance (Clausen et al. 1991, Bryant et al. 1993). These studies suggest that defoliation driven declines in host plant quality may be a general phenomenon in deciduous trees.

During outbreaks of forest insects, host trees are often partially or completely defoliated for at least two successive years (e.g., Mattson et al. 1991). However, the majority of studies documenting DIR have been based on only a single defoliation event, followed by phytochemical analyses and larval bioassays in the following year

(Kaitaniemi et al. 1999b). Single year defoliation studies have been used repeatedly despite the speculation of Haukioja et al. (1988) that consecutive years of defoliation might have cumulative negative effects on the quality of trees for herbivores. Kaitaniemi et al. (1999b) suggested that the effects of multiple years of defoliation on trees and their reciprocal effects on herbivores may be one of the least understood aspects of induced resistance to herbivory in trees. Increasingly negative effects on insect performance with each successive year of defoliation have been shown in gray birch, *Betula populifolia* Marsh, paper birch, and black oak, *Quercus velutina* Lamarck (Werner 1979, Wallner and Walton, 1979, Valentine et al. 1983). More recently, successive years of defoliation were shown to have only minor cumulative effects on phytochemistry and insect performance in mountain birch and hybrid poplar, *Populus* × *euramericana* c.v. 'Eugeneii' (Kaitaniemi et al. 1999b, Parry et al. 2000a). Reasons for the disparity among the studies above have not been identified. The magnitude of the effects could be system specific or could result from differences in experimental methods such as insect-caused versus manually applied defoliation, background soil fertility, nutrient addition to compensate for the lack of frass in manual defoliation experiments, and the timing or severity of the defoliation. The lack of congruence among the studies also highlights the gaps in our knowledge on the effects of long term defoliation on trees.

Outbreaks of the forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae) on trembling aspen rank among the largest in the world with contiguous areas of defoliation exceeding 13 million ha in a single season (e.g., Mattson et al. 1991). The majority of this defoliation is concentrated in the vast trembling aspen forests found across much of Canada and in the Great Lake States of Michigan, Wisconsin, and

Minnesota. In these regions of North America, forest tent caterpillar exhibits cyclical population dynamics with outbreaks occurring every 9-12 years, although at smaller scales, there is considerable variability around the mean cycle length (Hildahl and Reeks 1960, Sippell 1962, Hodson 1977). The length of outbreaks can be protracted with aspen trees often defoliated for three consecutive years although longer outbreaks are not uncommon (Hildahl and Reeks 1960, Sippell 1962, Ives 1971, Witter et al. 1975, Hodson 1977). Surprisingly, the effects of such sustained, severe defoliation on phytochemistry, and the potential effects that any changes in host quality may have on forest tent caterpillar population dynamics have received little attention.

Trembling aspen is typical of early succesional trees, quickly colonizing recently disturbed landscapes and relying on rapid growth to out-compete other vegetation (Barnes 1969, Peterson and Peterson 1992). Although aspen produces copious quantities of seed, reproduction is primarily by clonal suckering from parental rootstock (Barnes 1969, Peterson and Peterson 1992). This reproductive strategy may result in vast areas of even-aged stands with relatively little genetic diversity, especially following fire, clear cut logging, or other large disturbances. The low inter- and intra-specific diversity in aspen dominated environments led Mattson et al. (1991) to suggest that herbivore outbreaks are inevitable in these forests. From theory, fast growing, shade intolerant early succesional tree species such as aspen, should invest relatively little in constitutive resistance mechanisms, favoring instead resistance mechanisms that are inducible (Herms & Mattson 1992). Given the frequency of large-scale defoliation events in environments where most competition is intraspecific, trees such as aspen are also predicted to have evolved strategies allowing tolerance of high herbivory (Mattson et al. 1991).

Factors underlying the outbreak and collapse of forest tent caterpillar populations have been the subject of much speculation. Weather, parasitoids, and pathogens may all play a role, although linkage of these factors to changes in population density has not been shown. A component of population dynamics that has received relatively little attention is the marked declines in forest tent caterpillar fecundity that characterize the latter years of an outbreak. This decline can be large with egg masses being as much as 50% smaller at the end of an outbreak (Ives 1971, Witter et al. 1975, Parry, unpublished). In western tent caterpillar, *Malacosoma californicum* Dyar, fecundity remained high through the early years of outbreaks when densities were highest before declining and remaining low even as populations collapsed (Myers and Kukan 1995). These data suggest that rather than a simple shortage of food, a delayed-density dependent factor is operating on fecundity. Some authors have suggested that sublethal pathogens maybe primarily responsible for declines in fecundity (Rothman and Myers 1994, Myers and Kukan 1995, Rothman 1997), although the evidence is equivocal. The possibility that reductions in forest tent caterpillar fecundity over the course of an outbreak could be driven by DIR, manifested through changes in aspen primary or secondary phytochemistry, as has been suggested from studies of the large aspen tortrix (Clausen et al. 1991), has not been investigated.

Increasing attention has been focused on the effects of induced-resistance on the natural enemies of herbivorous insects. A number of studies have shown that plants damaged by herbivores emit chemical signals that are attractive to natural enemies of caterpillars (e.g., Eller et al. 1988, Turlings et al. 1993, Thaler 1997). Thus, increased emphasis has been placed on the study of plant attributes such as nutritional quality,

production of secondary compounds, and physical structure that may enhance or interfere with the activity of natural enemies (Agrawal 2000). While the focus of such research has generally been with agricultural plants, there is increasing evidence that similar tritrophic interactions occur in trees (e.g., Havill and Raffa 2000).

Conceptually, the idea that induction of volatiles attractive to natural enemies following herbivory is not new. More than 60 years ago, it was suspected that some tachinid flies (Diptera: Tachinidae) attacking caterpillars were attracted to volatiles from host trees because they would only oviposit in the presence of caterpillar damaged leaves (Bess 1936). This has now been documented for tachinids in both agricultural and forested environments (e.g., Monteith 1964, Roland et al. 1995). Two species of leaf-ovipositing tachinids, *Leschenaultia exul* (Townsend) and *Patelloa pachypyga* (Aldrich and Weber) dominate the larval parasitoid complex across the northern range of forest tent caterpillar (Sippell 1957, Witter and Kulman 1979, Parry 1995, Parry et al. 1997). Experiments have shown that both species are attracted to volatiles released when tent caterpillars feed on leaves (Mondor and Roland 1997, 1998) confirming the hypothesis proposed by Bess (1936). To my knowledge, the effect of induced responses in trees (RIR or DIR) on the activity of parasitoids has not been investigated. A number of studies have shown that the fitness of dipteran and hymenopteran parasitoids can be reduced when utilizing hosts that have been feeding on diets high in secondary metabolites (Bourchier 1991, Roth et al. 1997, Havill and Raffa 2000). Thus parasitoids may make tradeoffs between attacking larger numbers of poor quality hosts on plants with induced-resistance, or searching for higher quality hosts which may be at lower density.

The objective of this study was to emulate the interactions that occur between forest tent caterpillar and aspen during outbreak conditions. I incorporated as much realism as is possible under the constraints imposed by the nature of a manipulative experimental field study. Defoliation treatments were applied by inoculating trees with outbreak densities of caterpillars over a three-year period. Trees used in the experiment were ontogenetically mature belonging to two different clones growing in a forest environment. In 2000, the fourth year of the study, half of the previously defoliated trees were left undefoliated and half were defoliated again. In addition, another set of trees was defoliated for the first time in 2000. A fourth set of undefoliated trees served as the control. These treatments allowed the determination of the relative effects of RIR, DIR, and the combined effects of both, on growth, pupal mass, fecundity, and survival of forest tent caterpillars. I also examined the effects of the defoliation treatments on parasitism by two species of tachinid flies that are attracted by volatiles released by aspen leaves damaged by forest tent caterpillar feeding.

MATERIALS AND METHODS

EXPERIMENTAL SYSTEM

The study was conducted in an early successional forest (ca. 15 yr old) growing in an abandoned field at the edge of a mature wood-lot on the Michigan State University campus, East Lansing. I identified six aspen clones at this site based on leaf morphology, timing of leaf drop in autumn, and phenology of bud break. I selected two of the clones for the experiment because of the six, they contained the most ramets which afforded me

the greatest opportunity to standardize the experimental trees with respect to height, aspect, exposure, and foliar volume. These clones were separated by ca. 20 m of mixed white ash, *Fraxinus americanus* L., red maple, *Acer rubrum* L. and red oak, *Quercus rubra* L., saplings and were growing on similar sites with respect to drainage and insolation. The timing of bud break between the clones differed by approximately five calendar days each year. At the onset of the experiment in 1997, I selected 20 trees in each clone and permanently identified each tree with a metal identification tag. In the spring of 1997, trunks of the trees averaged 3.4 cm in diameter at 45 cm above ground (below first major branching point) and ranged from 3.6 to 4.5 m in height.

To my knowledge, no significant defoliation had occurred on these trees prior to this study. The Baker Woodlot, adjoining the plots on one edge, is a heavily utilized recreational and instructional area on campus and significant defoliation would likely have not gone unnoticed. Herbivory occurred at background levels (<10%) in the aspen stands in 1996, the year before the study began. Although I can not rule out significant herbivory occurring prior to the experiments, given the long time frame of the study, control trees would have been free of herbivory for at least 5 years previous to the bioassays in 2000.

IMPLEMENTATION OF TREATMENTS

In 1997, the initial year of the study, I randomly assigned half of the trees in each clone to the forest tent caterpillar defoliation treatment. These trees were defoliated in 1997, 1998, and 1999. In 2000, half of the trees defoliated from 1997-1999 were not defoliated and half were defoliated again (Table 20). In addition, five trees in each clone not previously

Table 20. History of defoliation treatments implemented between 1997-2000 to assess the effects of RIR and DIR on forest tent caterpillar performance. Short-term bioassays using second and final (fifth) instars and a life-long egg-adult bioassay were conducted in 2000. The same 40 trees first selected in 1997 were used throughout the study.

			Defoliation			
Treatment	Clone	N (trees)	1997	1998	1999	2000
CTRL	1	5	No	No	No	No
	2	5				
RIR	1	5	No	No	No	Yes
	2	5				
DIR	1	5	Yes	Yes	Yes	No
	2	5				
RIR/DIR	1	5	Yes	Yes	Yes	Yes
	2	5				

defoliated, were defoliated for the first time (Table 20). This experimental design allowed me to simultaneously test the effects of RIR (trees defoliated for the first time concurrent with insect bioassays = RIR), DIR (trees defoliated for the previous 3 years but with no defoliation in the year of the insect bioassays = DIR), and the combined effects of DIR and RIR (trees with 3 years of defoliation as well as defoliation concurrent with the insect bioassays = RIR/DIR). Control trees (CTRL) were always maintained free of herbivory from tent caterpillars.

To inoculate trees with caterpillar densities capable of creating high levels of defoliation, large numbers of forest tent caterpillar eggs were obtained from outbreak populations. I used eggs collected near Cochrane, Ontario, Canada in 1997 and 1998, Flin Flon, Manitoba, Canada in 1999, and Ontonagon, Michigan in 2000. Nuclear polyhedrosis virus (NPV) is a major concern with field collected eggs. To remove viable NPV from egg surfaces, spumaline, a frothy secretion from the female accessory glands used to cover the eggs, was scraped off using a razor blade. Egg bands were then immersed in household bleach, diluted to approximately 4%, for 3 minutes. In 1999 and 2000, I added a small amount of household dish detergent to the bleach and agitated the eggs using a toothbrush which appeared to give better results than the bleach soak alone. This method was very effective in eliminating pathogen problems and despite many thousands of tent caterpillars on each defoliation treatment tree, only a few cases of NPV mortality were observed. No NPV deaths were recorded in any of the bioassay larvae.

After surface sterilization, packages of eggs were held at ca. 4°C over the winter, and misted occasionally with water to prevent desiccation. Trees were monitored daily as spring approached and once buds began to swell, I placed 5-10 egg bands in cheesecloth

packages and warmed them at room temperature in the laboratory until approximately one day prior to hatch. Eggs were then returned to the refrigerator until bud break. Forest tent caterpillar cannot initiate feeding until buds soften and the scales begin to separate (D. Parry, unpublished data). Once bud scales began to separate, I removed the eggs from the refrigerator and attached them to treatment trees with plastic twist ties. Depending on the size of the tree, 3-6 packages were placed throughout the canopy. Eggs began to hatch within two days of being place on the trees, closely approximating the natural synchrony between aspen and forest tent caterpillar (see Parry *et al.* 1998).

Upon eclosion, groups of caterpillars moved to the buds and initiated feeding on the newly expanding leaves. Because forest tent caterpillars is a highly gregarious species and exhibits considerable fidelity to their 'maternal tree' until at least the middle of the fourth instar (Batzer et al. 1995, D. Parry, personal observation), larvae do not need to be restrained. Unlike other *Malacosoma* species, forest tent caterpillar does not make a tent, and exhibits a nomadic feeding behavior where larval groups wander throughout the canopy feeding at different locations. The density of caterpillars placed on the trees was sufficient to cause 50-70% defoliation by the end of the fourth instar, levels that are similar to those observed in many natural outbreaks (D. Parry, personal observation). After the fourth molt, forest tent caterpillars generally leave the maternal tree and wander extensively, irrespective of population density or defoliation level. In natural outbreaks, densities of these wandering final instar larvae are often high enough that any remaining aspen foliage is completely defoliated, along with many other tree and shrub species. However, in my plots, unrestrained larvae dispersed into the surrounding forest and disappeared. To achieve the higher levels of defoliation characteristic of outbreaks, I

enclosed groups of 30 to 60 final instars in fine mesh sleeve cages and moved the cages throughout the canopy of each treatment tree on nearly a daily basis to simulate the wandering behavior of larvae. Caterpillars in these bags were used solely to defoliate the trees and were not part of any bioassay. This method also allowed me to equalize the defoliation among trees by removing or adding cages of larvae to achieve the desired levels of herbivory. Unrestrained larvae were prevented from ascending control trees by a band of tangle trap placed over paper tree wrap at the base of the trunk.

