AN ABSTRACT OF THE THESIS OF

Cathy L. Rose for the degree of Doctor of Philosophy in Forest Science presented on November 30, 1989

Title: Application of the Carbon/Nitrogen Balance Concept to Predicting the Nutritional Quality of Blueberry Foliage to Deer in Southeastern Alaska

Abstract Approved: Signature redacted for privacy.

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Sitka black-tailed deer (Odocoileus hemionus sitkensis Sm.) prefer understory forages growing beneath a forest canopy despite a greater abundance of the same plant species in forest clearings. This research examined responses of the deciduous shrub - blueberry (Vaccinium ovalifolium), to test the hypotheses that 1) forage is less nutritious and less palatable when grown in clearings than in forest understories, and that 2) changes in the plant carbon/nitrogen (C/N) ratio in response to light and nitrogen supply determine forage nutritional quality. Responses to irradiance and nitrogen supply were examined with respect to plant physiology, morphology, biochemistry and nutritional quality in three phases : 1) under controlled conditions in a growth room, 2) with manipulations in a field experiment and 3) along natural gradients of light and nitrogen in the native forests.

The results were highly consistent from the growth room to the field. Light strongly affected plant physiological

responses, including photosynthesis, relative growth rates and growth efficiency, whereas nitrogen had little effect. In regression analyses, leaf morphological properties, including specific leaf weight and leaf succulence, were the best predictors of relative growth rates (R^2=.67). Irrespective of nitrogen supply, the biochemical properties of sun leaves included higher concentrations of starch, nonstructural carbohydrates and % lignin + cutin in the cell wall, but lower concentrations of structural polymers, total nitrogen, free amino acids, and ratio of free amino acids : total N, compared to shade leaves. Sun leaves also were slightly higher in digestible energy, much lower in digestible nitrogen and presumably less palatable due to higher tannin concentrations, compared to shade leaves. Tannins were directly correlated to specific leaf weight (R^2=.89). Regression equations based upon specific leaf weight, leaf succulence and leaf structural polymers accurately predicted field values for digestible nitrogen (R^2=.91) and digestible energy (R^2=.96) in foliage. Nutritional properties of blueberry forage grown under variable irradiance in the natural stands matched predictions based upon results from the growth room and field. Compared to even-aged stands and oldgrowth, leaves of plants grown in clear-cuts were similar in digestible energy, much lower in digestible nitrogen, and presumably less palatable due to higher tannin concentrations.

APPLICATION OF THE CARBON/NITROGEN BALANCE CONCEPT TO PREDICTING THE NUTRITIONAL QUALITY OF BLUEBERRY FOLIAGE TO DEER IN SOUTHEASTERN ALASKA

by

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INTRODUCTION

The intensified management of forests wordwide is disrupting the habitat relationships of many wildlife species (Richards and Tucker 1988, Wilson 1988). Furthermore, silvicultural practices which maximize timber yield can alter understory forage resources to the detriment of wildlife. Forest microenvironments strongly affect the productivity and chemical composition of understory vegetation, yet the physiological mechanisms determining understory growth and nutritional responses to forest disturbance are poorly understood.

Population responses of deer to their habitat have traditionally been linked directly to dramatic changes in forage abundance during forest stand development (Cowan 1945, Einarsen 1946, Brown 1961, Gates 1968, Jones 1974, Wallmo and Schoen 1980, Hanley 1984, Hanley et al. 1987). Forage biomass generally is highest in young open stands, lowest in dense even-aged stands, and intermediate in oldgrowth. Yet for unknown reasons, populations of Sitka black-tailed deer (Odocoileus hemionus sitkensis Sm.) have not responded favorably to an increased abundance of understory forages in regenerating forests created by the recent clearcutting of old-growth forests in southeastern Alaska. And in some heavily-logged regions deer populations

may be declining (Wallmo and Schoen 1980).

Forage quantity has been suggested to limit the carrying capacity or maximum sustainable populations of deer in coastal Alaska (Hanley and McKendrick 1985). By contrast, the nutritional quality of forage has been demonstrated to be important in determining the size, growth rates, and reproductive success of Alaskan deer (Klein 1965). Because natural mortality rates are periodically high for deer in this ecosystem, it is plausible that forage quality determines the observed population densities of deer by regulating reproductive success.

Critical nutritional factors for Alaskan deer include the concentrations of digestible energy and nitrogen in forage, as well as carbon-based chemical defenses such as condensed tannins which reduce forage palatability and inhibit the efficiency of forage digestion by deer (Hanley and McKendrick 1985, Robbins et al. 1987a, b). Observational studies have indicated that the nutritional properties of forages vary markedly among forests of different ages in southeastern Alaska. Open-grown forages seem to be lower in nutritional quality compared to the same plant species growing beneath a forest canopy (Rose 1982, Hanley and McKendrick 1983, Hanley et al. 1987, Van Horne et al. 1988). Consequently, poor nutritional quality of forages in forest openings may explain the preference of Alaskan deer for forages growing in forest environments compared to young clear-cuts.

The objectives of this study were to identify the physiological mechanisms of understory plant responses to changing environments in managed forests of southeastern Alaska, and to examine the consequences of management practices for the nutritional quality of forages for deer. Plant responses to light and nitrogen availability were examined in blueberry (Vaccinium ovalifolium, Sm.), an abundant deciduous shrub in the Alaskan rainforests and an important forage species for deer.

