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Title: Thinning and Urea Fertilization Effects on Emerging Grand Fir (Abies grandis) Foliage and Western Spruce Budworm (Choristoneura occidentalis) Larval Growth

Signature redacted for privacy.

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The objective of this study was to determine how the balance of carbon to nitrogen in a grand fir ecosystem affects the chemistry of emerging grand fir foliage and the growth of western spruce budworm larvae. Forest plots in the grand fir zone of eastern Oregon were thinned, thinned and fertilized, fertilized without thinning, or left as a control to determine how increased nitrogen availability alters the efficacy of carbon-based chemical defenses at different light levels.

Thinning did not significantly alter any of the foliar chemical fractions measured while fertilization increased the concentration of foliar nitrogen and free amino acids. Thinning increased tree vigor (g wood produced per m^2 foliage), but fertilization only increased vigor in the thinned plots. Fertilization increased the weights of western spruce budworm pupae; thinning had no effect.

Male and female pupal weights correlated with foliar free amino acid concentration and the ratios of foliar free amino acids to foliar nitrogen, available carbohydrates, and lignin, but stepwise regression analysis showed that foliar free amino acid concentration alone explained most of the variation in pupal weights.

The correlation of foliar free amino acid concentration with male and female pupal weights, and the lack of correlation of any indices of carbohydrate availability suggest that changes in available nitrogen rather than changes in the carbon/nitrogen balance were associated with changes in larval growth. This can be attributed to either a lack of defensive capability in the emerging foliage or a failure to measure or manipulate the variables responsible for controlling foliar defense. However, larval growth is only one aspect of plant susceptibility to insects; changing the carbon/nitrogen balance in the grand fir ecosystem may ultimately affect the susceptibility of grand fir to western spruce budworm by changing the balance between plant growth and levels of plant herbivory.

Thinning and Urea Fertilization Effects on Emerging Grand Fir (*Abies grandis*) Foliage and Growth of Western Spruce Budworm (*Choristoneura occidentalis*) Larvae

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THINNING AND UREA FERTILIZATION EFFECTS ON EMERGING GRAND FIR (*ABIES GRANDIS*) FOLIAGE AND WESTERN SPRUCE BUDWORM (*CHORISTONEURA OCCIDENTALIS*) LARVAL GROWTH

INTRODUCTION

Plant defense interests us because of its economic importance and its critical role in ecological interactions. An understanding of why plants have different systems of defense is required to successfully manipulate ecosystems to increase benefits from either plants or consumers. Large losses of forest productivity due to insect herbivory demand an understanding of tree defense so that foresters can successfully manipulate forests to reduce herbivory and maintain suitable forest growth.

In one of the first theories explaining why plants have different types and levels of chemical defense, Feeny (1976) and Rhodes and Cates (1976) proposed that a plant's defensive response depends on the plant's apparency to insects. Feeny predicted that the proportion of resources allocated to defense of apparent plants is greater than that of unapparent plants, that apparent plants have high concentrations of digestive inhibitors effective on both specialist and generalist herbivores while less apparent plants have low concentrations of toxins only effective on unadapted generalist herbivores, that defense of plant components also reflect their apparency, and that there is less diversity of

defensive compounds in communities dominated by apparent plants than those dominated by unapparent plants.

More recently, other investigators have hypothesized that plant defense adaptations depend on light and nutrient availability rather than plant apparency. Bryant et al. (1983) proposed that plants with relatively high resource availability had high growth rates, high leaf turnover, and less carbon allocation to plant defense while plants with relatively low resource availability allocated more resources to defense and had much lower leaf turnover and growth rates.

Coley et al. (1985) mathematically formulated this hypothesis, showing how the proportion of photosynthate allocated to defense that is optimum for realized growth varies with the inherent growth rate which, in turn, is a function of resource availability. They argued that resource allocation for defense would be higher in resource-limited plants because (1) the nutrient loss due to herbivory is more costly to replace in low-nutrient environments than high-nutrient environments, (2) herbivory impact as a percentage of production is much higher for slow-growing plant species than fast-growing plant species, and (3) if the percentage of photosynthate allocated to defense were the same for fast and slow-growing species, the absolute reduction in growth would be greater in fast-growing species. In addition, they argued that the relative cost of defense is minimized with immobile quantitative defenses in long-lived leaves and with mobile

qualitative defenses in short-lived leaves.

While this model eloquently relates total resource availability to the optimal level of plant defense, the balance of light and nitrogen available to a plant and the subsequent balance of carbon and nitrogen in the plant may also affect the optimal level of plant defense. Coley's model assumes that herbivory decreases strictly as a function of the relative allocation of carbon resources to defense. However, the level of nitrogen in the plant may also regulate herbivory by providing a limiting resource for herbivore growth and synthesis of enzymes capable of detoxifying plant defensive compounds.

Herbivory is affected not just by the level of plant defense, but by both the level of plant nitrogen and the level of chemical defense. That is to say, herbivory is a function of the balance of defensive compounds with plant nitrogen. This balance can relate to herbivory either as an additive combination of the positive effect of plant nitrogen with the negative effect of plant defense, or as a ratio of plant nitrogen to plant defense.

Indeed, plant nitrogen has been shown to regulate herbivory in many plant/herbivore interactions (for reviews see Mcneill and Southwood 1978 and Mattson 1980). White (1984) argues in a review that the availability of nitrogen is the single factor that relates a variety of environmental stresses to herbivore outbreaks.