An essential element of realism in this study is that comparisons of treatment effects were made with control trees that had background levels of herbivory in addition to any effects caused by the 30 caterpillars used in the on-tree bioassays. The low level herbivory on the control trees is important because if induced-responses are to have any role in the population dynamics of forest tent caterpillar, they must increase beyond background levels caused by groups of this gregarious species, as well as herbivory from other species. Unlike on-tree bioassays done with solitary species where density effects can confound results, forest tent caterpillar forage in clusters, thus rearing in groups is consistent with the species natural behavior. A single-family group consists of 100-500 caterpillars depending on latitude and population density (Parry et al. 2000b). In many previous studies, researchers compared the effects of defoliation treatments against control trees with no herbivory. This is unrealistic, with respect to gregarious species at least, because even in low-density populations, larvae will experience the induction effects of their own feeding, as well as feeding by the family group. Thus, the correct comparison for this and other gregarious species is between trees experiencing severe

defoliation and those affected by background herbivory and the feeding of individual family groups, a design element that I have utilized throughout this experiment.

I estimated defoliation by assigning trees to 10% classes based on the amount and degree of damage to the remaining foliage. In a study using hybrid poplar (Parry et al. 2000a), similar visual estimates of defoliation were highly correlated with damage assessments done on individual leaves. Because of its severity, defoliation in the three induction treatments was relatively easy to estimate. However, herbivory on control trees was very low and difficult to estimate. To further quantify defoliation on control trees, I estimated feeding damage on two dominant lateral branches in the mid-canopy, the maximum height accessible from a 3 m ladder. The first 50 leaves from the short shoots beginning at the branch tip were classified as undamaged if they were free from herbivory, suffered minor blemishes, or occasional small shot holes. Leaves with more than ca. 20% of the surface removed were classified as damaged. This showed that 18% of the leaves on undefoliated trees in Clone 1 (controls and DIR trees) had some damage. In Clone 2, the damage on the undefoliated trees (controls and DIR) was 20%. The majority of the damaged leaves had more than 70% of their total area remaining. In contrast, 100% of the leaves on all trees in the defoliated treatments were classified as damaged and most had lost 60% or more of the total leaf area.

INSECT BIOASSAYS

Lifelong Bioassay – To estimate the effects of the defoliation treatments on fitness, I reared forest tent caterpillars from egg hatch through adult on each of the experimental trees. Experimental larvae were obtained from egg bands collected the previous fall from

a moderately high, expanding population in Ontonagon County, Michigan. The collected egg bands were large, containing 150-200 eggs, indicating little previous stress and the population was growing (ratio of current year egg bands to previous year egg bands 128:27 for three sampled trees). Eggs were kept in an environmental chamber in plastic bags at ca. 4°C and 70% RH for the duration of the winter. Shortly prior to bud break, 45 egg bands were removed from cold storage, surface sterilized as above, and placed at room temperature to hatch. The progeny of each egg band, representing the full reproductive compliment of a single female, were allowed to mingle in a large plastic container for 24 hours. I used a fine paintbrush to randomly allocate 30 neonates to petri dishes assigned to each of the 40 trees. Neonate larvae are diminutive and could pass through the mesh in the screen cages. Thus, each group of 30 larvae was reared in the laboratory on foliage clipped from the assigned tree until the end of the first instar. Twigs were placed in florist's aquapicks to maintain leaf turgor. I reared the first instars at 18°C with a 16:8 photoperiod. At this temperature, growth of larvae kept pace with that of larvae in the field. At the time of molt to second instar, each group of 30 was transferred to the field and placed within a large (60 × 30 cm) mesh sleeve cage which enclosed foliage and was attached to the appropriate experimental tree. While the larvae were small (L2-L4), sleeves were moved to new branches every 2-3 days to simulate the movement of the nomadic feeding groups through the trees. Extensive field observations on the natural foraging patterns of forest tent caterpillars suggest that larvae often utilize a single branch for several days before moving elsewhere on the tree, particularly during early instars. Once the larvae entered the fourth instar, sleeves were moved more frequently, and nearly daily through the final (L5). Foliage within the bioassay sleeves

was never allowed to become depleted. I did not protect any foliage from non-bioassay caterpillars defoliating the trees although I did remove caterpillars during the final instar to prevent trees from becoming completely defoliated prior to the bioassay larvae completing their development. Therefore, the bioassay caterpillars were feeding on foliage representative of the damage levels on each tree, which added an essential degree of realism to the study, and also highlights the critical importance of using disease-free larvae to defoliate the trees. When moving the sleeve cages, an effort was made to provide the bioassay caterpillars with the best foliage remaining on a tree under the assumption that foraging caterpillars discriminate between severely damaged and partially consumed leaves. Even so, caterpillars in the defoliated treatments were completing development on leaves that had suffered considerable damage.

When caterpillars had finished feeding and were spinning cocoons in preparation for pupation, the sleeve cages were removed from the trees and returned to the laboratory. Within 24-48 hours of pupation, the silk cocoons were carefully cut away with scissors and the pupae were weighed on an electronic balance (to 0.1 mg) and then placed individually on squares of paper towel in plastic snap cap vials. Pupae were held at 22°C and 16:8 and checked daily for emergence of adults, which were sexed and the emergence date recorded.

To estimate the effects of the treatments on fecundity, caterpillars from the same population used in the bioassay were reared from egg hatch to pupation on aspen trees near the experimental plots. At pupation, pupae were weighed and individually placed in vials for emergence. Using the methods of Parry et al. (2000b), adult females were dissected, the number of eggs counted and a least-squares regression equation fitted to the

relationship between fecundity and pupal mass. This regression equation ($y = 390.85x - 19.11$, $r^2 = 0.88$, $p < 0.0001$, df = 24) was used to estimate fecundity changes based on the pupal mass data from the lifelong bioassay.

Short-term bioassay – Short-term bioassays were conducted using second (L2) and fifth (L5) instars, respectively in 2000. The progeny of 40 egg bands collected the previous year in Ontonagon County, Michigan were allowed to mingle for 24 hours and then several hundred were placed in the field on aspen trees outside of the plots. These larvae served as the stock for both the L2 and L5 short-term bioassay and were from the same population as those used in the long-term bioassay above. Because hatch was synchronous and caterpillars highly gregarious through the first four instars, sufficient numbers of similar aged larvae could easily be collected for bioassays. For the L2 bioassay, I retrieved caterpillars from the field when the first instars stopped feeding and congregated on silk pads in preparation for molt. Forest tent caterpillar is sensitive to phenological changes in foliar quality (Parry et al. 1998). Thus, using caterpillars reared in the field in close proximity to the plots ensured that they were synchronized with the host plant and larvae used in a life-long bioassay below. Following collection, caterpillars were returned to the laboratory and held in petri dishes on moist paper towels without food. Within 12 hours of collection, most of the collected caterpillars had molted to the second instar. Foliage was then obtained by clipping representative samples of leaves at the base of the petiole throughout the mid-canopy of each experimental tree. Clipping leaves in this manner does not induce phytochemical changes in aspen (Mattson and Palmer 1988). Aspen is heterophyllous, producing both determinate shoots from leaf primordia at bud break and indeterminate shoots that continue to develop new leaves

throughout the growing season. To standardize the collections, only leaves from determinate shoots were used in the short-term bioassays.

Collected leaves were placed in zip-lock bags on ice, returned to the laboratory, weighed on an electronic balance and placed in 125 x 50 mm plastic petri dishes. The bottom third of each dish had been filled with plaster saturated with water, which maintained humidity and prevented leaf turgor loss over the 2-3 day duration of the bioassays. I randomly allocated groups of second instars (n = 12) to foliage from each of the 40 trees. Fifteen larvae were individually weighed fresh and then dried to provide an initial dry weight estimate. The bioassay was conducted at in an environmental chamber at 23°C with 16:8 photoperiod until the most rapidly developing groups finished feeding in preparation for the second molt (51 hours for Clone 1 and 42 hours for Clone 2). At the conclusion of the bioassay, caterpillars were placed individually in vials and the remaining portions of leaves and frass were collected then dried at 40°C in a drying oven for five days prior to weighing. Relative growth and consumption rates were calculated for the L2 caterpillars from the weights of insects and foliage consumed as described below.

For the fifth instar bioassay, the same methods as for the L2 bioassay were used. Fourth instars were collected from silk molting mats in the field when their head capsules showed signs of slippage, and were returned to the laboratory to complete the molt. I reared four caterpillars individually on leaves collected from each of the trees for 72 hours at 24°C. Observations from previous experiments suggest that this time period brackets the period of maximum growth in this instar. Leaves were weighed prior to presentation to caterpillars. Sub-samples of 15 larvae were dried prior to the bioassay and

linear regression was used to estimate the initial dry weight of the experimental insects. In this Michigan population, newly molted fifth instar females were larger (ca.190-250 mg) than males (ca.125-190 mg) so I allocated two females and two males (as identified from initial weight) to foliage from each tree. After the termination of the bioassay, larvae were frozen for a few hours and then examined microscopically to confirm their sex using diagnostic characters described in Stehr and Cook (1968). Caterpillars were then placed individually in vials and dried. Frass was collected into 1g plastic vials, and uneaten leaf portions were placed in small paper envelopes prior to drying.

For L5 caterpillars, I calculated relative growth and consumption rates, and standard estimates of efficiency of digestion of ingested food (ECI), approximate digestibility of ingested food (AD), and efficiency of conversion of digested food to biomass (ECD) using the gravimetric methods of Waldbauer (1968). Caterpillars that died or did not feed were not included in any analyses. Relative growth rate was calculated for individual caterpillars as $RGR = \ln(weight_f) - \ln(weight_i)/T$ where ln is the natural logarithm, $weight_f$ = final weight, $weight_i$ = initial weight, and T is the elapsed time in days (Gordon 1968). Estimates of initial dry weight of larvae were obtained from a subset of insects weighed wet and dried at the start of the experiment. To estimate initial leaf dry weight, a portion of the leaves collected from each tree was dried before the experiment started.

Survival and parasitism – Overall survival from hatch to pupation was assessed by counting the number of larvae from the original 30 that successfully pupated. To assess treatment effects independently of parasitism, I included in this analysis all caterpillars that were parasitized. The effects of parasitism on survival were addressed in a separate analysis (described below). Two sleeve cages ripped during a windstorm and some early

instars were lost. For these groups, the remaining larvae were transferred to a new sleeve, and survival to pupation estimated as a proportion of the larvae that were transferred, recognizing that this introduces a small amount of error to the estimate.

I was interested in the contribution that induced resistance could make to population growth, relative to the effects of other contemporaneous factors such as natural enemies. For each tree, sources of mortality were partitioned among parasitoids and unknown causes. The two dominant parasitoids of forest tent caterpillar larvae place microtype eggs on foliage and thus are not greatly affected by the presence of sleeve cages. Other parasitoid species can attack the larvae through the sides of the screen cages although rates of parasitism are often lower than in unrestrained larvae (D. Parry, personal observation). When sleeve cages were removed from the trees at pupation, a careful search was made of each bag for fly puparia and hymenopteran cocoons that had emerged from bioassay caterpillars. These were returned to the laboratory and placed in vials until adult emergence. Parasitoids emerging from tent caterpillar pupae or collected from inside the sleeve cages were identified using the keys of Sippell (1961) and Williams et al. (1996).

To address the relative contribution of induced resistance and parasitism to population dynamics, I estimated the approximate net reproductive rate (R_0) of forest tent caterpillar in each treatment. To simplify the calculations, I first converted males to female equivalents using the regression equation $y = 2.0798x - 0.1484$ ($r^2 = 0.67$, $p < 0.001$, df = 40) determined from the mean male pupal mass for each experimental tree. The number insects dying from unknown causes were subtracted from the original 30 insects in each cohort to estimate survival, and then multiplied by the mean fecundity for

each treatment to determine R_0 (Southwood 1991). Fecundity was determined from the regression equation calculated above ($y = 390.85x - 19.11$, df = 24, $r^2 = 0.88$, $p < 0.0001$). Because parasitized individuals were included in the survival calculation, their effect could be determined from R_0 with and without including parasitized individuals in the calculations. In addition to estimates obtained from my experiment, I calculated parasitism rates from first, third, and fourth year outbreaks of forest tent caterpillar in Alberta, Canada (Parry 1995), which are similar to levels recorded elsewhere across the northern range of forest tent caterpillar (e.g., Sippell 1957, Witter and Kulman 1979). I calculated R_0 using these values in place of those estimated in my experiment. The relative impact of parasitoids in natural populations could then be compared under the assumption that the induced-resistance levels found in my experiment are representative of aspen forests elsewhere.