Alaback (1982) has suggested that light is the principal factor influencing understory plants in southeastern Alaska. Light intensities beneath the canopy of the Alaskan forests typically are very low, reaching less than 1% of open, and small canopy gaps created by windthrow are the major feature of environmental heterogeneity. In addition to light interception by the forest canopy, persistent maritime cloudcover further reduces ambient light intensity in the region to less than one-half that of more temperate climates. Blueberry is capable of acclimating to a far greater range in light intensity in the Alaskan coastal forests than most vascular plants (Berry and Downton 1982). Hence, blueberry was expected to exhibit strong physiological responses to light intensity.

Nutrients, especially nitrogen, also frequently limit plant growth in northern ecosystems (Chapin and Shaver 1985, Oechel and Lawrence 1985). Limited soil nutrient availability in the Alaskan rainforest soils has been

attributed to cool temperatures, nutrient leaching by heavy rainfall, poor soil drainage, and the high organic content of the soils (Sidle and Shaw 1983). However, nitrogen availability may increase after forest disturbance (Vitousek et al. 1982). Deciduous plants generally exhibit improved growth with high levels of nutrient availability (Bigger and Oechel 1982); therefore blueberry also was expected to respond positively to nitrogen addition.

A major evolutionary response of plants to low nutrient availability has been the reduction in maximum growth rates (Chapin 1980a). Slow inherent growth rates (genetic limitation on growth) reduce nutrient demand, thereby preventing nutrient deficiencies in plants on infertile soils. According to the resource availability hypothesis of Coley et al. (1985), slow inherent growth rates have favored the evolution of greater amounts of carbon-based chemical defenses (such as condensed tannins) in plants from infertile soils, because the cost of chemical defense in terms of reduced growth is less than the cost of replacing tissues consumed by herbivores. This hypothesis applies strictly to evolutionary (genetic) variation in allocation among plant species, not to changes in allocation resulting from plastic or acclimatory responses of plants to environmental gradients.

Allocation patterns associated with acclimatory responses in plants are better explained by the carbon/nutrient balance hypothesis of Bryant et al. (1983).

This concept holds that changes in the availability of resources (primarily carbon or light and nitrogen) alter the fluxes of C- and N- based metabolic substrates in plants, which determine allocation.

And because N generally limits plant growth more than photosynthesis, N-limitation produces a high plant C/N ratio which favors the accumulation of surplus photosynthate and stimulates the production of carbon-based compounds, including chemical defenses such as condensed tannins. Conversely, because light limitation reduces photosynthesis more than growth, light limitation produces a low plant C/N ratio which favors the accumulation of N in tissues but reduces tannins and carbon-based compounds.

In addition to the indirect limitations on plant processes by metabolic substrates, direct effects of environment on plants must also be considered to interpret plasticity in allocation (Loomis 1932). Examples of direct effects include temperature effects on enzyme activity, effects of photoperiod and light spectra on gene expression, and hormonal control of metabolic activity.

The incorporation of Bryant et al.'s (1983) carbon/ nutrient balance concept into the integrated physiological framework of Loomis (1932) produces a holistic basis for understanding acclimatory variation in growth and allocation in plants (Fig I.1). Accordingly, allocation is mediated by the environment indirectly by the supply of metabolic substrates (upper arrow, Fig.I.1), and/or

directly by the activity of different metabolic pathways and plant organs (lower arrow, Fig.I.1).

Understory vegetation in regenerating forests of southeastern Alaska fluctuates widely in biomass but not in species composition as characterizes temperate forests in more southerly latitudes. Consequently, the native understory flora of the Alaskan coastal forests were expected to possess a broad capability for acclimating to changing light and nutrient availability. I hypothesized that variation in the C/N ratio of understory plants in response to changing availability of resources (light and nitrogen) during forest regeneration in southeastern Alaska determined changes in biomass production, chemical defense, and nutritional quality of understory forage to deer. In the context of this research, the term C/N ratio refers to the fluxes of C and N in a plant, as inferred from physiological and biochemical variables.

Chapter I examines the response of blueberry shrubs to controlled irradiance and nitrogen supply in a growth room, and develops equations to predict forage quality as a function of easily-measured biochemical and morphological properties of leaves. Chapter II tests the principles and predictive equations derived from the growth-room study in a field experiment employing gradients in irradiance and nitrogen supply. And finally, Chapter III examines plant nutritional properties in natural forests encompassing a range of sites, stand ages, and resource availability.

CHAPTER I.

THE INFLUENCE OF IRRADIANCE AND NITROGEN SUPPLY ON THE NUTRITIONAL QUALITY OF BLUEBERRY FOLIAGE TO DEER :

I. A CONTROLLED ENVIRONMENT STUDY

by

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ABSTRACT

A growth room experiment was conducted on blueberry seedlings (Vaccinium ovalifolium Sm.), to test the hypothesis that changes in the plant C/N ratio in response to light and nutrient availability alter the nutritional quality of forage.

Net photosynthetic rates, net photosynthetic uptake (whole plant basis), relative growth rates and growth efficiency increased directly with irradiance, but nitrogen effects were minor. Sun leaves had a higher C/N ratio as indicated by higher concentrations of starch, % lignin + cutin in the cell wall and condensed tannins, but lower cell wall fiber, free amino acids and total nitrogen compared to shade leaves. Leaf concentrations of digestible nitrogen were highest at low irradiance (low C/N ratio), but digestible energy peaked at moderate irradiance (intermediate C/N ratio). At high irradiance, leaf digestible energy decreased because tannin accumulation reduced energy digestibility.

Condensed tannins were closely related to specific leaf weight (R^2 = .89). Regression equations for digestible nitrogen (R^2 = .91) and energy (R^2 = .96) were based upon specific leaf weight, leaf succulence, and structural polymers and served as a foundation for a field experiment in Chapter II.