Fewer studies have considered how the balance between defensive compounds and nitrogen affects herbivory. Coley (1983) found a positive correlation of percent leaf nitrogen and a negative correlation of leaf fiber content with herbivory; Fox and Macauley (1977) found a positive correlation of percent leaf nitrogen with herbivore biomass. Neither study found any correlation of herbivory with ratios of total nitrogen to total phenols and tannins.

Other studies have considered how nitrogen and light interact to affect the balance between defensive compounds and nitrogen in the foliage and subsequent herbivory. Fertilization of willow clones (Salix dasyclados) increases foliar nitrogen and free amino acid concentrations but does not increase herbivory by Galerucella lineola in light or under shade. However, increased light allows foliar phenols and lignin (but not tannins) to increase and herbivory decreases (Larsson et al. 1986, Waring et al. 1985). Matson and Waring (1984) found that fertilization with shading increases susceptibility of mountain hemlock (Tsuga mertensiana) to laminated root rot (Phellinus weirii). Mitchell et al. (1983) and Waring and Pitman (1985) found that thinning alone or thinning with fertilization, but not fertilization alone, increases tree vigor and reduces susceptibility of lodgepole pine stands (Pinus contorta) to mountain pine beetle (Dendroctonus ponderosae).

Both direct and indirect evidence suggest that plant

carbon/nitrogen balance may regulate spruce budworm development. First, defoliation is related to the site characteristics and percent crown closure of the forest (Fauss and Pierce 1969, Johnson and Denton 1975). Second, nitrogen fertilization increases the pupal weights of eastern spruce budworm (Choristoneura fumiferana) (Shaw and Little 1972, Shaw et al. 1978). Third, laboratory populations of western spruce budworm fed Douglas-fir (Pseudotsuga menziesii) foliage grown in the sun has greater larval and pupal mortality, longer larval development times, lower pupal weights, and reduced fecundity than those fed foliage grown in the shade (Waddell 1983). Finally, Cates et al. (1983) found that increased foliar nitrogen increases larval success and reduces the negative impact of terpenes on larval success, although Redak and Cates (1984) found female adult dry weight correlates negatively with certain terpenes and increasing foliar nitrogen.

The objective of this study was to determine how the balance of carbon to nitrogen in a grand fir ecosystem affects the chemistry of emerging grand fir foliage and the growth of western spruce budworm larvae. Increasing the availability of nitrogen by fertilization was expected to increase the amount of available nitrogen in the foliage and promote the growth of budworm larvae. Thinning was expected to increase the light available to the remaining trees, permitting them to produce defensive compounds and inhibit

larval growth. Thus, spruce budworm larval growth was expected to correlate with the balance of available nitrogen to available carbon in the grand fir foliage.

MATERIALS AND METHODS

Study Area

The experiment was conducted on a nearly level 10 ha study area at 1850 m (6100 ft) elevation in the Malheur National Forest (43°49′ N, 118°52′ W) about 50 km north of Burns, Oregon. Although the forest is located in the *Abies grandis* vegetation zone of Franklin and Dyrness (1973), grand fir is a recent dominant on the site. Only after 1900, when fire control stopped periodic fires did grand fir begin to dominate the understory under a fire-resistant forest of ponderosa pine (*Pinus ponderosa*), (Filip and Goheen 1984). In 1982 the forest was characterized by a scattered ponderosa pine overstory with the grand fir understory, but in 1983 and in the summer of 1984 the Forest Service harvested most of the ponderosa pine, leaving a nearly pure stand of dense grand fir.

The site receives approximately 50 cm precipitation annually, most of which occurs as snowfall in the winter. The average January temperature is -6.5° C and the average July temperature is 17° C, but diurnal variations are extreme. (Carlson 1974)

A gravelly loam soil 30 to 75 cm deep overlays hard basalt and andesite with some soft to moderately hard tuffaceous interflow material. The soil surface is 10 to 30

percent covered with flat and angular rock fragments; the litter layer is 0 to 2.5 cm thick and covers 40 to 60 percent of the soil surface. Soil pH ranges from 5.6 to 6.5. (Carlson 1974)

Experimental Design

A split-plot experiment was replicated on four 0.6 ha blocks within the study area with thinning as the main-plot factor and fertilization as the sub-plot factor (figure 1). In the summer of 1984, a random half of each block was thinned by U.S. Forest Service operational procedures and the remaining half was left alone. Trees were thinned to an 18 ft (5.5 m) spacing with the following restriction: grand fir trees greater than 5 in (13 cm) diameter at breast height (DBH) and any remaining ponderosa pine trees greater than 7 in (18 cm) DBH could not be removed, although trees up to 12 in (30.5 cm) that could not yield a 13 ft (4 m) log due to defect could be removed. Due to this restriction, the spacing throughout the thinned plots was not consistent. A random half of each thinning treatment (both thinned and unthinned) was hand-fertilized with 350 kg/ha nitrogen in the form of urea, so that each block contained a control, fertilized only, thinned only, and thinned and fertilized sub-plot.