STATISTICAL ANALYSES

In all statistical analyses, I used a mixed-model ANOVA where $Y_{ijk} = \mu + C_i + D_j + T_k(C_i) + (C_i \times D_j) + \in_{ijk,}$ where C_i = aspen clone, Dj = defoliation treatment, and T_k = tree. In this model, aspen clone was a random effect and defoliation treatment was a fixed effect. *F*-tests for defoliation treatment, clone × defoliation treatment, and tree(clone) were over the mean square error. Clone was tested over the C_i x D_j interaction. I used the PDIFF option following the LSMEANS statement (PROC GLM, SAS Institute, 1997) to make preplanned *a priori* pairwise comparisons of treatment means within a clone and between same treatments among clones. Prior to analysis, data were checked for normality and homoscedasticity and appropriately transformed if

necessary. To improve the normality, proportional and percentage data (nutritional indices, survival, parasitism) were arcsine-square root transformed prior to analysis.

Some have cautioned that each ramet in an aspen clone ('Tree' in my analysis) may not be entirely independent (Osier et al. 2000), presumably because of concerns about common root connections. This may be more of a problem with young trees than with older trees which have well developed root systems and may sever root connections to parental and adjoining trees entirely (Peterson and Peterson 1992). To my knowledge, only Mattson and Palmer (1988) have looked at the effect of defoliation of individual aspen trees on surrounding ramets. They found that potassium concentration varied with distance, but that there was no effect on any other primary or secondary compound. Individual ramets are used as the experimental unit throughout my study.

RESULTS

EFFECTS OF DEFOLIATION ON CATERPILLARS

Life-long bioassay – In egg to adult bioassays, both aspen clone and defoliation treatments had significant effects on forest tent caterpillar pupal mass. Female pupae were heavier when reared on trees in Clone 1, whereas there was no effect of clone on male pupal mass (Fig 14, Table 21). Pupal mass of both sexes was reduced relative to controls in all three defoliation treatments. However, there was no difference in pupal masses among the three defoliation treatments for females or males. Among trees, male and female pupal masses were highly correlated ($r = 0.82$, $p < 0.001$, n = 40) indicating

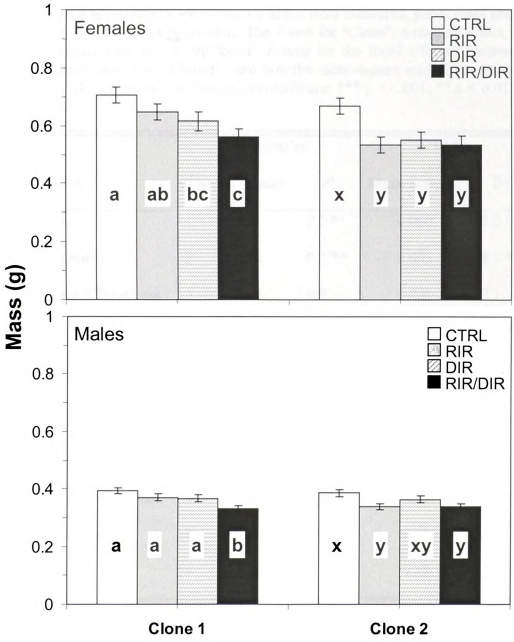


Figure 14. Pupal mass (mean ± SE) for female and male forest tent caterpillars reared from egg hatch to pupation on trees in each of the treatments. Different letters indicate significant pairwise differences between means ($p < 0.05$) following a significant treatment effect in ANOVA. Treatments are CTRL = Control (undefoliated), RIR = one year of defoliation concurrent with the bioassay, DIR = defoliation for three consecutive years previous to the bioassay, and RIR/DIR = defoliation for the three previous years and one year of defoliation concurrent with the bioassay.

Table 21. Results of ANOVA for an egg to adult, on tree bioassay. Thirty caterpillars were reared on each experimental tree. Two variables were measured, pupal mass and the duration of development from egg to adult. The *F*-test for 'Clone', a random effect, was over the mean-square term for 'Tree(Clone)'. *F*-tests for the fixed effects 'Treatment', 'Clone × Treatment', and 'Tree (Clone)' were over the mean-square error term. Analyses were done separately for males and females. Symbols are: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.5$, † $p < 0.10$.

			Females		Males	
Variable	Source	df	Mean Square	*F*	Mean Square	F
Pupal Mass	Clone	1	0.035	7.2 *	0.000	0.6
	Treatment	3	0.036	9.2 **	0.005	8.5 **
	Clone × Treatment	3	0.004	0.9	0.001	0.1
	Tree (Clone)	8	0.005	1.2	0.001	2.0 †
	Error	24	0.004		0.001	
Duration	Clone	1	53.20	62.3 ***	63.06	68.2 ***
	Treatment	3	1.90	1.4	1.78	1.7
	Clone × Treatment	3	1.38	1.0	0.55	0.5
	Tree (Clone)	8	0.85	0.6	0.92	0.9
	Error	24	1.34		1.03	

that defoliation had similar effects on the performance of both sexes. Relative to controls, females were 8 and 18% smaller after a single concurrent defoliation of Clones 1 and 2, respectively, reflecting effects of RIR. In both clones, female pupae were 15% smaller on trees with three previous years of defoliation, reflecting the effects of DIR. Three years of previous defoliation, coupled with an additional concurrent year of defoliation, manifests the combined effects of RIR and DIR. In this treatment, female pupal mass was reduced by 17% and 20% in Clone 1 and 2, respectively. Although pupal masses of both male and female forest tent caterpillars were reduced by defoliation, the duration of development from egg to adult was not affected for either males or females (Fig. 15, Table 21). There were significant clonal effects however, with the development times of both males and females longer on Clone 2.

Using the regression equation derived for this forest tent caterpillar population, the effects of the treatments on female pupal mass can be estimated as decreases in fecundity. In the single year defoliation treatment (RIR), fecundity was reduced by 23.0 eggs (8.5%) and 56.3 eggs (21.7%) in Clones 1 and 2, respectively. Three years of previous defoliation (DIR) decreased egg production in Clone 1 females by 37 eggs (13.5%) and by 49 eggs (18.7%) in Clone 2 females. The combined effects of DIR and RIR reduced fecundity by 59 eggs (21.5%) and 55 eggs (21.3%) in females feeding on trees in Clones 1 and 2, respectively. While reductions in pupal mass of similar magnitude to that of females occurred in males, no direct effect on male fitness was assessed in our study.

Short term bioassays - I found no significant effect of the defoliation treatments on relative growth (RGR) of second instars (Fig. 16, Table 22). Caterpillars fed foliage from Clone 2 had significantly higher RGR than those feeding on Clone 1. There was no

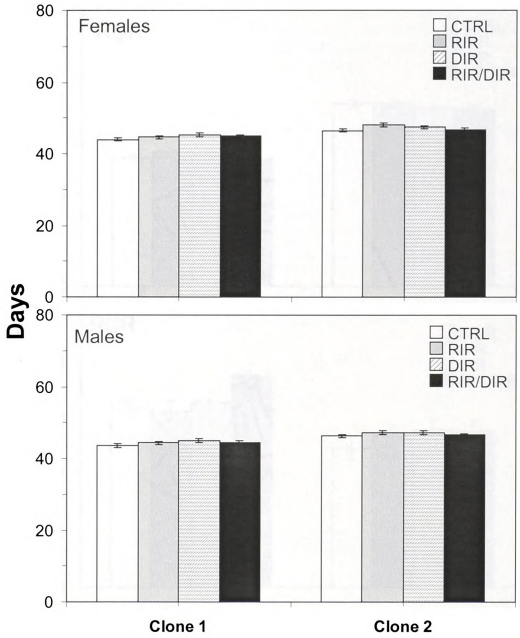


Figure 15. Development time (mean ± SE) for female and male forest tent caterpillars reared from hatch to pupation on trees from each of the treatments. Means comparisons done only following significant treatment effect ($p < 0.05$) in overall ANOVA. Treatments are CTRL = Control (undefoliated), RIR = one year of defoliation concurrent with the bioassay, DIR = defoliation for the three years previous to the bioassay, and RIR/DIR = defoliation for the three previous years and one year of defoliation concurrent with the bioassay.

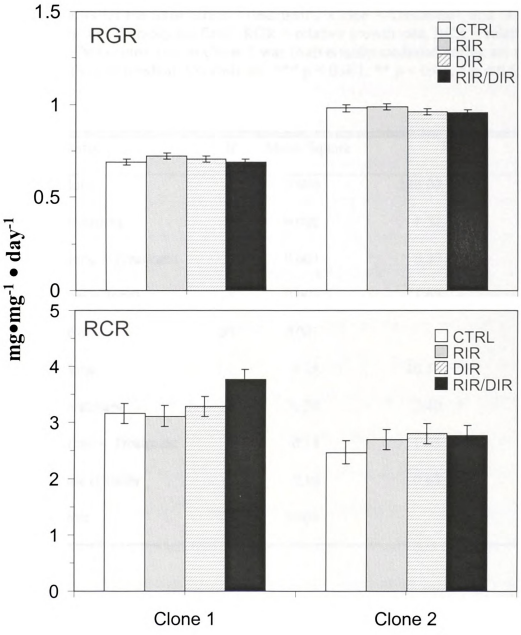


Figure 16. Mean (± SE) relative growth rates (RGR) and relative consumption rates (RCR) for second instar forest tent caterpillar. Treatments are CTRL = Control (undefoliated), RIR = one year of defoliation concurrent with the bioassay, DIR = defoliation for three consecutive years previous to the bioassay, and RIR/DIR = defoliation for the three previous years and one year of defoliation concurrent with the bioassay.

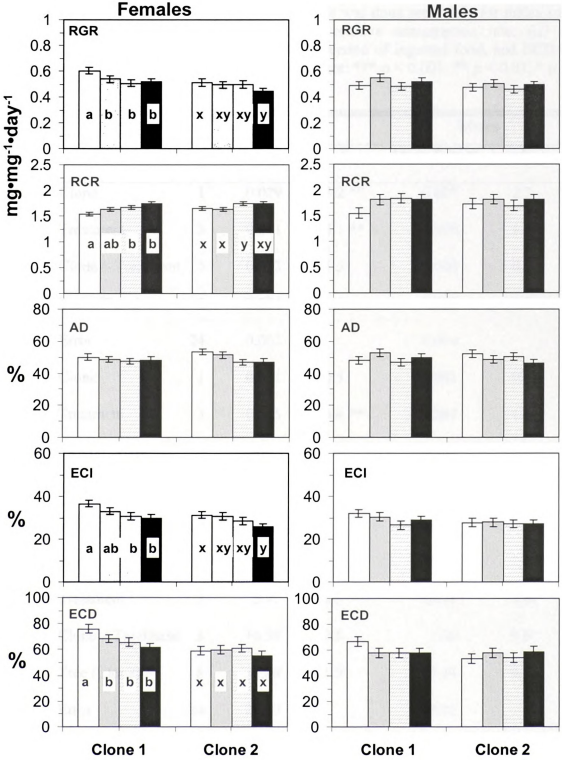
Table 22. Results of ANOVA for short-term bioassay using second instar forest tent caterpillar larvae. The F-test for 'Clone', a random effect, was over the mean-square for 'Tree(Clone)'. *F*-tests for the fixed effects 'Treatment', 'Clone × Treatment', and 'Tree (Clone)' were over the Mean-Square Error. RGR = relative growth rate, RCR = relative consumption rate. One control tree in Clone 2 was inadvertently excluded during set up, resulting in 38 degrees of freedom. Symbols are: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.5$, † $p < 0.10$.

Variable	Source	df	Mean Square	*F*	
RGR	Clone	1	0.696	288.09	***
	Treatment	3	0.002	1.32	
	Clone × Treatment	3	0.001	0.37	
	Tree (Clone)	8	0.001	1.63	
	Error	23	0.001		
RCR	Clone	1	4.11	40.30	***
	Treatment	3	0.39	2.40	†
	Clone × Treatment	3	0.18	1.03	
	Tree (Clone)	8	0.10	0.63	
	Error	23	10.08		

interaction between treatment and clone, or any effect of trees within clones, on second instar relative growth rates. As with relative growth rate, relative consumption (RCR) was significantly affected by aspen clone (Fig 16, Table 22). Relative consumption was higher for larvae feeding on foliage from trees in the defoliated treatments although the difference was only marginally significant. The elevation in RCR suggests that even early instars may compensate for defoliation-induced changes in host quality, since RGR remained constant across treatments. Alternatively, any changes in the young leaves induced by previous and current year defoliation by early instars were relatively slight, and had only small effects on the feeding behavior and growth of second instars.

The effects of the defoliation treatments on caterpillars were more pronounced on fifth than on second instars. Females were more sensitive to treatments than males. Female RGR in the fifth instar was significantly affected by clone and in contrast to the second instars was higher on Clone 1 foliage (Fig. 17, Table 23). Relative growth rate of females was significantly reduced when fed foliage from defoliation treatment trees. Conversely, RGR of males was unchanged among clones and was not significantly affected by defoliation treatments. Relative consumption rates did not differ among clones for either males or females. Female RCR was significantly lower on control tree foliage than on leaves from treatment trees. There was no significant effect of clone or defoliation treatments on approximate digestibility (AD) of either females or males. The efficiency of conversion of ingested food (ECI) varied significantly among clones for males and marginally so for females. Both sexes had lower ECI when reared on foliage from all of the defoliated treatment when compared to undefoliated controls. There was

Figure 17. Mean (± SE) relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of ingested foliage to biomass (ECI), and efficiency of conversion of digested foliage to biomass (ECD) for final (fifth) instar female and male forest tent caterpillars. Treatments are CTRL = Control, RIR = one year of defoliation concurrent with insect bioassay, DIR = defoliation for the three years previous to insect bioassay, and RIR/DIR = defoliation for the three previous years and in the year concurrent with the insect bioassay.