INTRODUCTION

Deer in southeastern Alaska demonstrate a distinct preference for forage growing beneath a forest canopy compared to the same forage species growing in recently harvested clear-cuts (Wallmo and Schoen 1980, Rose 1982, Hanley et al. 1987). This preference may be explained by the lower nutritional quality of forages grown in clear-cuts compared to the forest understory (Rose 1982, Hanley and McKendrick 1985, Van Horne et al. 1988). Concentrations of nutrients and plant chemical defenses are important factors in the diet selection and nutrition of many herbivores (Bryant and Kuropat 1980, Mattson 1980, White 1984, Cooper and Owen-Smith 1985). Although understory plants can respond dramatically to disturbances in the forest overstory, relatively little is known about the nature or physiological mechanisms of such responses in the rainforest ecosystem of southeastern Alaska.

Bryant et al. (1983) proposed a mechanism in the carbon/nutrient balance hypothesis, whereby changing light and mineral nutrient availability in the environment alters fluxes of carbon and nutrient substrates (especially nitrogen) in plants. And as the plant internal carbon to nitrogen (C/N) ratio changes, shifts occur in the allocation of limiting resources to different metabolic pathways and plant parts. Allocation shifts produce variation in the growth, nutrient concentrations and

chemical defenses of plants along environmental gradients (Bazzaz et al. 1987, Chapin et al. 1987, Pearcy et al. 1987).

This hypothesis recognizes a basic physiological principle that nutrient shortages (mainly N) generally limit plant growth more than they limit photosynthesis. Accordingly, the limitation of growth relative to photosynthesis leads to the accumulation of surplus carbohydrates which stimulates the production of carbon-based chemical defenses such as condensed tannins. Thus, nutrient limitations increase the plant C/N ratio and produce plant tissues low in nitrogen, but high in carbon-based compounds, including storage carbohydrates and tannins. In contrast, light limitations inhibit photosynthesis more than either growth or nutrient absorption. This reduces the plant C/N ratio and produces plant tissues with high concentrations of nitrogen, but low concentrations of carbohydrates and tannins (Waring et al. 1985, Larsson et al. 1986).

This hypothesis focuses on the indirect effects of resource availability on the plant internal C/N ratio and allocation, yet environment also can affect allocation by directly controlling the activity of different metabolic sinks (Loomis 1932, 1953), as outlined in Figure I.1. In the present study, the carbon/nutrient balance hypothesis of Bryant et al. (1983) is interpreted in the broader physiological context of Loomis (1932, 1953) as the

carbon/nutrient balance concept. The important distinction is that allocation is determined not only by the indirect effects of limiting resources (light and nitrogen), but also by direct environmental effects, such as hormone induction, gene expression and enzyme activation or inhibition.

The purpose of this study was to determine how plant physiological responses to light and nitrogen (the C/N ratio) influence growth, nutrient composition, and chemical defense in blueberry (Vaccinium ovalifolium, Sm.) an important forage for deer in southeastern Alaska. I hypothesized that the changing nutritional quality of blueberry forage in terms of digestible energy and nitrogen was related to the C/N ratio of the plant under different regimes of irradiance and nitrogen supply.

In a growth room experiment I investigated : 1) the effects of irradiance and nitrogen supply on the plant C/N ratio and responses in terms of photosynthesis, nutrient concentrations and allocation to growth and defense and 2) whether easily-measured biochemical or morphological properties of the foliage could accurately predict forage nutritional quality to deer.

METHODS

Growth room experiment

Blueberry seedlings were grown from seed collected during September 1983, in a young clear-cut near

Petersburg, Alaska. The seed were immediately planted with potting soil into styrofoam blocks and placed in a greenhouse at the US Forest Service's Heintzelman Nursery near Petersburg, Alaska. Greenhouse conditions included ambient light, with high moisture and nutrient supply.

In May 1986, the three-year-old seedlings were transplanted for a duration of three months into an Environmental Growth Chamber (EGC, Chagrin Falls, OH, 3m x 4m x 2.5 m). The chamber was equipped with temperature and humidity controls, and Sun-Brella lighting composed of G.E. Multivapor and Lucalox (sodium vapor) lamps. Chamber conditions matched the native environment during mid-summer (June-July) in southeastern Alaska, including a 20-h photoperiod, relative humidity of 85-95%, and day/night temperatures of 14 /10 °C (NOAA 1988). The lamps provided light in a spectral range from 300 to 1100 nm at an intensity of 600 uE m^{-2} s^{-1}, PAR. Approximately one-quarter of the total incident radiation was in the spectral range of ultraviolet and near infrared (unpubl. data, Duke Phytotron).

Irradiance to the plants was measured with a Li-Cor 190s PAR sensor (LiCor Inc.) at 1 m distance from the lamps. Shading provided by tents of polypropylene cloth was chosen to simulate the range of light conditions typical of the natural forests with overcast skies (Chapter III). Irradiance treatments (in uE m^{-2} s^{-1}) ranged from a low of 5, characteristic of dense even-aged regenerating stands,

to a maximum of 450 in open clear-cuts, with two intermediate shade levels of 100 and 200.

The experimental design consisted of a split-plot factorial with three nutrient levels (subplots) randomly assigned within each of four light levels (whole plots), and eight seedlings (replications) per each of twelve treatments (total = 96 seedlings, with one light treatment per tray). Individual seedlings were regarded as valid replicates because conditions were checked for uniformity throughout the growth chamber, and because seedling trays were relocated regularly to randomize possible effects of location within the chamber.