Five trees with diameters at breast height (DBH) ranging

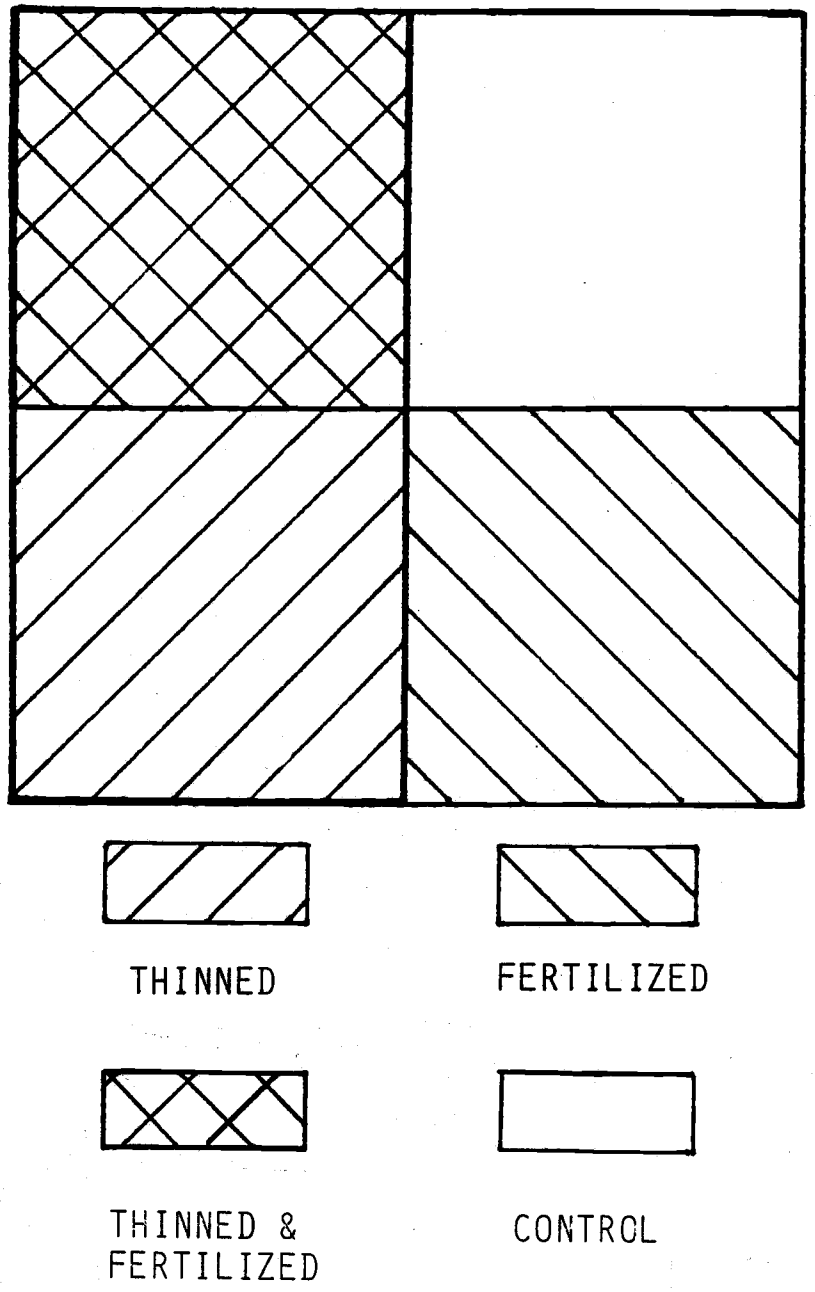

Figure 1. Layout of treatments to one 0.6 ha block. A random half of each block was thinned and the remaining half was left unthinned. A random half of each thinning treatment (thinned and unthinned) was fertilized and the remaining half of the thinning treatment was left unfertilized.

from 5 to 20 cm were selected in each sub-plot as sample trees for foliar analysis and vigor measurements. Insects were collected from these trees and five additional trees per sub-plot. Sample trees were not picked at random due to the heterogeneity of the thinning treatment. Trees receiving full sunlight were selected in the thinned sub-plots while trees shaded by adjacent trees were selected in the unthinned sub-plots. Foliar chemistry and vigor measurements were analyzed for each sample tree in a sub-plot then averaged to obtain a sub-plot mean. However, the insects collected from the all the sample trees in a sub-plot were pooled first, then weighed individually, and these individual insect weights were averaged to obtain an average value for each sub-plot.

Foliar Chemistry

On June 23-24, 1986, 5-10 g fresh weight of emerging current-year foliage was collected from the mid-crown of each sample tree (except one from which all the new foliage had been consumed), then placed in a cooler with dry ice, brought to the laboratory in Corvallis, Oregon, where samples were stored at -40^{o}C until analyzed.

Soluble sugar, free amino acid (FAA), starch, holocellulose, and lignin content of emerging foliage from each tree was analyzed from a 1 g fresh weight subsample of

frozen foliage weighed and placed in 35 ml 80 % ethanol. The needles in the ethanol were homogenized with a Polytron for 30 s then centrifuged for 10 min at 10,000 rpm. The supernatant containing the soluble sugars and free amino acids was decanted and combined with a second extraction of 35 ml 80% ethanol. After bringing the volume of the combined supernatant to 100 ml with 80% ethanol, approximately 2 g of insoluble PVPP was added to remove phenolic compounds (Sanderson and Perera, 1966) and the sample was evaporated to 10-20 ml to remove most of the ethanol. The PVPP was filtered from the sample, and distilled de-ionized water was added to bring the volume back to 100 ml.