Females
Males
RGR
RCR
AD
ECI
ECD
mg•mg-1•day-1
%
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%
a b b b
x xy xy y
a ab b b
x x y xy
a ab b b
x xy xy y
a b b b
x x x x
Clone 1
Clone 2
Clone 1
Clone 2

Table 23. Results of ANOVA for short-term bioassay of fifth instar forest tent caterpillar larvae. The *F*-test for 'Clone', a random effect, was over the mean-square for 'Tree(Clone)'. *F*-tests for the fixed effects 'Treatment', 'Clone × Treatment', and 'Tree (Clone)' were over the Mean-Square Error. Analysis was done separately for males and females. RGR = relative growth rate, RCR = relative consumption rate, AD = approximate digestibility, ECI = efficiency of conversion of ingested food, and ECD = efficiency of conversion of digested food. Symbols are: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.5$, † $p < 0.10$.

			Females		Males	
Variable	Source	df	Mean Square	*F*	Mean Square	*F*
RGR	Clone	1	0.029	8.2 **	0.007	2.3
	Treatment	3	0.011	5.1 **	0.006	1.4
	Clone × Treatment	3	0.003	1.5	0.000	0.1
	Tree (Clone)	8	0.004	1.7	0.003	0.8
	Error	24	0.002		0.004	
RCR	Clone	1	0.021	0.5	0.001	0.0
	Treatment	3	0.045	6.4 **	0.069	1.4
	Clone × Treatment	3	0.009	1.3	0.044	0.9
	Tree (Clone)	8	0.043	6.2 **	0.057	1.1
	Error	24	0.007		0.048	
AD	Clone	1	118.89	3.2	46.09	2.5
	Treatment	3	5.97	0.3	53.38	1.6
	Clone × Treatment	3	16.39	0.8	1.26	0.0
	Tree (Clone)	8	37.29	1.9	18.44	0.5
	Error	24	20.19		34.02	
ECI	Clone	1	182.91	4.5 †	46.70	10.7 *

Cont'd

Table 23 cont'd

	Treatment	3	54.38	6.2 **	13.75	0.7
	Clone × Treatment	3	5.06	0.6	12.60	0.6
	Tree (Clone)	8	40.51	4.6 **	4.38	0.2
	Error	24	8.78		19.83	
ECD	Clone	1	228.88	2.3	27.57	1.4
	Treatment	3	340.71	7.2 **	121.69	1.6
	Clone × Treatment	3	65.53	1.4	62.96	0.8
	Tree (Clone)	8	101.85	2.1 †	19.24	0.3
	Error	24	47.67		78.22	

no effect of clone on efficiency of conversion of ingested food (ECD) for either sex but females had significantly lower ECD when consuming foliage from defoliated trees.

There was no correlation between the mean RGR for second instars on each tree and pupal mass of males (r = -0.14, $p < 0.41$, n = 39) but second instar RGR was negatively correlated with pupal masses of females (r = -0.34, $p < 0.04$, n =39). Male pupal mass and fifth instar RGR was not correlated (r = -0.13, $p < 0.42$, n = 40) but fifth instar RGR was significantly correlated with female pupal mass (r = 0.40, $p < 0.02$, n =40). The low proportion of the variance in pupal mass that was explained by RGR over the first three days of the final instar suggests that the treatment effects on pupal mass may be due primarily to foliage quality experienced in the latter part of the final instar.

EFFECTS OF TREATMENTS ON SURVIVAL AND PARASITISM

Survival of larvae from hatch to pupation was generally high (937 out of 1200 successfully pupated) and did not vary among the treatments although there was a trend toward lower survival on defoliated trees (Fig. 16, Table 24). Mortality in the sleeve cages was classified as unknown if the cocoons or puparia of parasitoids were not found. Observations suggested that the primary cause of the unknown mortality were pentatomid bugs that preyed on caterpillars resting on the sides of the sleeve cages. Due to the obvious liquefaction of caterpillars that succumb to NPV, I am confident that this pathogen killed none of the bioassay caterpillars.

Larvae were attacked by several species of parasitoids. Low levels of parasitism (<1%) were caused by the braconid parasitoids *Meteorus* sp. and *Hypositor fugivitus*. I was concerned that the sleeve cages might interfere with these species, but similar levels

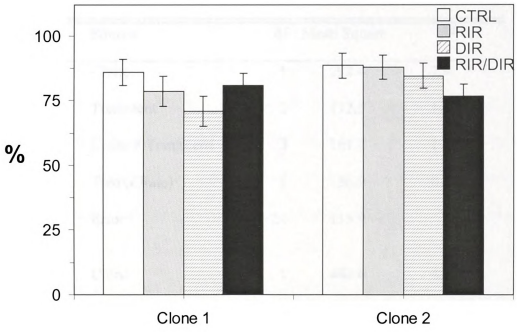


Figure 18. Percent survival (mean ± SE) of forest tent caterpillar reared from hatch to pupation on trees from each of the treatments. Analysis were done on arcsine-square root transformed data. Survival data includes caterpillars that were parasitized. Means comparisons done only following a significant ($p < 0.05$) treatment effect in overall ANOVA. Treatments are CTRL = Control, RIR = one year of defoliation concurrent with the bioassay, DIR = defoliation for the three years previous to the bioassay, and RIR/DIR= defoliation for the three previous years and one year of defoliation concurrent with the bioassay.

Table 24. Results of ANOVA for survival and parasitism of forest tent caterpillar in the egg to adult bioassay. To determine if tree-mediated treatment effects influenced survival, individuals that were parasitized were counted as surviving and the treatment effects on parasitism were analyzed separately. Analyses were done using arcsine-square root transformed data to meet assumptions of normality. The *F*-test for 'Clone', a random effect, was over the mean-square for 'Tree(Clone)'. *F*-tests for the fixed effects 'Treatment', 'Clone × Treatment', and 'Tree (Clone)' were over the Mean-Square Error. Symbols are: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.5$, † $p < 0.10$.

Parameter	Source	df	Mean Square	*F*	
Survival	Clone	1	292.4	7.9	*
	Treatment	3	172.5	1.5	
	Clone × Treatment	3	161.2	1.4	
	Tree (Clone)	8	36.9	0.3	
	Error	24	115.9		
Parasitism	Clone	1	482.6	4.5	†
	Treatment	3	681.2	9.2	***
	Clone × Treatment	3	96.1	1.3	
	Tree (Clone)	8	107.9	1. 5	
	Error	24	74.29		

were recorded from samples of caterpillars from outside of the sleeve cages. A larger source of mortality in the experiment was due to the multivoltine and highly polyphagous tachind *Lespesia frenchii*. This fly attacks caterpillars directly by cementing eggs to larval setae. Parasitism rates were much greater in caterpillars collected from outside of the bags (30%) than in the bioassay larvae (2%). Of the mortality attributable to parasitoids in bioassay larvae, 78% was due to the tachinid flies *Leschenaultia exul* and *Patelloa pachypyga*, which are specialists on forest tent caterpillar (see Parry 1995, Parry *et al.* 1997 for the biology of these species). Although they emerge from pupae, both species are actually parasitoids of fourth and fifth stage larvae. Adults deposit microtype eggs on foliage, which must be ingested for parasitism to occur. In my experiment, virtually all of the mature maggots emerged from pupae, although *L. exul* maggots may also emerge from prepupational larvae (Parry 1995). A proportion of the unknown mortality may be due to this fly, as the maggots are capable of escaping by burrowing through the mesh of the sleeve cages. Since these two species have nearly identical lifecycles, their parasitism was pooled for analysis.

There were significant differences in parasitism rates among treatments and also a marginal significant difference among clones (Fig. 17, Table 24). The highest parasitism rates occurred on trees in the RIR and RIR/DIR treatments, where large numbers of larvae were defoliating the trees concurrent with estimates of parasitism rates. In the DIR treatment, caterpillar densities were equivalent to controls because only the bioassay larvae were feeding on the foliage in the year that parasitism was assessed. There was no difference in the rate of parasitism among control trees and the DIR treatment. This

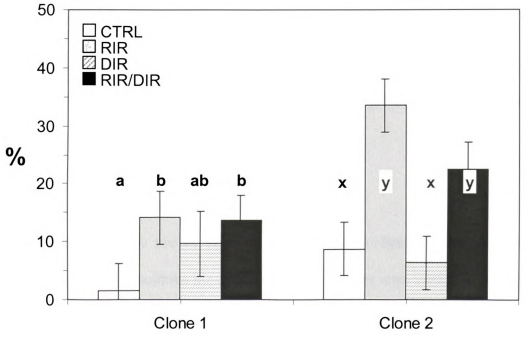
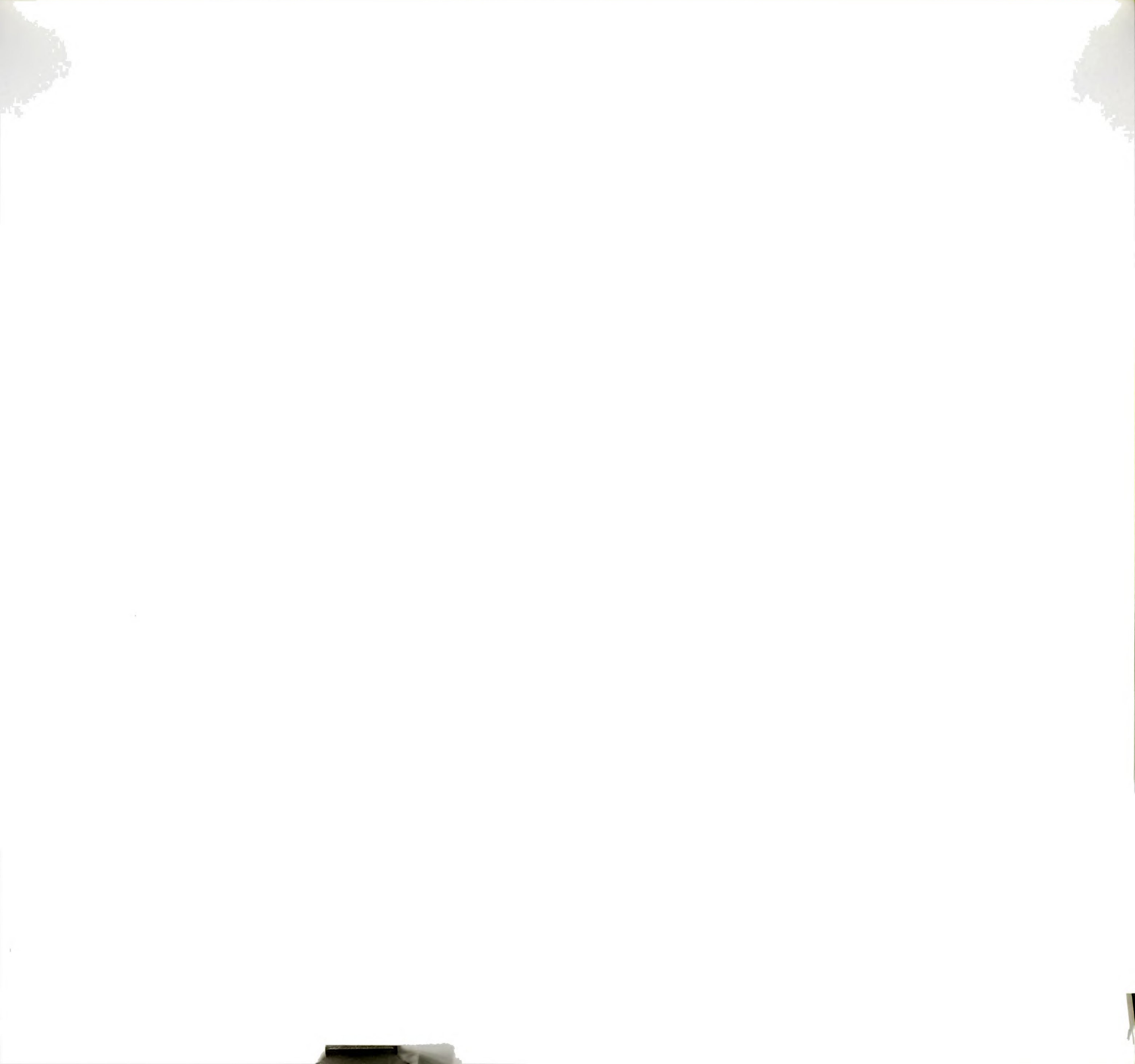


Figure 19. Percent parasitism rate (mean ± SE) of forest tent caterpillar reared from hatch to pupation on trees in each treatment. Analysis was done on arcsine-square root transformed data. Different letters indicate significant pairwise differences ($p < 0.05$) among means following significant treatment effects in ANOVA. Treatments are CTRL = Control, RIR = one year of defoliation concurrent with the bioassay, DIR = defoliation for the three years previous to the bioassay, and RIR/DIR= defoliation for the three previous years and one year of defoliation concurrent with the bioassay.



suggests that the parasitoids were responding in a spatially-density dependent manner and were not influenced by the treatment history of the trees.