Seedlings were planted in a sand-perlite mixture (Hewitt 1952), fertilized daily and the soil was flushed weekly with distilled water to prevent salt accumulation. The balanced nutrient solution contained three concentrations of nitrogen (mg N /l) : low = 5; medium =20; high = 100. Ingestad (1973) found these three solutions provided (sequentially), suboptimal, optimal, and supra-optimal N nutrition to a closely related Vaccinium species.

Gas exchange, seedling harvest, and growth analysis

Net photosynthetic rates were measured on leaves at two-months of age, with a Licor 6000 portable flow-through gas exchange system and infrared gas analyzer (Licor, Inc.) at light saturation. Gas exchange measurements were made at this time because at age two months when the leaves are

fully expanded, the physiological and biochemical properties of the leaves have reached relative stability. The maximum lifetime of leaves on this deciduous shrub is about 6-7 months.

Measurements of net CO_2 exchange were made at mid-day by attaching a cuvette to a group of three mature leaves on the distal end of the terminal leader of each seedling. Projected leaf areas were then determined on a leaf area meter (Licor, Inc.) to determine the net CO_2 exchange per unit leaf area. Plant photosynthetic uptake was calculated as the rate of CO_2 gain per plant by multiplying the photosynthetic rate per unit leaf area times the total leaf area per plant.

Plants were harvested on July 28, 1986 after 3 months in the growth room. This was considered sufficient time for the acclimatory responses to take place because responses to disturbance in the natural habitat are apparent almost immediately in newly-flushed leaves. For growth analysis, the initial fresh weight of each plant was measured, and additional plants were weighed, dried, and re-weighed to obtain an estimate of the initial fresh:dry weight ratios. At harvesting, roots and shoots were weighed, fresh:dry weight ratios were again determined, and plant growth rates were calculated as the total biomass increase per gram dry weight of original plant biomass.

Additional measurements made at harvest time included leaf mortality, specific leaf weight and leaf succulence.

Variables calculated from the data included growth efficiency (% change in biomass/ mg CO_2 cm^{-2} leaf) and the root/shoot biomass ratio. Fresh leaf material was stored in air-tight plastic bags at -40 °C prior to chemical analysis. Mature leaves only were used for measurement of physiology, morphology, and biochemistry.

Biochemical analyses

Sequential chemical analysis of the fresh, frozen leaf material followed the laboratory protocol described in Appendix A. Protein precipitating capacity of the foliage was measured with the BSA technique of Martin and Martin (1982) as modified by Robbins et al. (1987a). Protein precipitation by leaf extracts in blueberry was expressed in terms of amounts of condensed tannins because Van Horne et al. (1988) found that concentrations of condensed tannins were directly proportional to protein-precipitating activity of leaf extracts in this species.

Sugars were measured by colorimetric reaction with anthrone (Yemm and Willis, 1954) on fresh leaf tissue homogenized and extracted in 80% ethanol (Harborne 1984). For starch analysis, the plant material was dried and ground in liquid nitrogen according to procedures described by Sandstrom and Loomis (1987). Starch was quantified by colorimetric reaction with anthrone, using a perchloric acid extract of plant tissue (Viles and Silverman 1949, McCready et al. 1950).

Holocellulose and hololignin were determined by modifying the standard methods of forage analysis (Goering and Van Soest 1970) to separate the leaf fiber into fractions digestible and nondigestible to ruminant herbivores such as deer. Holocellulose (the digestible fiber), composed of cellulose and acid-labile hemicellulose, was determined by weight loss of the tissue fiber component after hydrolysis in 72% sulfuric acid. Hololignin (the nondigestible fiber), composed of lignin, acid-resistant hemicellulose, and cutin, was determined by sample weight difference after ashing the cellulose-extracted residue in a muffle furnace (550 °C, 4 h).

Total Kjeldahl nitrogen was measured with a Technicon Autoanalyzer II on dried foliage ground to pass a 40-mesh screen, and digested in a Technicon block digestor using a sulfuric acid-Se/CuSO4 catalyst (Technicon Instruments Corp. 1975, 1976).

Total free amino acids were determined by the ninhydrin reaction (Moore and Stein 1954) using the same ethanol extract used for sugar analysis.

NDF (Neutral Detergent Fiber = hemicellulose, cellulose, lignin, and cutin), NDS (Neutral Detergent Solubles = 1 - NDF), and the percentage lignin + cutin in the NDF were quantified by standard sequential detergent analysis techniques (Goering and Van Soest 1970), as modified by Mould and Robbins (1982). These variables then were used in equations supplied by Robbins (1983), Robbins and Moen

(1975), and Robbins et al. (1987a, 1987b) to calculate in-vivo concentrations of digestible protein and energy digestibility as follows:

Digestible protein

$$Z = -3.87 + 0.9283 \text{ (crude protein)} - 11.82 \text{ (bsa)} \; ; \; R^2=.99$$

where: Z = digestible protein; g/ 100 g forage
crude protein = % total N * 6.25; g/ 100 g forage
bsa = bsa protein precipitated; g/ 100 g forage

Digestible energy

$$Y_1 = [(0.9231e^{-0.0451x})(NDF)]; \; R^2 = .98$$
$$+ [-16.03 + (1.02\ NDS) - 2.8\ P]; \; R^2 = .99$$

where: Y_1 = % dry matter digestibility; g/ 100 g forage
x = % lignin + cutin in the NDF
NDF = % of cell wall in tissue; g/ 100 g forage
NDS = 100 - % NDF; g/ 100 g forage
P = 11.82% * bsa = in vivo correction for bsa

$$Y_2 = -0.71 + 0.99 * Y_1; \; R^2 = .94$$

where: Y_2 = % energy digestibility; g/ 100 g forage

$$Y_3 = Y_2 * 4.65 \text{ kcal/ g forage; kal /g forage}$$

where: Y_3 = digestibile energy in kcal/ g forage

Values for digestible protein were then converted to units of digestible nitrogen (by dividing by 6.25) to make the results easier to compare to the % total N values. Digestible energy (Y_3) was determined by multiplying the % energy digestibility (Y_2) by an estimated average caloric value of 4.5 kcal/g leaf tissue (Hanley and McKendrick 1985, Williams et al. 1987).