Soluble sugar content of the supernatant solution was determined colorimetrically by the anthrone reaction (Yemm and Willis, 1954). An 80 ml aliquot of the sample solution was treated with 100 ul of saturated lead acetate followed twenty minutes later with 500 ul of saturated sodium oxalate. Lead acetate and sodium oxalate remove phenolic anions and that interfere with the reaction of the sugars with the anthrone (Joslyn 1970). A 0.5 ml aliquot of the sample solution was added to 5.0 ml of 9.01 mM anthrone in 55 % H_2SO_4. The reaction tube was heated in a boiling water bath for 12 min, cooled for 10 min, then the absorbance at 625 nm was read. The sugar content was calculated using a standard curve derived from absorbance readings of glucose standards in water concurrently reacted with anthrone.

Free amino acid content of the supernatant solution was determined colorimetrically using a ninhydrin reaction modified from Moore and Stein (1954) by Margolis (1884). A 20 ml aliquot of the supernatant was filtered through Celite to remove high molecular weight lipids and proteins. Two ml of the filtrate was added in a test tube to a 7.09×10^{-2} M ninhydrin solution in a 1.15 N sodium acetate buffer (ph=5.5) of 68.6 % methylcellosolve with 2.7×10^{-4} M KCN (an antimicrobial agent). The tube was heated for 20 min in a boiling water bath, cooled for 10 min, and diluted with 5 ml 60 % ethanol. Absorbance was read at 570 nm and free amino acid content calculated using a standard curve derived from absorbance readings of simultaneously reacted leucine standards.

Starch, holocellulose, and lignin concentrations of the foliage was determined from a sequential digestion of the pellet left after the 80% ethanol extraction. Perchloric acid-soluble polysaccharides (mostly starch, but see Hansen and Moller 1975) were extracted by shaking the pellet at room temperature for 24 h with 35 ml 35 % perchloric acid in an 125 ml Erlenmeyer flask. The sample was filtered through a 30 ml gooch crucible and rinsed with hot distilled de-ionized water until the volume of the filtrate was brought to 100 ml. The glucose equivalent of the starch content of the filtrate was determined colorimetrically with the same anthrone procedure used for sugar determination, except that glucose

standards were mixed in 12.25 % perchloric acid because perchloric acid affects the level of color development (Hansen and Moller 1975). Starch content was calculated by multiplying the glucose equivalent by 0.9 to account for the weight of the water molecule added during the starch hydrolysis that occurs during the anthrone reaction.

The residue left in the gooch crucible following the perchloric acid extraction was used to determine holocellulose and lignin concentration of the foliage. After the rinse with hot distilled de-ionized water, the sample was washed with acetone to remove any remaining lipids and to promote drying. The sample in the gooch crucible was dried for 24 h in an oven at 70° C and weighed to the nearest 10^{-4} g. Holocellulose was then extracted by adding approximately 30 ml 72% H_2SO_4 to the crucible, stirring with a glass rod, and allowing it to drain from the crucible for 8-10 h. The residual sulfuric acid was filtered off, the sample was re-rinsed with hot water and acetone, and the sample in the gooch crucible was dried and re-weighed. Holocellulose content was calculated from the weight difference before and after the sulfuric acid extraction.

The sample residue remaining in the gooch crucible was ashed at 500° C for 5 h, then the gooch crucible with the ash residue was weighed. Lignin content (Klason lignin) was calculated from the difference between the weight of the sulfuric acid extraction residue and the ashing residue

(Swain 1979).

Total nitrogen (N) concentration and percent water content of foliage from each sample collected was determined from a separate 0.5-1.0 g fresh weight subsample of frozen foliage. After weighing, the foliage was dried in a 70° C oven then reweighed to determined water content. Total nitrogen content of the dried foliage was determined using standard micro-Kjeldahl techniques.

The concentration of each chemical component in the foliage is reported per unit dry weight of foliage.

Vigor

Vigor, xylem production per unit leaf area (Waring et al. 1980), was estimated from allometric equations that related grand fir diameter to xylem mass (Gholtz et al. 1979) and grand fir sapwood basal area to leaf area (Waring et al. 1982):

$$\ln(\text{stemwood biomass(kg)}) = 2.6825\ \ln(\text{DBH (cm)}) - 3.7389$$

$$\text{leaf area}(m^2) = 0.48(\text{sapwood area}(cm^2))$$

Diameter growth and sapwood thickness of each sample tree were estimated from wood cores drilled completely through the diameter at breast height on September 19-20, after the 1986 growing season. Sapwood was identified either by its transluscence or with a bromocresol green stain

(Kutscha and Sachs 1962). Diameter of the tree before and after the current increment was estimated by adding 5 % as an estimate of bark thickness to each year's diameter without bark measured from the increment core. These diameter measurements were used with the stemwood biomass equation to calculate stemwood biomass for 1986 and 1985, and stemwood biomass production was calculated by difference. Stemwood biomass production was then divided by the leaf area calculated using the leaf area equation to obtain the vigor estimate in g wood per m^2 leaf area for 1986.

Spruce budworm pupal weights

Pupal weights were used to index larval growth. Cooperators from the U.S. Forest Service Forest and Range Experiment Station based in LaGrande, Oregon collected pupae from small branches pruned from a total of ten trees per sub-plot, including the five foliar chemistry sample trees. All the branches from a sub-plot were beaten with a stick over a cover cloth so that the pupae were knocked on the cover cloth. The pupae were collected, immediately refrigerated, and transported back to the laboratory in LaGrande, Or., where they were sexed and weighed to the nearest milligram.