Relative to the effects of induced-resistance, the effect of larval parasitism on approximate reproductive rates was variable (Table 25). In Clone 1, the additional negative effects of parasitism on R_0 ranged from 9-14% and values remained very high. In Clone 2, parasitism reduced R_0 below the effects of induced-resistance by an additional 40% in RIR and additional 30% in RIR/DIR. The data from natural populations yielded reductions in R_0 similar to the values obtained from the experimental populations. The very high R_0 values in all treatments suggest that large additional reduction in fecundity and/or increases in mortality are required to slow population growth to rates observed in natural populations.

DISCUSSION

This study was the first to use an experimentally created outbreak to emulate natural defoliation processes and examine the effects of short and long term induction on a North American defoliator. The experiment was run for four years, an appropriate time span for emulating the effects of natural forest tent caterpillar outbreaks on trees. Furthermore, the unique design allowed me to isolate the relative contributions of RIR and DIR to herbivore performance. I found that forest tent caterpillar defoliation elicited both short-term and long-term induced responses in aspen. All three of the defoliation treatments reduced the growth, pupal mass, and fecundity of forest tent caterpillars relative to control trees. Contrary to my hypothesis however, the effects of three and four

Table 25. Approximate reproductive rates (R_0) for each treatment. Reproductive rates were calculated for surviving females with and without parasitism. Initial cohorts had 30 female equivalents. Numbers in parentheses are percentage decreases from control values in each row. Larval parasitism rates in natural outbreak populations were obtained from Parry (1995) (see text). † Data on parasitism in natural low-density populations corresponding to the controls are not available so values from my control plots were substituted.

Clone	Comparison	CTRL	RIR	DIR	RIR/DIR
1	No parasitism	114.7	95.0 (-17.2)	78.4 (-31.6)	86.4 (-24.7)
	Parasitism	112.7	77.4 (-31.3)	66.8 (-40.7)	71.3 (-36.7)
2	No parasitism	112.3	88.8 (-20.9)	93.7 (-16.5)	78.7 (-29.9)
	Parasitism	105.5	53.7 (-49.1)	86.7 (-17.8)	55.1 (-47.8)
1	Parasitism rates in natural populations	112.7†	69.7 (-38.1)	66.8 (-40.1)	38.3 (-66.0)
2	Parasitism rates in natural populations	105.5†	79.5 (-24.6)	82.9 (-21.4)	44.8 (-57.5)

consecutive years of defoliation were not significantly greater than the reduction in caterpillar performance caused by a single year of defoliation.

Rapid-induced resistance had surprisingly strong effects on forest tent caterpillar growth. In previous studies, the effects of RIR on the forest tent caterpillar have been variable. For example, Parry *et al.* 2000 found that gypsy moth defoliation of *Populus* reduced pupal masses of forest tent caterpillar feeding on the same trees by 10%. Similarly, Robison and Raffa (1997) found that partial defoliation of two different poplar cultivars significantly reduced the growth rates of second instar tent caterpillars. In contrast, Roth et al. (1998) found no significant effect of defoliation on forest tent caterpillar feeding on aspen. Cappuccino *et al.* (1995) found no deleterious effects of herbivory on forest tent caterpillar, but rather observed increased pupal mass of males feeding on damaged birch leaves. Differences in defoliation levels among these studies could account for the variable results. The data of Roth et al. (1998) indicated that most of the changes in aspen phytochemistry induced by forest tent caterpillar defoliation occurred in response to feeding by final instar (fifth) caterpillars which account for approximately 80% of the total larval consumption (Hodson 1941). At the time of their fourth instar bioassays, little phytochemical change was evident between control and defoliated trees, and by the end of the experiment, trees were only ca. 50% defoliated. In my study, no treatment differences in relative growth rates of second instars were found. Growth rates of female fifth instars were only modestly correlated with female pupal mass while male growth rate and male pupal mass were not correlated. In addition, development time was not significantly lengthened by the defoliation treatments for either sex. This suggests that RIR is primarily a response to feeding damage in the latter

part of the final instar where, in my experiment, defoliation exceeded 70% on all treatment trees and was higher than 90% on some trees. As Roth et al.'s (1998) data also suggested, lighter defoliation levels (30-60%) have little effect on forest tent caterpillar growth, at least in one-year studies (D. Parry and D.A. Herms unpublished). Removal of some aspen leaves has been shown to increase nitrogen in the remaining leaves (Mattson and Palmer 1988) which could account for the lack of negative effects at lower levels of defoliation. Conversely, the late onset of RIR may account for the reduced performance of gypsy moth, white-marked tussock moth, *Orgyia leucostigma*, and tiger swallowtail, *Papilio canadensis,* larvae feeding on birch, sugar maple, and aspen trees moderately defoliated by forest tent caterpillar earlier in the season (Dankert et al. 1997).

Forest tent caterpillar may compensate for reductions in foliar quality by increasing relative consumption rates (RCR). Williams et al. (1998) found that FTC increased consumption rates by 8% on white oak foliage with lower nitrogen levels. I found that on aspen, both early and late instars were capable of increasing consumption rates. In second instars, elevation of RCR on defoliated treatments was only of marginal statistical significance although it may have served to maintain equal RGR across all treatments. In final instars, female consumption rates were 6% higher in response to RIR in Clone 1 and were unchanged in Clone 2, while male consumption rates were 15% higher in Clone 1 and 5% higher in Clone 2. Although females increased consumption by 9-12% and 5-12% in Clone 1 and Clone 2, respectively, in response to DIR and the combined effects of RIR and DIR, the changes in RCR by final instars were insufficient to prevent a significant reduction in pupal mass.

Change in nitrogen utilization is an aspect of compensatory feeding behavior in forest tent caterpillar not investigated in my study. Williams et al. (1998) found that although RCR increased on low nitrogen foliage, most of the forest tent caterpillar's ability to compensate for poor quality food was through a large increase in nitrogen utilization efficiency. Final instars were able to increase nitrogen utilization efficiency by as much as 20% when consuming low nitrogen leaves. The mechanism underlying this physiological behavior is not understood, although it would undoubtedly be adaptive for an outbreak species that frequently must process damaged foliage with low nitrogen levels.

The effects of DIR on herbivore performance have been best studied in the mountain birch systems of northern Fennoscandia. In the year following defoliation, the quality of leaves of mountain birch are reduced for its primary herbivore, the autumnal moth, as well as other species and these effects may linger for several years (Tuomi et al. 1990). Mechanisms underlying the reduction in performance of caterpillars on previously defoliated mountain birch are not fully understood. Elevation of secondary phenolics including gallotannins and proanthocyanidins as well as decreases in foliar nitrogen may contribute to observed reductions in pupal mass and fecundity (Haukioja et al. 1985, Kaitaniemi et al. 1998). Some research has suggested that a suite of traits may interact and undergo phenological shifts so that no single measure of leaf quality had significant effects on the growth of both early and late instars (Kause et al. 1999). Instead, gallotannins, proanthocyanidins, foliar nitrogen, and water all contributed at different times to the performance of larvae. Reductions in autumnal moth fecundity exhibit large variation depending on the year and the outbreak, although more than 90% of the studies

reviewed showed some DIR related reduction in fecundity (Ruohomaki et al. 1992, Kaitaniemi et al. 1999a).

Only one study in the mountain birch system has examined the effects of multiple seasons of defoliation on autumnal moth performance, a species with outbreaks that persist from 1-3 years at any one locality (Bylund 1995). Kaitaniemi et al. (1999b) found that two consecutive years of defoliation did not have greater effects than a single season of 75% defoliation on the growth and fecundity of either autumnal moth or the winter moth, *Operophtera brumata* (Lepidoptera: Geometridae). In contrast, defoliation of trees for two or more years in succession in other systems has had additive deleterious effects on the herbivores assayed. With each successive year of defoliation of black oak, Valentine et al. (1983) found increasingly negative effects on gypsy moth pupal mass. Similarly, Werner (1979) found that survival of black-marked spear moth decreased with consecutive years of defoliation of Alaska paper birch, while Clausen et al. (1991) found that defoliation of aspen from 1-3 years had additive negative effects on pupal mass of the large aspen tortrix. I found that three consecutive years of defoliation reduced fecundity by 13.5% and 18.7% in Clones 1 and 2, respectively, translating into 37 and 49 fewer eggs per female. However, these effects were not significantly lower than those caused by RIR resulting from a single defoliation. Furthermore, although the reduction in fecundity was greatest in the four-year defoliation treatment (ca. 22% in both clones), the combined effects of RIR and DIR did not differ from the single-year defoliation treatment. This suggests that there may be an upper threshold for induced resistance in aspen beyond which additional response is not physiologically possible.

The basis for induced-resistance in aspen is not well understood. Levels of primary compounds, including water and nitrogen, decrease in damaged leaves (Roth et al. 1998). Clausen et al. (1991) suggested that leaf size was one of the best predictors of large aspen tortrix performance on defoliated trees. Reductions in leaf size are a common response of trees in the year following severe defoliation and are thought to be a function of the decrease in available nitrogen (Tuomi et al. 1984, 1990). Smaller leaf sizes are often accompanied by increase in secondary phenolics and in some cases leaf toughness. Studies have shown that the constitutive level of nitrogen is the best predictor of forest tent caterpillar performance among aspen clones (Hwang and Lindroth, 1995, 1997), thus changes in foliar nitrogen levels may also determine performance on defoliated trees. In other *Populus* species, defoliation-induced reductions in nitrogen were associated with reduced performance of forest tent caterpillar (Robison and Raffa 1997, Parry et al. 2000a).

Phenolic glycosides and condensed tannins, products of the shikimic acid pathway, dominate the secondary chemistry of aspen. Clausen et al. (1989) suggested that RIR in aspen was due to increased production of phenolic glycosides and developed a pathway to describe the phytochemical changes involved in the RIR response following herbivory (see also Clausen et al. 1991, Lindroth and Hwang 1996). They suggested that the phenolic glycosides salicortin and tremulacin increase significantly in the first 24 hours following feeding damage. When ingested by caterpillars, these unstable compounds are rapidly converted to salicin, tremuloiden and the byproduct 6-HCH. When salicortin, tremulacin, and the by-product 6-hydroxy-2-cyclohexenone (6-HCH) were incorporated in to artificial diets, they had deleterious effects on the growth of large aspen tortix.

However, in subsequent experiments, inducibility has been inconsistent. Roth et al. (1998) showed that forest tent caterpillar herbivory had no significant on levels of salicortin, while tremulacin increased initially but then decreased to levels below that of controls. In other *Populus* species, the response of phenolic glycosides to defoliation has also been unpredictable with significant increases in one year and no change in the following year (Parry et al. 2000a). In contrast to the instability of phenolic glycosides, condensed tannins concentrations were elevated by defoliation in all of these experiments. However, condensed tannins in aspen and other poplars while readily inducible, appear to have little effect on a variety of herbivorous insects (Roth et al. 1998, Ayres et al. 1997, Lindroth and Kinney 1998, Parry et al. 2000a). Clausen et al. (1991) suggested that RIR and DIR in aspen are related processes. My experiment agrees with this premise because RIR and DIR individually had effects on larval performance of similar magnitude to that of RIR and DIR combined.

The costs of induction relative to any accrued benefits is an aspect of induced-resistance in aspen and other trees that has received little attention. In large-scale outbreaks of the forest tent caterpillar in boreal forests, virtually all woody deciduous species are defoliated, and few if any aspen trees are spared even in areas where hundreds of aspen clones/hectare occur. This suggests that despite wide variability in constitutive and induced resistance in aspen clones, the end result may still be complete defoliation during outbreaks (Mattson et al. 1991). The proposition that variability in defoliation intensity is due to clonal differences in phenolic glycosides (e.g., Lindroth and Hwang 1996), is speculative and has not been confirmed experimentally. In extensive ground and aerial surveys of forest tent caterpillar outbreaks in Canada, variation in defoliation

intensity appeared to be driven more by features of the landscape than any distinct clonal attributes (D. Parry, personal observations). Tree phenology, oviposition preferences by females, and spatial heterogeneity in mortality from natural enemies may all contribute to variation in defoliation levels among trees, and should be investigated before differences in herbivory are ascribed to any phytochemical attribute. Thus, while there are phytochemically based differences in the performance of forest tent caterpillar among clones, at outbreak densities, trees are defoliated irrespective of biochemical components.

In aspen, chemical defenses are costly in terms of their trade-off with growth (Hwang and Lindroth (1997). If defoliation is inevitable for 2-5 years in succession, trees with the greatest investment in resistance may actually incur the highest fitness costs since they will be defoliated anyway, but will grow more slowly and be less competitive in the absence of defoliation (i.e., Herms and Mattson 1992). Mattson et al. (1991) suggested that in aspen forests susceptible to frequent forest tent caterpillar outbreak, tolerance to defoliation might represent the optimal life-history strategy. Assessing the benefits and costs of resource allocation to resistance during widespread outbreaks is likely to be a productive area of future research.