Assessing the plant C/N ratio

The term C/N ratio functionally relates to the net flux of photosynthates (carbon) and nitrogenous substrates into

a plant. Fluxes of carbon and nitrogen are difficult to measure in plants, therefore researchers have frequently used tissue concentrations of carbon and nitrogen to estimate the ratio. However, interpretations based upon the C/N ratio inferred from C and N concentrations are subject to large errors unless data on growth and other physiological responses are known (Waring et al. 1985, Chapin et al. 1987). Furthermore, correct interpretation of the ratio is dependent upon the recognition that plants have inherent (genetically-fixed) allocation priorities (Waring 1985). For example, when plant growth is limited to a greater extent than photosynthesis, surplus carbon may accumulate as storage reserves or defense compounds in plant tissues even though the total carbon flux is reduced (Bryant et al. 1983, Waring et al. 1985, Larsson et al. 1986). This is true because growth has a higher priority for carbon than either storage reserves or chemical defense. In this study, complementary physiological and biochemical data are collected which permit the interpretation of the C/N ratio in terms of resulting changes in allocation.

Statistical analysis

Statistical analyses were performed with SAS statistical software for personal computers (SAS Institute, Inc 1985). All variables having unequal variances were log-transformed and results reported for variables in the untransformed

state. The data were analyzed as a factorial, not as a split-plot design because replications within light treatments were not significant as calculated by the SAS LSMEANS procedure. Two-way analysis of variance was conducted using the GLM procedure. In order to compare all treatment means and minimize the probability of Type II error despite significant interaction effects of light and nitrogen, the more conservative Scheffe's test was used. Quantitative relationships between selected variables were examined using linear and nonlinear regression analysis.

RESULTS

Growth and physiology

Most of the variation in plant responses was explained by irradiance. Nitrogen effects and irradiance x nitrogen interactions were generally significant in the ANOVA, but explained only a small amount of the variation in plant responses compared to the main effect of irradiance (Appendix B). However, using the more conservative Scheffe's test to compare treatment means (controls experiment-wise error rate), most significant plant responses were attributable to irradiance rather than to nitrogen supply or to irradiance x nitrogen interactions.

Rates of light-saturated net photosynthesis (photosynthetic capacity) doubled and growth efficiency quadrupled with increasing irradiance (Figs. I.2, I.3). Net photosynthetic uptake increased most dramatically in direct

response to irradiance, but also increased directly with nitrogen at 200 uE m^{-2} s^{-1} irradiance (Table I.1). Relative growth rates increased by a factor of 10 across the range of irradiance treatments, but tended to decrease with increasing nitrogen supply (Table I.1). Treatment means for the root:shoot ratio differed only between treatments combining the highest and lowest levels of irradiance and nitrogen supply (Table I.1). Leaf mortality increased with increased nitrogen supply only at the lowest irradiance (Table I.1). Total leaf area increased at higher irradiance, and moreso with increased N supply (Table I.1)

Leaf biochemistry and morphology

Leaves of plants grown under high irradiance accumulated C-based compounds and were depleted in nitrogenous compounds compared to shade leaves. Of the C-based compounds, tannin concentrations (proportional to protein precipitation, see Methods), responded directly to irradiance with a 5-fold increase, but did not respond to nitrogen (Fig. I.4a). Of the nonstructural carbohydrates, sugars increased with increased nitrogen supply at 200 uE m^{-2} s^{-1} irradiance. The most notable response in starch was a tripling of concentrations at maximum irradiance and low nitrogen supply (Fig. I.4b). Total nonstructural carbohydrates (sugar + starch) mirrored increasing trends in sugars at 200 uE m^{-2} s^{-1} irradiance and increasing starch at 450 uE m^{-2} s^{-1} irradiance (Table I.2).

Leaf structural polymers, responded mainly to irradiance (Table I.2). Although cellulose concentrations tended to be slightly lower at higher irrradiance treatments, the differences were not significant (Table I.2). Hololignin decreased significantly above 100 uE m^{-2} s^{-1} irradiance, and there was a trend for the decrease to be larger as nitrogen supply increased; these trends also were evident in total structural polymers (holocellulose + hololignin) (Table I.2).

With respect to nitrogenous compounds, irradiance had the major effect on the concentration of total N (Appendix B). The concentration of total nitrogen was inversely related to irradiance and varied by a factor of two across the range of treatments (Fig. I.5a). Main and interactive effects of light and nitrogen were significant for free amino acids and the ratio of free amino acids to total N. Free amino acids and the ratio of free amino acids to total nitrogen were inversely related to irradiance, but a direct response to nitrogen supply was evident at 200 uE m^{-1} s^{-1} irradiance (Figs. I.5 b,c).

Specific leaf weight increased directly with irradiance (Fig. I.4c). Succulence was inversely related to irradiance, but directly related to nitrogen supply at lower irradiance (Table I.2).

Forage nutritional quality

Variables relating to forage nutritional quality were

affected by irradiance but not by nitrogen (Appendix B). Similar to total structural polymers, NDF (total cell fiber) was inversely related to irradiance. NDS (total cell solubles) exhibited an direct response to irradiance (Table I.2). The proportion of lignin + cutin in the cell wall also increased slightly with irradiance (Table I.2).