Statistical Analyses

Several variables were derived from the primary analyses. Sugar and starch concentrations of each sample collected were added to obtain an index of the available carbohydrates in the foliage. The ratios of free amino acids to sugar + starch, free amino acids to lignin, and free amino acids to vigor, and the ratios of total nitrogen to sugars + starch, total nitrogen to lignin, and total nitrogen to vigor were also calculated for each sample collected.

I used split-block analysis of variance with the sub-plot means of all variables measured to test for thinning effects, fertilization effects, and interactions between thinning and fertilization (Steel and Torrie 1980). Main effect and interaction means were separated with standard t-tests.

Correlation analysis was used to relate the foliar chemistry and tree vigor to male and female spruce budworm pupae weights.

Stepwise regression analysis using a probability of F less than 0.05 as the selection criterion was used to determine whether foliar nitrogen content, foliar carbohydrate availability, the ratio of foliar nitrogen to foliar carbohydrate availability, or a combination of these variables could explain the most variation in male and female pupae weights. Six combinations were investigated using either free amino acid concentration or total nitrogen concentrationt as an index of nitrogen availability to the

insect and using (sugars + starch), lignin, or vigor as an index of carbohydrate supply in the plant.

RESULTS

Table 1 shows the means, standard deviations, and sample size of the foliar chemical fractions and tree vigor in the four different combinations of thinning and fertilization treatments.

The analysis of variance of the sub-plot means shows the significance of the thinning effects, fertilization effects, and interactions between thinning and fertilization. Thinning did not significantly affect any of the foliar chemical fractions measured (Table 2). Fertilization increased the concentration of free amino acids, total nitrogen content, the ratio of free amino acids to total nitrogen, and male and female pupal weights, but decreased foliar starch content (Table 3). Interactions between thinning and fertilization were significant for foliar lignin and tree vigor (Table 4). Thinning increased vigor in both fertilized and unfertilized plots, but fertilization increased vigor only in the thinned plots. Thinning increased foliar lignin only in unfertilized sub-plots.

Mean free amino acid concentration, and the ratios of free amino acid concentration to total nitrogen, sugars + starch, and lignin significantly correlate with male and female pupal weights (Table 5, 6). No other measured variables significantly correlated with either male or female pupal weights.

Variable	Unthinned Unfertilized	Unthinned Fertilized	Thinned Unfertilized	Thinned Fertilized
Free Amino Acids (umol Leucine eq./g)	20.9(1.2) n=20	77.7(53.0) n=20	18.8(6.5) n=19	75.4(39.1) n=20
Total Nitrogen (mg/g)	10.9(3.9) n=20	16.8(6.4) n=20	10.1(5.9) n=19	14.2(2.3) n=19
Free Amino Acids/ Total Nitrogen (umol Leucine eq./mg)	2.17(1.61) n=20	4.64(2.49) n=20	2.16(0.87) n=19	4.96(2.08) n=19
Sugars (mg/g)	36.3(10.6) n=20	39.0(10.1) n=20	39.5(6.3) n=19	48.5(10.8) n=20
Starch (mg/g)	95.0(30.1) n=20	60.9(13.4) n=20	88.9(21.9) n=19	76.3(25.7) n=20
Sugars + Starch (mg/g)	131.3(37.9) n=20	99.9(18.2) n=20	128.4(22.4) n=19	124.8(33.6) n=20
Holocellulose (mg/g)	148(82) n=20	141(70) n=20	151(79) n=19	152(65) n=20
Lignin (mg/g)	238(70) n=20	248(74) n=20	299(105) n=19	251(69) n=20
Vigor (g wood/m^2 leaf area/yr)	22.9(14.6) n=20	17.9(9.6) n=20	37.4(20.7) n=20	54.1(12.6) n=20

Table 1. Means, standard deviations (in parentheses), and sample size of tree vigor and foliar chemistry in the four different combinations of treatments.

Variable	Treatment: Unthinned	Thinned
Free Amino Acids (umol Leucine eq./g)	49.1	46.8
Total Nitrogen (mg/g)	13.9	12.3
Free Amino Acids/ Total Nitrogen (umol Leucine eq./mg N)	3.4	3.5
Sugars (mg/g)	37.7	43.9
Starch (mg/g)	77.9	82.7
Sugars + Starch (mg/g)	115.6	126.6
Holocellulose (mg/g)	144	151
Female Pupal Weight[1] (mg/individual)	104.9	107.3
Male Pupal Weight[1] (mg/individual)	77.3	77.1

[1] Pupal weight data courtesy of Boyd Wickman, Dick Mason, and Roy Beckwith.

Table 2. Mean foliar concentrations of chemical fractions and mean male and female pupal weights in thinned and unthinned forest plots. Differences between the means are not significant at the p=.05 level.

Variable	Treatment: Unfertilized	Fertilized
Free Amino Acids (mmol Leucine eq./g)	19.6	76.5 **
Total Nitrogen (mg/g)	10.6	15.5 **
Free Amino Acids/ Total Nitrogen (umol Leucine eq./mg N)	2.1	4.8 **
Sugars (mg/g)	37.7	43.8 *
Starch (mg/g)	92.2	68.6 *
Sugars + Starch (mg/g)	129.9	112.3
Holocellulose (mg/g)	150	146
Female Pupal Weight[1] (mg/individual)	98.4	113.8**
Male Pupal Weight[1] (mg/individual)	73.4	81.1 *

** Difference between means is significant at $p<.01$.

* Difference between means is significant at $p<.05$.