Parasitoids undoubtedly play an important role in the population dynamics of forest tent caterpillar (Sippell 1957, Hodson 1977, Witter and Kulman 1979, Parry 1995, Parry et al. 1997). While parasitoids probably do not initiate population decline, the action of larval and pupal parasitoids in older outbreaks may hasten the demise of populations and drive them to very low levels. I found no evidence of synergistic or antagonistic effects of either RIR or DIR and the dominant larval parasitoids. Highest parasitism levels occurred in the two treatments where, in addition to the bioassay larvae, large numbers of

caterpillars were used to defoliate the trees. In the year that parasitism rates were assessed, the control and DIR treatment had only the bioassay larvae feeding on the trees. Conversely, the RIR and COMB treatments had high densities of larvae used to defoliate the trees in addition to the bioassay larvae. Parasitism rates were equivalent among the two treatments with low larval densities (CNTL and DIR) and were significantly higher but equivalent between the two high-density treatments (RIR and COMB). This result indicates that the response of the tachinids was driven by spatial responses to host density rather than an effect of the induction treatments. Spatial density dependent responses have been shown previously for both *P. pachypyga* and *L. exul* (Parry et al. 1997).

Although only marginally significant, there were intriguing differences in parasitism rates among the two clones. Since the aspen clones were separated by only a short distance, were similar in foliar area, tree size, proximity to clearings, and insolation, and caterpillar densities were approximately equal, the markedly higher rates of parasitism in Clone 2 may reflect an interaction between phytochemistry and tachinid olfaction. Both *L. exul* and *P. pachypyga* locate tent caterpillars using volatiles given off by leaves damaged by larval feeding (Bess 1936, Mondor and Roland 1997, 1998). Thus, there may be subtle differences in the attractiveness of clone specific combinations of the phytochemicals important in host-detection. To my knowledge, mediation of interactions between parasitoids and hosts through intraspecific variation in chemistry of trees has not been explored in a field environment. An interaction between natural enemies and phytochemical variation in host plants could be an important component of spatial heterogeneity in herbivore population dynamics.

A longstanding criticism of the role of induced-responses in plants is their relevance for insect population dynamics. Fowler and Lawton (1985) suggested that many of the observed effects of induced resistance, while statistically significant, are small, easily overwhelmed, and rendered unimportant by other processes acting on insect populations. While their point is well taken, the criticism might be most valid for studies looking at RIR elicited by low-level herbivory and at the scale of individual leaves or branches. Such research has revealed large variability in the magnitude and direction of the effects (see Karban and Baldwin 1997 for review). In the context of outbreak species, however, few would argue that reductions in fecundity of 40% or more caused by DIR, documented in some experiments with autumnal moth on mountain birch (e.g., Ruohomaki et al. 1992), do not have measurable impact on population level processes.

For DIR to play an important role in population dynamics, significant decreases in fecundity must be manifested at the population level. It is well established that large reductions in fecundity are characteristic of declining populations of many outbreak folivores (Mason et al. 1977, Baltensweiler and Fischlin 1988, Carter et al. 1991). Similar trends are evident in forest tent caterpillar populations. Following several years of defoliation, forest tent caterpillar egg masses can be 50% smaller than early in an outbreak (Ives 1971, Witter et al. 1975, D. Parry, unpublished). However, DIR is not the only mechanism operating in outbreak population that can reduce fecundity, thus it is important to consider alternative hypotheses.

Undoubtedly, a lack of food contributes to reductions in the average egg mass size of defoliating Lepidoptera, especially in years of peak densities when virtually all edible foliage in a forest is consumed. Hodson (1941) found that partial starvation of final instar

forest tent caterpillar reduced fecundity by 45%. Field data also indicate that female pupal mass can be dramatically reduced in stands with complete defoliation (Hodson 1941, Parry *et al.* 2000b). In a study of forest tent caterpillar population dynamics in Minnesota, Witter et al. (1975) showed that fecundity was lowest in the year of highest density suggesting that intraspecific competition for foliage was a primary determinant of clutch size. In high-density populations of forest tent caterpillar, most of the aspen foliage is removed prior to the final larval molt. Final instars must then complete development on secondary host trees and shrubs after they completely consume all remaining aspen foliage. The quality of many secondary hosts is considerably lower than aspen. When final instars were switched from aspen to foliage from the five most abundant secondary host species in boreal aspen forests, fecundity was reduced by 5-31% (D. Parry & J.R. Spence, unpublished). Thus, even in forests where densities are not sufficient to cause starvation, fecundity can be reduced when aspen foliage is depleted prior to pupation. In outbreak species where adults of both sexes are capable of efficient dispersal, such as the coniferophagous budworms, *Choristoneura* spp., large aspen tortrix, and tent caterpillars, emigration of moths from large outbreaks may swamp processes operating in local populations. Thus low or high fecundity may be a property of the dispersing individuals and not of processes operating in the forest in which oviposition occurs.

While factors such as RIR and competition for resources can account for reductions in fecundity within a season, the effect is directly density dependent and fecundity should immediately rebound following population decrease. However, in some defoliator populations, fecundity does not appear to follow this pattern. Myers and Kukan (1995) showed that for western tent caterpillars, fecundity appeared to increase, or at least

remained stable, even as densities were increasing. Fecundity decreased in later outbreak years, but remained low even as populations collapsed. There is some evidence that similar patterns in fecundity may occur in forest tent caterpillar. A long-term study in Minnesota suggested that fecundity fluctuated considerably before declining during the last few years of the outbreak (Witter et al. 1975). Unfortunately, the study was initiated several years into the outbreak and values from the first few years were not recorded. Batzer et al. (1994) monitored populations for five years and found that fecundity decreased in three stands as outbreaks declined, but fecundity changed little in one stand while an increase was observed in another stand. During an outbreak in Alberta, Canada, fecundity was highest in the first two years of outbreak and was lower in later years (Ives 1971). These studies suggest that some time-lagged process or processes contribute to changes in forest tent caterpillar fecundity over the course of an outbreak.

A number of authors have suggested that reduced fecundity and survival caused by induced-resistance may be responsible for driving cycles of forest defoliators (e.g., Benz 1974, Haukioja 1980, Rhoades 1983). Others have been critical of an important role for DIR in the forest insect populations (e.g., Myers 1988a, b, 1993). The main criticism of hypotheses based on plant quality is that outbreak populations may collapse simultaneously across large regions irrespective of their defoliation histories. Furthermore, manipulative studies where populations were cropped or moved to undefoliated areas failed to prevent declines (Myers 1981, 1990).

Myers (1993) suggested that instead of induced-resistance, sublethal infection by baculoviruses such as NPV is a more parsimonious explanation for fecundity declines. This hypothesis proposes that virus loads increase in the environment when populations

are high. Increasing numbers of females with sublethal infection lowers average fecundity. Since NPV may persist for some years in the environment, there may be a time lag in the recovery of fecundity levels to preoutbreak levels. While NPV treatments are associated with modest reductions of western tent caterpillar in laboratory studies (Rothman and Myers 1994), there were no differences in fecundity among infected and uninfected high-density populations in a field study (Rothman 1997). Moreover, extensive research with autumnal moth and larch budmoth, two systems which show the most regular cycles, have failed to demonstrate that pathogens are of much importance (Baltensweiler and Fischlin 1988, Bylund 1995). There is also no evidence to suggest that sublethal NPV infections impact fecundity of gypsy moth (Murray and Elkinton 1989). Thus by themselves, sublethal levels of baculovirus does not appear to provide a satisfactory general mechanism for explaining cycles in forest defoliator populations.

Considerable research over the past three decades has focused on identifying a single causal agent that drives the population dynamics of cyclically outbreaking forest defoliators. I suggest that this effort for the most part has been fruitless. While most researchers now agree that delayed-density dependent factors must be involved (Turchin 1990, 1995), several processes are thought to be capable of producing cycles (e.g., Berryman et al. 1987, Ginzburg and Taneyhill 1994, Berryman 1996, Underwood 1999). In fact, recent studies have suggested that even within a species, the relative role of different agents may vary in different population peaks. For example, a granulosis virus infection thought responsible for the collapse of larch budmoth populations in Switzerland was not found to have any impact on previous or subsequent outbreaks in the same area (Baltensweiler and Fischlin 1988). Similarly, Bylund (1995) suggested that

different agents were responsible for the collapse of outbreak autumnal moth populations during three separate population peaks in 1955, 1965, and 1987. Clearly, induced-resistance plays a role in the population dynamics of some outbreak species but rather than operating independently, it should be viewed in context with contemporaneously acting natural enemies. In addition, the interactive effects of plant-quality and natural enemies need to be coupled with spatially synchronizing density-dependent and density-independent processes like dispersal and weather. I echo the call of other authors (e.g., Hunter and Price 1992, Hunter et al. 1997, Karban and Baldwin 1997) that plurality is needed and that viewing plant-quality driven and natural enemy driven hypotheses as mutually exclusive is artificial and counter productive.

I clearly showed that that delayed-density dependent declines in fecundity could be attributed solely to changes in tree quality in this study. Yet DIR induced by defoliation at most decreased fecundity by 22% which is insufficient to account for the 50% or more declines in fecundity that have been recorded in natural forest tent caterpillar outbreaks. Furthermore, parasitoids had a much greater impact on reproductive rates than the effects of induced-resistance. Neither factor was sufficient to slow the approximate reproductive rates of my experimental populations to levels found in nature. My results suggest that the integrating the effects of agents acting contemporaneously on populations is the next major hurdle in understanding the mechanisms underlying cycles of forest Lepidoptera.

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LITERATURE CITED

Agrawal, A.A. 2000. Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Current Opinion in Plant Biology* **3**: 329-335.

Ayres, M.P., T.P. Clausen, S.F. MacLean, Jr., A.M. Redman and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78**: 1696-1712.

Baltensweiler, W. and A. Fischlin. 1988. The larch budmoth in the Alps. Pp. 331-351 in A.A. Berryman, ed, Dynamics of Forest Insect Populations. Patterns, Causes, and Implications. Plenum Press, New York.

Barnes, B.V. 1969. Natural variation and delineation of clones of *Populus tremuloides* and P. grandidentata in northern lower Michigan. *Silvae Genetic* **18**: 130-142.

Batzer, H., M.P. Martin, W.J. Mattson and W.E. Miller. 1994. The forest tent caterpillar in aspen stands: Distribution and density estimation of four life stages in four vegetation strata. *Forest Science* **41**: 99-121.

Benz, G. 1974. Negative feedback by competition for food and space, and by cyclic induced changes in the nutritional base as regulatory principles in the population

dynamics of the larch budmoth, *Zieraphera dineana* (Guenee) (Lepidoptera: Tortricidae). *Zietschrift fur Angewandte Entomologie* **76**: 196-228.

Berryman, A.A., N.C. Stenseth and A.S. Isaev. 1987. Natural regulation of herbivorous forest insect populations. *Oecologia* **71**: 174-184.

Berryman, A.A. 1996. What causes population cycles of forest Lepidoptera? *Trends in Ecology and Evolution* **11**: 28-32.

Bess, H.A. 1936. The biology of *Leschenaultia exul* Townsend, a tachinid parasite of *Malacosoma americana* Fabricius and *Malacosoma disstria* Hübner. *Annals of the Entomological Society of America* **29**: 593-613.

Bryant, J.P., P.B. Riechardt, T.P. Clausen and R.A.Werner. 1993. Effects of mineral nutrition on delayed inducible resistance in Alaska paper birch. *Ecology* **74**: 2072-2084.

Bourchier, R.S. 1991. Growth and development of *Compsilura concinnata* (Miegan) (Diptera: Tachinidae) parasitizing gypsy moth larvae feeding on tannin diets. *The Canadian Entomologist* **123**: 1047-1055.

Byland, H. 1995. Long-term interactions between the autumnal moth and mountain birch: the role of resources, competitors, natural enemies, and weather. PhD. thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Cappuccino, N., H. Damman and J.F. Dubuc. 1995. Spatial behavior and temporal dynamics of outbreak and nonoutbreak species. Pp. 65-82 in N. Cappuccino and P.W. Price, eds,. Population Dynamics: New Approaches and Syntheses. Academic Press, San Diego.

Carter, M.R., F.W. Ravlin and M.L. McManus. 1991. Changes in gypsy moth (Lepidoptera: Lymantriidae) fecundity and male wing length resulting from defoliation. *Environmental Entomology* **20**: 1042-1047.

Clausen, T.P., P.B. Reichardt, J.P. Bryant, R.A. Werner, K. Post and K. Frisbee. 1989. A chemical model for short-term induction in quaking aspen (*Populus tremuloides*) foliage against herbivores. *Journal of Chemical Ecology* **15**: 2335-2346.

Clausen, T.P., P.B. Reichardt, J.P. Bryant and R.A. Werner. 1991. Long-term and short-term induction in quaking aspen: related phenomena? Pp.71-83 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Dankert, B.A., D.A. Herms, D. Parry, J.M. Scriber and L.A. Haas. 1997. Mediation of interspecific competition between folivores through defoliation induced changes in host quality. Pp. 71-88 in A. Ramen, eds, Ecology and Evolution of Plant Feeding Insects in Natural and Man-Made Environments. International Scientific Publications. New Delhi, India

Eller, F.J., J.H. Tumlinson and W.J. Lewis.1988. Beneficial arthropod behavior mediated by airborne semiochemicals: Source of volatiles mediating the host location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environmental Entomology* **17**: 745-753.

Fowler, S.V. and J. H. Lawton.1985. Rapidly–induced defenses and talking trees: the devil's advocate position. *The American Naturalist* **126**: 181-195.

Ginzburg, L.R. and D.E. Taneyhill. 1994. Population cycles of forest Lepidoptera – a maternal effects hypothesis. *Journal of Animal Ecology* **63**: 79-92.