Digestible nitrogen declined by more than half at high irradiance, and a significant response to maximum N supply also was evident at 200 uE m^{-1} s^{-1} irradiance (Fig. I.6a). Digestible energy remained fairly constant regardless of irrradiance or N supply (Fig. I.6b).

Equations to describe plant growth and nutritional quality

Plant relative growth rates were most closely correlated with leaf tannins (R^2=.62), and to the combined variables : specific leaf weight and leaf succulence (R^2 = .67), (Table I.3). Tannins were highly correlated with specific leaf weight (R^2=.89) (Table I.3), as depicted in Figure I.7. Digestible nitrogen was strongly related to the combined variables : specific leaf weight and leaf succulence (R^2=.91, Table I.3). Digestible energy also was closely related to leaf structure, including together : specific leaf weight and total structural polymers (R^2 =.96) (Table I.3).

DISCUSSION

Responses in the growth, physiology and biochemistry of

blueberry seedlings indicated that irradiance was the major limiting factor and N supply was relatively unimportant. Strong light limitations on metabolism in blueberry were evident in the higher net photosythetic rates per unit leaf nitrogen at higher irradiance (c.f. Seemann et al. 1987). Physiological stress was demonstrated by the low growth efficiency and net photosynthetic uptake of densely shaded blueberry plants (McLaughlin et al. 1982), resulting from concomitant reductions in total leaf area and photosynthetic rates.

Limited response to nitrogen is characteristic of slow-growing plants adapted to infertile soils (Chapin 1980a, Chapin and Shaver 1985), such as Ledum palustre in the Alaskan tussock tundra (Shaver 1981), and seven species of understory shrubs in Costa Rican rainforests (Denslow et al. 1989). Although N fertilization did not produce a large response in foliar N levels during the 3-month study, this experiment should be a fair test of blueberry response to an N gradient because Ingestad (1973) found large responses in Vaccinium in only 6 weeks.

The proportional and absolute amount of N in photosynthetic enzymes presumably increased despite decreasing concentrations of total nitrogen at high irradiance, a pattern frequently observed with understory species (Medina 1977, Seemann et al. 1987). This intepretation is supported by the lower ratio of free amino acids to total nitrogen at higher irradiances. Amino acid

accumulation in shade leaves of blueberry is symptomatic of surplus N resulting from inhibited growth and protein synthesis (Margolis and Waring 1986). Amino acid synthesis depends primarily upon substrates from glycolysis, hence amino acids may accumulate even when unfavorable growing conditions inhibit Krebs cycle respiration.

By contrast, plant species which are nitrogen-limited usually increase tissue levels of nitrogen at higher irradiance, presumably because more carbon is available to support root function and mycorrhizal development (White 1984, McDonald et al. 1986, Denslow et al. 1989). A threshold of light limitation may be denoted at 100 uE m^{-2} s^{-1} irradiance by the concurrent decline in growth and accumulation of nitrogen and amino acids (Perchorowicz et al. 1981, Pearcy et al. 1987). Since the increasing ratio of free amino acids to total N signals higher proportions of non-metabolizable N in densely shaded leaves, the ratio may serve as a sensitive biochemical index of plant stress related to metabolic imbalances in the plant C/N ratio.

Higher rates of net photosynthesis, relative growth and growth efficiency indicated increasing net fluxes of metabolic substrates in plants grown at the higher irradiance. However, concurrent changes in the foliar concentrations of carbon- and nitrogen-based compounds suggested that the net flux per leaf of carbon increased, whereas the net flux of nitrogen decreased at higher irradiance. Consequently, the C/N ratio in blueberry varied

primarily as a function of light limitations on photosynthesis and growth, and to a lesser degree as a function of genetic limitations on the maximum uptake and assimilation of nitrogen.

Maximum rates of net photosynthesis in blueberry were low, as expected for an ericaceous shrub (Sestak et al. 1971). The fact that specific leaf weight increased as irradiance increased from 200 to 450 uE m^{-2} s^{-1}, even though net photosynthetic rates stabilized at 200 uE m^{-2} s^{-1} irradiance suggests that maximum photosynthetic capacity of blueberry is reached near 450 uE m^{-2} s^{-1} irradiance. Low maximum photosynthetic capacity is characteristic of stress-tolerant plants with slower inherent growth rates (Chapin 1980a). This phenomenon has been attributed to genetic limitations on the production of rate-limiting catalysts in photosynthesis (Boardman 1977, Jurik et al. 1979, Oren et al. 1986). Self-shading or increasing biosynthetic costs in sun leaves also may have contributed to larger increases in specific leaf weight than net photosynthesis at maximum irradiance (Potter and Breen 1980, Jarvis and Leverenz 1983, Williams et al. 1987).

Although light was reduced 100-fold at the lowest irradiance treatment, shade leaves attained half the assimilation rate found at maximum irradiance. High photosynthetic efficiency at low irradiance has been reported for many understory species (Schulze 1970, Boardman 1977). Furthermore, assimilation rates in

blueberry were considerably higher than values reported for other another Vaccinium species at comparable irradiance levels (Karlsson 1985). High photosynthetic efficiency in blueberry leaves grown under dense shade probably reflect a strong adaptation to extremely low light levels in the natural habitat.