[1] Pupal weight data courtesy of Boyd Wickman, Dick Mason, and Roy Beckwith.

Table 3. Mean foliar concentrations of chemical fractions and mean male and female pupal weights in fertilized and unfertilized forest plots.

Variable	Unthinned		Thinned	
	Unfertilized	Fertilized	Unfertilized	Fertilized
Lignin (mg/g)	238[a]	248[a]	299[a]	251[b]
Vigor (g wood/m^2 leaf area)	22.9[a]	17.9[a]	37.4[b]	54.1[c]

Table 4. Mean lignin content and tree vigor at two different levels of thinning and two different levels of fertilization. Interactions between thinning and fertilization are significant (lignin, $p<0.10$; vigor, $p<.05$) and means followed by different letters are significant at $p<0.05$.

Variable	Female Pupal Weight (mg)	Male Pupal Weight (mg)
Free Amino Acids (mmol Leucine eq./g)	0.74 **	0.60 *
Total Nitrogen (mg/g)	0.28	0.24
Free Amino Acids/ Total Nitrogen (umol Leucine eq./mg N)	0.75 **	0.66 **
Sugars (mg/g)	0.34	0.21
Starch (mg/g)	-0.47	-0.57 *
Sugars + Starch (mg/g)	-0.27	-0.40
Holocellulose (mg/g)	-0.27	-0.15
Lignin (mg/g)	-0.31	-0.31
Vigor (g wood/m^2 LA/yr)	0.35	0.32

** correlation significant at $p<.01$

* correlation significant at $p<.05$

Table 5. Correlation coefficients between male and female spruce budworm pupae weight sub-plot means and foliar chemistry sub-plot means (n=16).

Variable	Female Pupal Weight (mg)	Male Pupal Weight (mg)
Free Amino Acids/ (Sugar+Starch) (umol Leucine eq./mg carbohydrate)	0.62 **	0.58 *
Free Amino Acids/ Lignin (umol Leucine eq./mg carbohydrate)	0.66 **	0.52 *
Free Amino Acids/ Vigor (umol Leucine eq./ g wood/m^2 leaf area)	0.24	0.11
Total Nitrogen/ (Sugar+Starch) (mg N/mg carbohydrate)	0.17	0.24
Total Nitrogen/ Lignin (mg N/mg carbohydrate)	0.24	0.23
Total Nitrogen/ vigor (mg N/ g wood/m^2 leaf area)	-0.20	-0.27

** correlation significant at $p<.01$

* correlation significant at $p<.05$

Table 6. Correlation coefficients between male and female spruce budworm pupae weight sub-plot means and sub-plot means of ratios between available nitrogen and available carbon (n=16).

While both FAA and the ratios of FAA to total N and carbohydrates correlated with male and female pupal weights, the only variable significant in the stepwise regression analysis of pupal weights was the concentration of free amino acids in the foliage (Table 7). Analysis of the residuals from the model selected suggested the relationship was logarithmic; a natural log transformation of free amino acid concentration increased the r^2 from 0.55 to 0.62 with female pupal weight and from 0.36 to 0.49 with male pupal weight (Figure 2). The regression equations are:

$$\text{female pupal weight} = 8.40(\ln(\text{FAA})) + 76.1 \quad (n=16,\ p<0.01)$$

$$\text{male pupal weight} = 4.84(\ln(\text{FAA})) + 59.9 \quad (n=16,\ p<0.01)$$

where pupal weights are in mg and FAA concentrations are expressed as umoles leucine equivalents per g dry weight of foliage.

Dependent Variable	Full Model	Variables Selected	Selected model r^2
Female pupal weight	FAA Sugar+Starch FAA/Sugar+Starch	FAA	0.55
	FAA Lignin FAA/Lignin	FAA	0.55
	FAA Vigor FAA/Vigor	FAA	0.55
	Total N Sugar+Starch Total N/Sugar+Starch	none	-
	Total N Lignin Total N/Lignin	none	-
	Total N Vigor Total N/Vigor	none	-
Male pupal weight	FAA Sugar+Starch FAA/Sugar+Starch	FAA	0.36
	FAA Lignin FAA/Lignin	FAA	0.36
	FAA Vigor FAA/Vigor	FAA	0.36
	Total N Sugar+Starch Total N/Sugar+Starch	none	-
	Total N Lignin Total N/Lignin	none	-
	Total N Vigor Total N/Vigor	none	-

Table 7. Model selected and R^2 from stepwise regression analysis of sub-plot mean male and female pupal weights using different indices of nitrogen and carbon availbility as independent variables.