Gordon, H.T. 1968. Quantitative aspects of insect nutrition. *Zoology* **8**: 131-138.

Haukioja, E. 1980. On the role of plant defenses in the fluctuation of herbivore populations. *Oikos* **35**:202-213.

Haukioja, E. 1982. Inducible defenses of white birch to a geometrid defoliator, *Epirrita autumnata*. Proceedings of the 5th International Symposium on Insect-Plant Relationships, Wageningen.

Haukioja, E. J. Suomela and S. Neuvonen. 1985. Long-term inducible resistance in birch foliage: triggering cues and efficacy on a defoliator. *Oecologia* **65**: 363-369.

Havill, N.P. and K.F. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecological Entomology* **25**: 171-179.

Hemming, J.D.C. and R.L.Lindroth. 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* **103**: 79-88.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants – to grow or defend. *Quarterly Review of Biology* **67**: 283-335.

Hildahl, V. and W.A. Reeks. 1960. Outbreaks of the forest tent caterpillar and their effects on stands of trembling aspen in Manitoba and Saskatchewan. *The Canadian Entomologist* **92**: 199-209.

Hodson, AC. 1941. An ecological study of the forest tent caterpillar, *Malacosoma disstria* Hbn. Minnesota Agricultural Experiment Station Technical Bulletin 148.

Hodson, A.C. 1977. Some aspects of forest tent caterpillar population dynamics. Minnesota Agricultural Experiment Station Technical Bulletin 310: 5-16.

Hunter, M.D. and P.W. Price. 1992. Playing chutes and ladders – heterogeneity and the relative role of bottom-up and top-down forces in natural communities. *Ecology* **73**: 724-732.

Hunter, M.D., G.C. Varley and G.R. Gradwell. 1997. Estimating the relative roles of top-down and bottom-up forces on insect herbivore populations: A classic study revisited. *Proceedings of the National Academy of Science.* **94**: 9176-9181.

Hwang, S.Y. and R.L. Lindroth. 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* **111**: 99-108.

Ives, W.G.H. 1971. The forest tent caterpillar in Alberta. Environment Canada, Canadian Forest Service, Northern Forestry Centre, Internal Report NOR-4.

Kaitaniemi, P. K. Ruohomaki, V. Ossipov, E. Haukioja and K. Pihlaja. 1998. Delayed induced changes in the biochemical composition of host plant leaves during an insect outbreak. *Oecologia* **116**: 182-190.

Kaitaniemi, P., K. Ruohomaki, T. Tammaru and E. Haukioja. 1999a. Induced resistance of host tree foliage during and after natural insect outbreak. *Journal of Animal Ecology* **68**: 382-389.

Kaitaniemi, P., S. Neuvonen and T. Nyyssonen.1999b. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. *Ecology* **80**: 524-532.

Karban, R. and I.T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago.

Kause A, V. Ossipov, E. Haukioja, K. Lempa, S. Hanhimaki and S. Ossipova. 1999. Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia* **120**:102-112.

Lindroth, R.L. and S.-Y. Hwang. 1996. Diversity, redundancy, and multiplicity in chemical defense systems of aspen.), Pp. 25-56 in T. Romeo, J.A. Saunders and P. Barbosa, eds, Phytochemical Diversity and Redundancy in Ecological Interactions. Plenum Press, New York.

Lindroth, R.L and K.K. Kinney. 1998. Consequences of enriched atmospheric CO2 and defoliation for foliar chemistry and gypsy moth performance. *Journal of Chemical Ecology* **24**:1677-1695.

Mason, R.R., R.C. Beckwith and H.G. Paul. 1977. Fecundity reduction in a collapse of a Douglas-fir tussock moth outbreak in Northeast Oregon. *Environmental Entomology* **6**: 623-626.

Mattson, W.J. and S.R. Palmer. 1988. Changes in levels of foliar minerals and phenolics in trembling aspen, *Populus tremuloides*, in response to artificial defoliation. Pp. 157-169 in W.J. Mattson, J. Levieux and C. Bernard-Dagan, eds, Mechanisms of Woody Plant Defenses against Insects, Springer, New York.

Mattson, W.J., D.A. Herms, J.A. Witter and D.C. Allen. 1991. Woody plant grazing systems: North American outbreak folivores and their host plants. Pp. 53-84 in Y.N. Baranchikov, W.J. Mattson, F.P. Hain and T.L. Payne, eds, Forest Insect Guilds: Patterns of Interactions with Host Trees (eds),. United States Department of Agriculture, Forest Service. General Technical Report NE-153.

Mondor, E.B. and J. Roland. 1997. Host locating behaviour of *Leschenaultia exul* and *Patelloa pachypyga*: two tachinid parasitoids of the forest tent caterpillar, *Malacosoma disstria*. *Entomologia Experimentalis et Applicata* **85**: 161-168.

Mondor, E.B. and J. Roland. 1998. Host searching and oviposition by *Leschenaultia exul*, a tachinid parasitoid of the forest tent caterpillar, *Malacosoma disstria*. *Journal of Insect Behavior* **11**: 583-592.

Monteith, L.G. 1964. Influence of the health of the food plant of the host on host-finding by tachinid parasites. *The Canadian Entomologist* **96**: 1477-1482.

Murray, K.D. and J.S. Elkinton. 1989. Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). *Journal of Invertebrate Pathology* **53**: 324-334.

Myers, J.H. 1981. Interactions between western tent caterpillars and wild rose: A test of some general plant-herbivore hypotheses. *Journal of Animal Ecology* **50**: 11-25.

Myers, J.H. 1988a. Can a general hypothesis explain population cycles of forest Lepidoptera? *Advances in Ecological Research* **18**: 179-242.

Myers, J.H. 1988b. The induced-defense hypothesis: does it apply to the population dynamics of insects. Pp. 345-366 in K.C. Spencer, ed, Chemical Mediation of Coevolution. Academic Press, San Diego.

Myers, J.H. 1990. Population cycles of western tent caterpillars: Experimental introductions and synchrony of fluctuations. *Ecology* **71**: 986-995.

Myers, J.H. 1993. Population outbreaks in forest Lepidoptera. *American Scientist* **81**: 240-251.

Myers, J.H. and B. Kukan. 1995. Changes in the fecundity of tent caterpillars – a correlated character of disease resistance or sublethal effect of disease? *Oecologia* **103**: 475-480.

Neuvonen, S. and E. Haukioja. 1991. The effects of inducible resistance in host foliage on birch-feeding herbivores. Pp. 277-291 in D.W. Tallamy and M.J. Raupp, eds, Phytochemical Induction by Herbivores. John Wiley & Sons, Inc., New York.

Osier, T.L., S.Y. Hwang and R.L. Lindroth. 2000. Effects of phytochemical variation in quaking aspen Populus tremuloides clones on gypsy moth, *Lymantria dispar*, performance in the field and laboratory. *Ecological Entomology* **25**: 197-207.

Parry, D. 1995. Larval and pupal parasitism of the forest tent caterpillar in Alberta. *The Canadian Entomologist* **127**: 877-893.

Parry, D., J.R. Spence and W.J.A. Volney. 1997. Responses of natural enemies to experimentally increased forest tent caterpillar populations. *Ecological Entomology* **22**: 97-108.

Parry, D., J.R. Spence & W.J.A Volney. 1998. Budbreak phenology and natural enemies mediate survival of early instar forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology* **27**: 1368-1374.

Parry, D., D.A. Herms, K.R. Kosola, J. M. Scriber and R. Julkenen-Tiitto. 2000a. Emulating Insect Outbreak: Experimentally Testing the Effects of Cumulative Defoliations on Induced-Resistance and Caterpillar Herbivory. *Ecology.* **In preparation**.

Parry, D., R.A. Goyer & G.A. Lenhard. 2000b. Macrogeographic variation in egg and neonate size of the forest tent caterpillar, *Malacosoma disstria. Ecological Entomology.* **In press**.

Peterson, E.B. and N.M Peterson. 1992. Ecology, management, and use of aspen and balsam poplar in the Prairie Provinces. Forestry Canada, Northwest Region, Northern Forestry Centre Special Report 1.

Rhoades, DF. 1983. Herbivore population dynamics and plant chemistry. Pp. 155-220 in R.F. Denno and M.S. McClure, eds, Variable Plants and Herbivores in Natural and Managed Systems, Academic Press, San Diego.

Robison, D.J. and K.F. Raffa.1997. Effects of constitutive and inducible plant traits of hybrid poplars on forest tent caterpillar feeding and population ecology. *Forest Science* **43**: 252-267.

Roland, J. K.E. Denford and L. Jimenz. 1995. Borneol as an attractant for *Cyzenis albicans*, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae). *The Canadian Entomologist* **127**: 413-421.

Roth S, C. Knorr R.L. Lindroth. 1997. Dietary phenolics affects performance of the gypsy moth (Lepidoptera: Lymantriidae) and its parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). *Environmental Entomology* **26**: 668-671.

Roth, S., R.L. Lindroth, J.C. Volin and E.L. Kruger. 1998. Enriched atmospheric CO2 and defoliation: effects on tree chemistry and insect performance. *Global Change Biology* **4**: 419-430.

Rothman, L.D. 1997. Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. *Ecology* **78**: 1481-1493.

Rothman, L.D. and J.H. Myers. 1994. Effect of nuclear polyhedrosis virus treatment on reproductive potential of western tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology* **23**: 864-869.

Ruohomaki, K., S. Hanimaki, E. Haukioja, L. Iso-Iivari, S. Neuvonen, P. Niemela and S. Suomela. 1992. Variability in the efficacy of delayed-inducible resistance in mountain birch. *Entomologia Experimentalis et Applicata* **62**: 107-115.

Sippell, W.L. 1957. A study of the forest tent caterpillar *Malacosoma disstria* Hbn. and its parasite complex in Ontario. PhD. thesis. University of Michigan, Ann Arbor, Michigan.

Sippell, W.L. 1961. Key to the puparia of common dipterous parasites of the forest tent caterpillar, *Malacosoma disstria* Hbn. in Ontario. *The Canadian Entomologist* **93**: 433-436.

Sippell, W.L. 1962. Outbreaks of the forest tent caterpillar *Malacosoma disstria* Hbn., a periodic defoliator of broad-leafed trees in Ontario. *The Canadian Entomologist* **94**: 408-416.

Southwood, T.R.E. 1991. Ecological Methods, 3rd Edition. Chapman and Hall.

Stehr, F.W. and E.F. Cook.1968. A revision of the genus *Malacosoma* Hübner in North America (Lepidoptera: Lasiocampidae): Systematics, biology, immatures, and parasites. United States National Museum Bulletin 276.

Thaler, J.S. 1997, Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* **399**: 686-688

Tuomi, J., P. Niemela, E. Haukioja, S. Siren and S. Neuvonen. 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. *Oecologia* **61**: 208-210

Tuomi, J., P. Niemela and S. Siren.1990. The Panglossian paradigm and delayed-inducible accumulation of foliar phenolics in mountain birch. *Oikos* **59**: 399-410.

Turchin, P. 1990. Rarity of density-dependence or population regulation with lags? *Nature* **344**: 660-663.

Turchin, P. 1995. Population regulation: Old arguments and a new synthesis. Pp. 19-40 in N. Cappuccino and P.W. Price, eds, Population Dynamics: New Approaches and Syntheses. Academic Press, San Diego.

Turlings, T.C.J., P.J. McCall, H.T. Alborn and J.H. Tumlinson. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* **19**: 411-425.

Underwood, N. 1999. The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist* **153**: 283-294.

Valentine, H.T., W.E. Wallner and P.M. Wargo. Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia* **57**: 298-302.

Waldbauer, G.P. 1968. The consumption and utilization of food by insects. *Advances in Insect Physiology* **5**: 229-289.

Wallner, W.E. and G.S. Walton. 1979. Host defoliation: a possible determinant of gypsy moth population quality. *Annals of the Entomological Society of America* **72**: 62-67.

Werner, R.A. 1979. Influence of host foliage on development, survival, fecundity and oviposition of the spear-marked black moth, *Rheumaptera hastata* (Lepidoptera: Geometridae). *The Canadian Entomologist* **111**: 317-322.

Williams, D. J. M., D. Parry and D.W. Langor. 1996. Sampling and identification of forest tent caterpillar parasitoids in the Prairie Provinces. Natural Resources Canada, Canadian Forest Service, Northwest Region, Northern Forestry Centre NOR-X-345.

Williams, R.S., D. E. Lincoln and R.J. Norby. 1998. Leaf age effects of elevated CO2-grown white oak leaves on spring-feeding lepidopterans. *Global Change Biology* **4**: 235-246.

Witter, J.A. and H.M. Kulman. 1979. The parasite complex of the forest tent caterpillar in northern Minnesota. *Environmental Entomology* **8**: 723-731.

Witter, J.A., W.J. Mattson and H.M. Kulman. 1975. Numerical analysis of a forest tent caterpillar (Lepidoptera: Lasiocampidae) outbreak in Northern Minnesota. *The Canadian Entomologist* **107**: 837-854.

CHAPTER 4:

CONCLUSIONS

My dissertation research focused on four main themes outlined in the introductory chapter. The primary objectives were to determine if (1) consecutive years of defoliation have cumulative effects on tree physiology and if this in turn successively reduces herbivorous insect performance; (2) induced-resistance has strength sufficient to account for the large (ca. 50%) reductions in fecundity found in many declining populations of outbreak Lepidoptera; (3) what are the indirect competitive effects of defoliation caused by a dominant outbreak species on other members of the folivorous lepidopteran community; (4) can RIR or DIR interact to enhance or reduce the susceptibility of caterpillar to mortality from higher trophic levels such as parasitoids?