Growth rates of blueberry were comparable to reported values for other species of deciduous shrubs native to high latitudes (Chapin 1980b). Slow inherent growth rates not only prevent nutrient deficiencies in plants adapted to nutrient-poor soils, but may also conserve storage reserves during conditions unfavorable for photosynthesis (Stewart and Bannister 1973). Because blueberry seedlings were carbon-limited, growth was closely related to dry matter accumulation in leaves, including specific leaf weight and succulence (R^2=.67).

Maintenance (dark) respiration associated with high amino acid concentrations in leaves may also have contributed to slow growth rates in shaded plants. Free amino acids are a storage form of surplus nitrogen that cannot be incorporated into proteins in light-limited plants (Miflin and Lea 1980, Bassham et al. 1981, Runge 1983, Amthor 1984, Journet et al. 1986). Concentrations of amino acids and rates of dark respiration were observed to increase dramatically in shaded and fertilized willow under controlled conditions in a growth room (Waring et al. 1985).

Stress-tolerant plants such as V. ovalifolium which regenerate in forest gaps frequently accumulate tannins during exposure to high light intensity (Langenheim et al. 1984), a response attributed to increasing carbon substrates and accumulation of phenylpropanoids, a precursor in tannin synthesis (Margna 1977, Bryant et al. 1983, Coley et al. 1985, Jensen 1986). Yet, the fact that tannins increased disproportionately to net photosynthesis and growth at maximum irradiance suggests that tannin synthesis may have been directly induced by the changing light conditions.

The primary enzyme involved in tannin synthesis (PAL phenylalanine ammonium lyase), is stimulated by red light and ultraviolet radiation (Wellman 1976, Woodhead 1981, Goodwin and Mercer 1983, Waterman et al. 1984, Larsson et al. 1986). Direct induction of PAL activity by light has been cited as a primary mechanism allowing facultative shade plants to acclimate to bright light (Robberecht et al. 1980, Langenheim et al. 1984). Phenolics protect leaf enzymes and membranes from damage by ultraviolet radiation (Lee and Lowry 1980, Larcher 1983), and also function as an "overflow" pathway preventing photoinhibition by metabolic intermediates of the Citric Acid Cycle (Philips and Henshaw 1977). The close relationship of tannins to specific leaf weight (R^2 =.96) is explained by the fact that increasing carbon availability at high irradiance stimulates dry matter accumulation in foliage in addition to tannin

synthesis.

The fairly constant concentrations of sugars probably resulted from accelerated metabolism of sugars at higher irradiance. By comparison, the large increase in starch concentrations at high irradiance more accurately reflects the improved carbon balance of the plants. No explanation is readily apparent for the peak in sugars with increased N supply at 200 uE m-2 s-1 irradiance, nor for the reduced starch levels with increased N supply at 450 uE m-2 s-1 irradiance. However, it is possible that nitrogen not only stimulated the conversion of starch to sugars, but also influenced sugar metabolism.

The small variation in cellulose concentrations across irradiance treatments likely resulted because Vaccinium species construct new leaves almost entirely from stored root reserves (Stewart and Bannister 1973). Similar responses have been documented elsewhere (Gulmon and Chu 1981, Chazdon 1986). The synthesis of cellulose and related polymers is controlled by complex and conservative biosynthetic processes which are well-buffered against changing environment. Conservative mechanisms of cellulose synthesis are thought to prevent structural inadequacies in the cell wall that could seriously compromise cell functioning.

However, concentrations of lignin + cutin in the cell wall increased slightly at higher irradiance. Lignin synthesis is controlled by a simple enzyme (PAL) capable of

greater flexibility in response to environment. Because lignin synthesis is part of secondary cell development continuing as leaves age, plasticity in lignin formation serves to fine-tune cell wall structure to environmental conditions (Northcote 1985).

Certain labile components of leaves, such as sugars or starch vary widely over periods of minutes or hours, whereas structural polymers integrate environmental effects over the lifetime of the plants. Therefore, changes in structural polymers may most accurately reflect long-term physiological stress when expressed on the basis of leaf area rather than weight (Smith et al. 1981, Stachurski and Zimka 1975). Structural polymers in blueberry increased directly with irradiance on a leaf area basis, due to the greater thickness of leaves and cell walls exposed to high irradiance (Nygren and Kellomaki 1983, Spalinger et al. 1988).

High values of digestible nitrogen in shaded blueberry leaves were a result of both the high concentrations of total nitrogen and the high digestibility of the nitrogen due to from low tannin concentrations.

High concentrations of nonstructural carbohydrates and other cell solubles (NDS) in rapidly-growing sun leaves were expected to yield greater amounts of digestible energy. Yet digestible energy remained fairly constant because higher energy concentrations were balanced by lower digestibility of sun leaves, a phenomenon also reported by

Bryant and Kuropat 1980, and Waterman et al. 1984. Physiologically, this is explained by the disproportionate increase in digestibility-reducing tannins and other phenolics compared to total gross energy of the forage at high irradiance (Bryant and Kuropat 1980, Waterman et al. 1980). Leaf morphology (specific leaf weight and succulence) was the independent variable most closely related to forage quality, both for digestible nitrogen (R^2 = 0.91) and for digestible energy (R^2 = 0.96). In other studies, specific leaf weight was found to be a good integrator of environmental limitations on dry matter accumulation (Marini and Marini 1983, Oren et al. 1986). Light limitations in this experiment reduced plant carbon gain and produced large changes in biochemistry as well as growth. Consequently, leaf morphology also was a good index to changes in forage nutritional quality.

From this growth room experiment I have demonstrated how changes in the plant carbon/nitrogen ratio under different regimes of irradiance and nitrogen supply affect the growth, physiology, biochemistry and leaf nutritive properties in an understory shrub. Further, I have shown that easily measured morphological and biochemical properties of leaves mirror the environment under which the leaves were grown. With this background, a field experiment was initiated to examine plant responses in the natural environment and to test predictive models of forage quality.