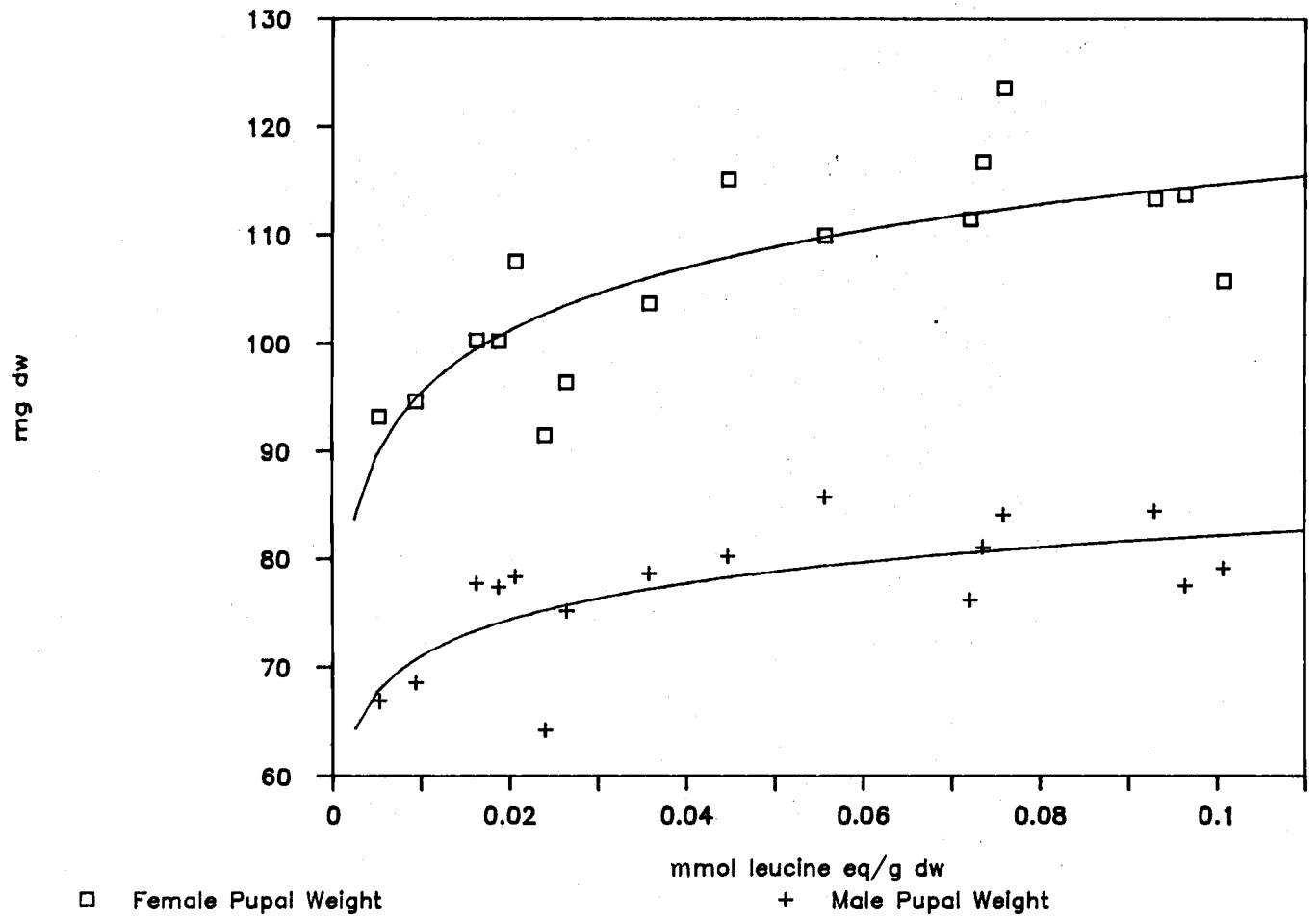

Figure 2. Plot of sub-plot mean male and female pupal weights vs. sub-plot mean foliar free amino acid concentrations. Free amino acid values above 40 umol/g are from fertilized plots.

DISCUSSION

The regression analyses (Table 7, Figure 2) showed that growth of western spruce budworm larvae was best related to foliar free amino acid concentration rather than to a balance of readily available forms of carbon and nitrogen. None of the indices of carbohydrate availability, sugar + starch, lignin, or vigor, was significantly related to pupal weights either alone or in a ratio with nitrogen after the contibution of free amino acids was accounted for in the model.

The significant correlation of free amino acid concentration with pupal weights and the lack of correlation of total nitrogen with pupal weights suggest that the form of nitrogen is very important in insect nutrition. Many studies have shown that increased total nitrogen concentration in host tissue favors herbivory, but few studies have separately evaluated the relative importance of free amino acids. Population density and fecundity of the whitefly Aleurotrachelus jelinekii feeding on Viburnum tinus and fecundity of the aphids Brevicoryne brassicae and Myzus persicae feeding on Brussels Sprouts are related to free amino acid concentration of the foliage but, importantly, are not related to total nitrogen of the foliage (Mcneill and Southwood 1978). In addition, the seasonal fluctuation of population densities of several herbivores correspond to the seasonal fluctuation of soluble nitrogen in their hosts

(Mcneill and Southwood 1978). Amino acids also stimulate feeding by locusts and short-horned grasshoppers on artificial diets (Bernays and Chapman 1978).

Previous studies of spruce budworm nutrition also suggest that nitrogen form and content is important for insect performance. Heron (1965) found that amino acids stimulate feeding of eastern spruce budworm on artificial diets either alone or mixed with sugars. Lending support to the probable greater importance free amino acids over total nitrogen, Redak and Cates (1984) found that the total nitrogen concentration of Douglas-fir foliage negatively correlates with the dry weight of female adult western spruce budworm in a regression model that includes several defensive terpenoids. The soluble nitrogen:total nitrogen ratio is not a significant variable in their analysis, but the importance of the absolute amount of soluble nitrogen or the amount of free amino acids was not tested. Under experimental conditions with an artificial diet using casein as a nitrogen source, Cates et al. (1983) did find that higher total nitrogen increased larval success and reduced the negative effect of terpenes on larval success.