Three consecutive years of defoliation did not have cumulative effects on the growth, development time, and fecundity of lepidopterans in the hybrid poplar system. A single year of severe defoliation had the same magnitude of effect on gypsy moth growth rate, pupal mass, and fecundity as two consecutive years. This was evident from the comparison of short and long-term bioassays in 1997, which were preceded by one year of mostly light defoliation, and the 1998 assays that were preceded by severe defoliation in 1997 in addition to light defoliation in 1996. Similarly in the aspen system, reductions in growth, pupal mass, and fecundity experienced by forest tent caterpillar was no greater following four consecutive years of defoliation than after a single year of defoliation. These results concur with those of Kaitaniemi et al. (1999) who found that the effects of two years of 75% defoliation of mountain birch on two lepidopteran folivores was greater than a single year of defoliation. Conversely, cumulative defoliations of black oak did

have cumulative negative impacts on gypsy moth pupal mass (Wallner and Walton 1979, Valentine et al. 1983) and on survival of black-marked spear moth (Werner 1979). Further experiments on other species of trees will be required to determine if there are general response of trees to successive years of defoliation or if the variability in results to date reflect system specific responses.

In outbreaks of several different species of Lepidoptera, reductions in fecundity in declining populations can approach 50% (e.g., Carter et al. 1991, Witter et al. 1975, Myers and Kukan 1995). Since DIR has been shown to reduce pupal mass and fecundity, it has been proposed as mechanism to account for changes in fecundity. In both of my studies, the effects of induced resistance could not account for more than a portion of the declines observed in natural populations. For example, in declining gypsy moth populations, declines in fecundity of 52% relative to low-density populations have been recorded (Carter et al. 1991). Similar changes in fecundity have been documented in forest tent caterpillar populations (Ives 1971, Witter et al. 1975). In my study, the effects on gypsy moth fecundity were maximally 14% and furthermore, the effects were directly dependent on density. In the forest tent caterpillar study, there were delayed effects of 13-19%, which account for approximately a third of the changes seen in natural outbreak populations. This indicates that factors in addition to DIR are driving fecundity changes.

An interesting aspect of the hybrid poplar study was that, even though the physiology of the trees responded as theory predicted, the insect herbivores did not. No residual effects of previous defoliation were detected on four species of Lepidoptera assayed in 1999, one year after the cessation of treatments. Elevated levels of condensed tannins as well as some minor phenolics appeared to have little impact on the

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An interesting aspect of the hybrid poplar study was that, even though the physiology of the trees responded as theory predicted, the insect herbivores did not. No residual effects of previous defoliation were detected on four species of Lepidoptera assayed in 1999, one year after the cessation of treatments. Elevated levels of condensed tannins as well as some minor phenolics appeared to have little impact on the

performance of the herbivores. This was most evident in 1999 where the trees continued to exhibit an induced-response to defoliation with elevated levels of total phenolics and condensed tannins. However, in 1999 there was no effect of three previous years of defoliation on the growth of gypsy moth, forest tent caterpillar and white-marked tussock moth, and even an apparent increase in performance of the mid-season feeding poplar tent maker. This result suggests that simplistic cause and effect statements about secondary compounds and herbivore performance need to be reevaluated. It also lends credence to the results of Ayres et al. (1997), which showed that tannins had little effect on many herbivorous insects, leading the authors to suggest that the function of condensed tannins is enigmatic and may not necessarily be related to insect resistance.

Trees have physiological limits to their responses to defoliation. In the hybrid poplar system, even though there was no cumulative effect on herbivores, there was a lagged response on tree physiology. Kosola et al. (2000) found that there was no difference in tree mortality among the treatments through the first three years of the experiment. However, increases in mortality became evident in 1999 with defoliated, unfertilized treatments suffering the highest losses. Furthermore, after coppicing in the winter of 1999, height and mortality measurements on the stump sprouts in 2000 showed continued residual effects of the fertilizer and defoliation treatments applied last in 1998 (A.A. Agrawal, K.R. Kosola and D. Parry, unpublished manuscript). In the aspen system, the response of the trees to repeated defoliation was different from the patterns seen in the hybrid poplar experiment. Even four successive years of defoliation did not increase tree mortality. Over the course of the study, none of the experimental trees died. While not

quantified, there appeared to be many more dead branches on the repeatedly defoliated aspen trees, especially in the lower third of the canopy.

The response of hybrid poplars to defoliation and fertilization generally fit the predictions of the CNB hypothesis, at least with respect to condensed tannins, the most prevalent phenolic compound. With the exception of 1997, N-fertilizer mitigated the production of condensed tannins in defoliated trees. The predicted allocation of carbon to growth in the presence of nitrogen fertilizer appeared to be validated because tree growth was higher in defoliated plots with added fertilizer than in defoliated unfertilized plots. The effects of fertilizer on induced-responses of poplars to defoliation corroborate many previous experiments addressing the constitutive responses of condensed tannins to fertilization (e.g., Koricheva et al. 1998).

My study showed that the effects of defoliation caused by an outbreak species has significant effects on the performance of other species in the folivorous lepidopteran community. The magnitude of the effects was similar for each of the species reared in 1998 with the exception of fall webworm. This result was unexpected because of the variety of life histories represented among the herbivores. While Agrawal (2000) found that induced-responses of wild radish to defoliation had variable effects on four caterpillars assayed, the results from my poplar experiment suggest that performance of these caterpillars may be driven by similar determinants of foliar quality. This result also indicates that the effects of defoliation on competitors in natural outbreaks may be driven more by direct competition for foliage or displacement from preferred habitat than by phytochemical induction.

Parasitoids have often been identified as one of the most important mortality factors in populations of outbreak Lepidoptera (e.g., Bylund 1995, Parry et al 1997, Gould et al. 1990). Despite current interest in tritrophic relationships in agricultural crops, little research has examined the interaction between trees, parasitoids, and caterpillars. In agricultural plants such as corn and tomatoes, specific compounds are elicited in response to caterpillar feeding and these are in turn used by parasitic Hymenoptera to locate potential hosts (Turlings et al. 1993, Thaler 1997). In laboratory tests, Havill and Raffa (2000) found that a parasitoid of gypsy moth responded to specific wound-related volatiles released following larval feeding on poplar leaves. Some tachinids appear to respond similarly to caterpillar feeding on the leaves of forest trees (Roland et al. 1995, Mondor and Roland 1997).

The response of the tachinids *Leschenaultia exul* and *Patelloa pachypyga* to forest tent caterpillar was mediated by the aspen clone on which they were feeding. Intraspecific differences in tree physiology have not previously been shown to alter susceptibility of caterpillars to parasitism. While preliminary in nature, this result could have significant bearing on the nature of host-parasitoid relationships. For example, it could contribute to spatial heterogeneity in susceptibility to parasitism, creating, if not enemy-free space, at least enemy reduced space. Considering that one of the tachinid species, *L. exul*, is an important source of mortality in low-density populations of forest tent caterpillar (Parry 1995, Parry et al. 1997), variance in susceptibility to parasitoids among clones over large forest landscapes could have important implications to population dynamics.

Future Research

My dissertation research has suggested a number of potentially interesting directions for future research. The lack of DIR in the hybrid poplar system and the presence of DIR in the aspen experiments is interesting because the trees are similar in phytochemistry and life-history strategy. This indicates that as Tuomi et al. (1990) hypothesized, DIR varies along environmental gradients in soil fertility. Experiments utilizing clones varying in inducible traits coupled with gradients in fertility regimes would be make a nice contribution to our understanding of induced-resistance. Aspen would be a particularly good experimental species in this regard because of its extremely wide distribution and occurrence over a range of site productivity.

Competition between outbreak species and other phytophages has not been well researched. Although biodiversity surveys suggest that some species do decrease following gypsy moth defoliation (e.g., Sample et al. 1996), the mechanisms underlying the changes in populations is not known. The scale and duration of forest tent caterpillar outbreaks (e.g., Mattson et al. 1991) suggest that populations of some competitors, particularly species such as contemporaneous leaf-rollers are likely to decline. My study has suggested that reductions in competitors could be due to changes in host plant chemistry although other mechanisms likely contribute significantly. Identifying the mechanisms responsible for determining the outcome of competition under experimental conditions in natural outbreaks might be a productive avenue of research. Such effects have the potential to cascade through ecosystems because several of the dominant defoliators in North America (gypsy moth, forest tent caterpillar) are not preferred prey

species of many forest dwelling birds that may regulate populations of other forest insects.

Finally, the integration of top-down processes with bottom-up is critical. My studies have shown that while the effects of induced-resistance can reduce fecundity by appreciable amounts, these reductions only account for a fraction of the total change observed in natural populations. Thus, full understanding of complex ecological processes such as the population dynamics of outbreak species can not be achieved until all of the relevant processes are included.

LITERATURE CITED

Agrawal, A.A. 2000. Specificity of induced-resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. *Oikos* **89**: 493-500.

Ayres, M.P., T.P. Clausen, S.F. MacLean, Jr., A.M. Redman and P.B. Reichardt. 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology* **78**: 1696-1712.

Byland, H. 1995. Long-term interactions between the autumnal moth and mountain birch: the role of resources, competitors, natural enemies, and weather. PhD Dissertation. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Carter, M.R., F.W. Ravlin and M.L. McManus. 1991. Changes in gypsy moth (Lepidoptera: Lymantriidae) fecundity and male wing length resulting from defoliation. *Environmental Entomology* **20**: 1042-1047.

Gould, J.R., J.S. Elkinton and W.E. Wallner. 1990. Density dependent suprresion of experimentally created gypsy moth, Lymantria dispar (Lepidoptera: Lymantriidae) populations by natural enemies. *Journal of Animal Ecology* **59**: 213-233.

Havill, N.P. and K.F. Raffa. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecological Entomology* **25**: 171-179.

Ives, W.G.H. 1971. The forest tent caterpillar in Alberta. Environment Canada, Canadian Forest Service, Northern Forestry Centre, Internal Report NOR-4.

Kaitaniemi, P., S. Neuvonen and T. Nyyssonen.1999. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. *Ecology* **80**: 524-532.

Koricheva J, S. Larsson, E. Haukioja and M. Keinanen. 1998. Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos* **83**: 212-226.

Kosola, K.R., D.I. Dickmann, E.A. Paul and D. Parry, 2000. Repeated insect defoliation effects on growth, nitrogen acquisition, carbohydrates and root demography of poplars. *Oecologia*. **In press**.

Mattson, W.J., D.A. Herms, J.A. Witter and D.C. Allen. 1991. Woody plant grazing systems: North American outbreak folivores and their host plants. Pp. 53-84 in eds Y.N. Baranchikov, W.J. Mattson, F.P. Hain and T.L. Payne, eds, Forest Insect Guilds: Patterns of Interactions with Host Trees. United States Department of Agriculture, Forest Service. General Technical Report NE-153.

Mondor, E.B. and J. Roland. 1998. Host searching and oviposition by Leschenaultia exul, a tachinid parasitoid of the forest tent caterpillar, *Malacosoma disstria*. *Journal of Insect Behavior* **11**: 583-592.

Myers, J.H. and B. Kukan. 1995. Changes in the fecundity of tent caterpillars – a correlated character of disease resistance or sublethal effect of disease? *Oecologia* **103**: 475-480.

Parry, D. 1995. Larval and pupal parasitism of the forest tent caterpillar in Alberta. *The Canadian Entomologist* **127**: 877-893.

Parry, D., J.R. Spence and W.J.A. Volney. 1997. Responses of natural enemies to experimentally increased forest tent caterpillar populations. *Ecological Entomology* **22:** 97-108.

Roland, J. K.E. Denford and L. Jimenz. 1995. Borneol as an attractant for Cyzenis albicans, a tachinid parasitoid of the winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae). *The Canadian Entomologist* **127**: 413-421.

Sample BE, L. Butler, C. Zivkovich, R.C. Whitmore and R. Reardon. 1996. Effects of Bacillus thuringiensis Berliner var Kurstaki and defoliation by the gypsy moth [*Lymantria dispar* (L)(Lepidoptera: Lymantriidae)] on native arthropods in West Virginia. *The Canadian Entomologist* **128**: 573-592.

Thaler, J.S. 1997, Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* **399**: 686-688.

Turlings, T.C.J., P.J. McCall, H.T. Alborn and J.H. Tumlinson. 1993. An elicitor in caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* **19**: 411-425.

Tuomi, J., P. Niemela and S. Siren.1990. The Panglossian paradigm and delayed-inducible accumulation of foliar phenolics in mountain birch. *Oikos* **59**:399-410.

Valentine, H.T., W.E. Wallner and P.M. Wargo. Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia* **57**: 298-302.

Wallner, W.E. and G.S. Walton. 1979. Host defoliation: a possible determinant of gypsy moth population quality. *Annals of the Entomological Society of America* **72**: 62-67.

Witter, J.A., W.J. Mattson and H.M. Kulman. 1975. Numerical analysis of a forest tent caterpillar (Lepidoptera: Lasiocampidae) outbreak in Northern Minnesota. *The Canadian Entomologist* **107**: 837-854.