The logarithmic form of the pupal weight = f(free amino acid content) curve suggests a threshold. Similarly, Lii et al. (1975) found that increasing the free amino acid concentration from 2.4 % to 3.6 % of an artificial diet fed to Argyrotaenia velutinana larvae increased weight gain and

the Efficiency of Conversion of Ingested matter (ECI - the ratio of larval weight gain to weight of food ingested), but that increases above 4.2 percent decreased weight gain and ECI. In my study, only unfertilized plots had foliar free amino acid concentrations below the threshold suggested by the curve (Figure 2), indicating that natural levels of free amino acids may play a significant part in regulating budworm larval growth.

The correlation of free amino acids with pupal weights in this study joins the already substantial evidence that supports White's (1984) contention that increased availability of nitrogen is the single most important factor that relates a variety of environmental stresses to outbreaks of herbivore populations.

Several scenarios could explain the lack of correlation of the indices of carbohydrate availability with budworm larval growth. First, a tree may allocate its carbon resources to growth rather than defense in expanding foliage. Perhaps, grand fir is a high-resource adapted plant that maximizes its growth by minimizing its allocation to plant defense in accordance with the Coley model (Coley et al. 1985). The correlation of pupal weights with the concentration of free amino acids in the foliage suggests that any defensive compounds produced would be less effective in trees with high levels of amino acids than in trees with low levels of amino acids. A plant with high resource

availability and high levels of nitrogen available to herbivores would not allocate its carbon resources to defense not only because, as Coley et al. argued, nutrient loss from herbivory is easily replaced and defense allocation is at the cost of a significant amount of growth, but also because the plant defense would not be effective when a large amount of nitrogen is available to herbivores in the plant.

I cannot discard the possibility, however, that the indices of carbohydrate availability did not correlate with pupal weights because they were not adequate measures of the the tree's ability to produce defensive compounds in the foliage. While one might expect an increase in carbon availability with thinning or fertilization, sugar + starch levels did not increase (Tables 2,3). The sugar and starch content of expanding foliage changes markedly as the foliage expands (Shaw and Little 1977, Shaw et al. 1978) so that differences due to leaf development may have obscured differences due to light or nutrient status. Indeed, Waring et al. (1985) showed that willow plants grown with high light intensity have greater photosynthetic rates, phenolic concentrations, and tannin concentrations than plants grown with low light, but that starch concentrations were the same or less in the more rapidly growing plants. Vigor also may not measure the carbohydrate status of the expanding foliage; it measures the photosynthetic capacity of the total leaf area (Waring et al. 1980) which may not match the

photosynthetic capacity of the new, expanding foliage.

The final explanation for the lack of effect of foliar carbohydrates on the weight of budworm pupae is that the vigor of these trees may be so low that the trees do not have sufficient carbon resources to meet their full defensive potential. The vigor on the thinned and fertilized plots averaged 54 g wood/ m^2 leaf area; lodgepole pine (Waring and Pitman 1985) and other conifers (Christiansen et al. 1987) require a vigor of 100 g/m^2 before they can totally protect themselves against bark beetles (Waring and Pitman 1985).

Climate, rather than light or nutrients, may ultimately limit the ability of grand fir in this region to produce enough carbon for defense against insects and pathogens. Wagg (1958) reported that growth of grand fir and western spruce budworm is related to accumulated degree days. Filip and Goheen (1984) reported that grand fir trees in this climatic zone are very susceptible to root disease. The exclusion of fire may have allowed grand fir to spread slightly out of its normal range so that it lacks the marginal resources required for defense against insects and pathogens.

Changes in foliar chemistry with thinning and fertilization suggest how trees and herbivores respond differently to changes in light and nutrient availability. In a series of studies on the effect of fertilization on the chemical content and physiology of douglas-fir foliage, van

den Driessche and Webber (1975, 1977) found, as this study found (Table 3), that the the percentage increase of ninhydrin-positive compounds (mostly amino acids) with fertilization in current-year foliage was much greater than the percentage increase of total nitrogen. Brix (1971) showed that fertilization increases chlorophyll content of current-year foliage, but that photosynthesis is increased only when the light intensity is greater than 1500 ft - c. The increase in free amino acid concentration in both thinned and unthinned plots suggests that fertilization increases the nutritive quality of foliage regardless of light availability, but that only in thinned plots are the trees able to use the added nitrogen to increase photosynthesis. This explains why vigor increases with fertilization only on the unthinned plots (Table 4) while the budworm larvae weigh more on both thinned and unthinned fertilized plots (Table 3).

Thinning (Waring et al. 1981) and fertilization (Brix and Ebell 1969) increase both the photosynthetic capacity per unit leaf area and the total leaf area per tree. Tree growth responds to both these increases while leaf-eating insects may benefit only from the increased foliar nitrogen concentrations that accompany fertilization. Thus, changes in tree susceptibility with changing light and nutrient status must consider both the effect on tree growth as well as the effect on herbivory; herbivory may increase slightly

due to changes in leaf quality, but growth may overcome that herbivory due to increases in both leaf quality and leaf quantity.

In this experiment, changes in available nitrogen rather than changes in the carbon/nitrogen balance were associated with changes in budworm larval growth. This can be attributed either to a lack of defensive capability in the emerging foliage or a failure to measure or manipulate the variables that are responsible for inducing or limiting foliar defense. However, larval growth is only one aspect of plant susceptibility to insects; changing the carbon/nitrogen balance in the grand fir ecosystem may ultimately affect the susceptibility of grand fir to western spruce budworm by changing the balance between plant growth and levels of plant herbivory.

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