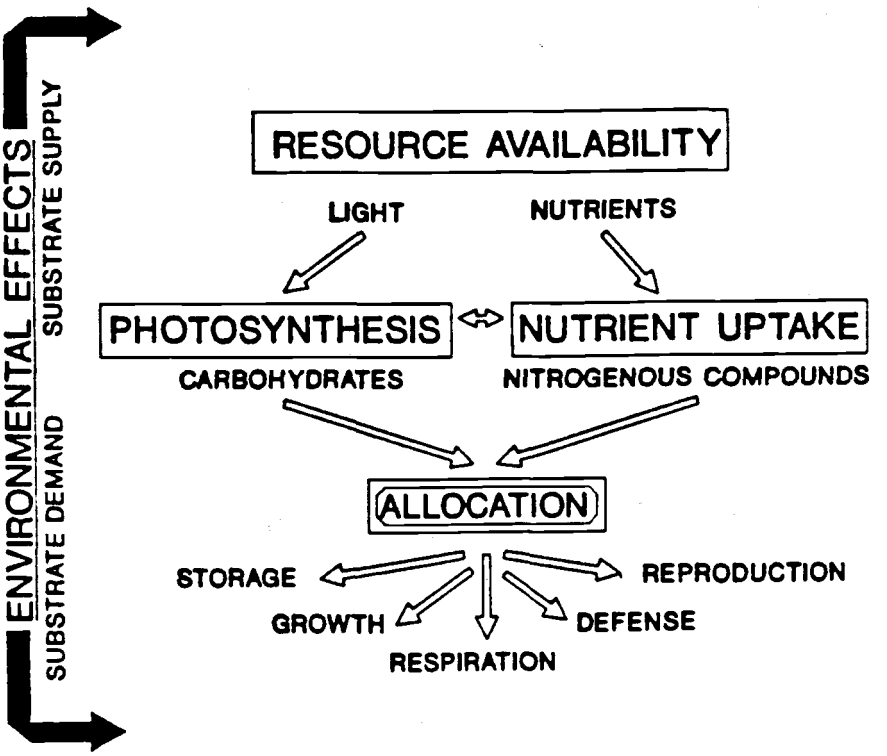

Figure I.1. Environmental influences on intraspecific variation in resource allocation by plants include indirect effects on the flux of photosynthates and nutrients (bold arrow, upper left), as well as direct effects on the growth of different plant organs and the activity of various metabolic pathways (bold arrow, lower left).

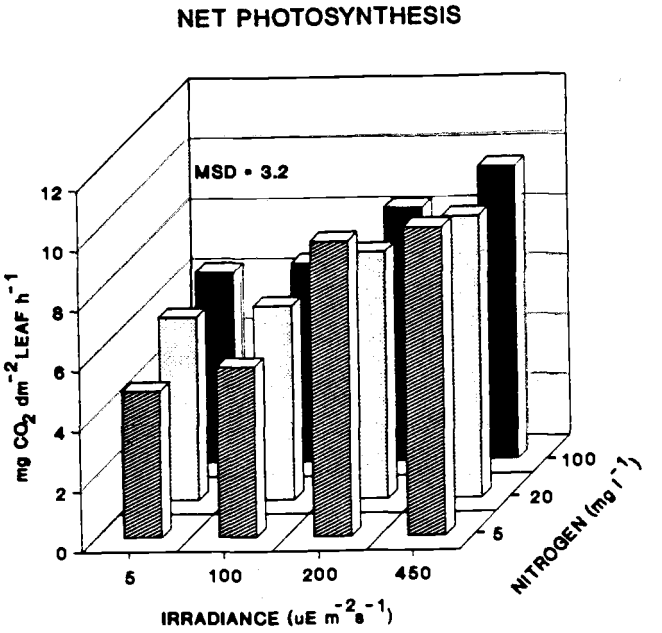

Figure I.2. Light-saturated rates of net photosynthesis in blueberry seedlings acclimated to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 3$ plants per treatment mean.

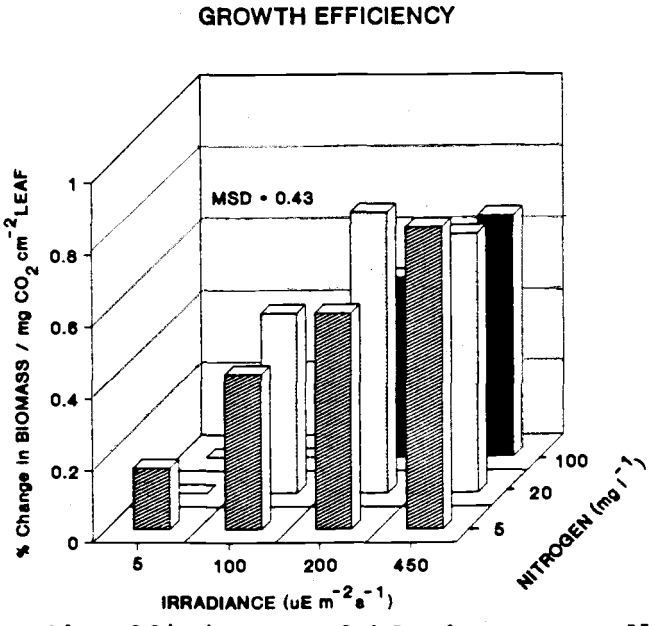

Figure I.3. Growth efficiency of blueberry seedlings in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 3$ plants per treatment mean.

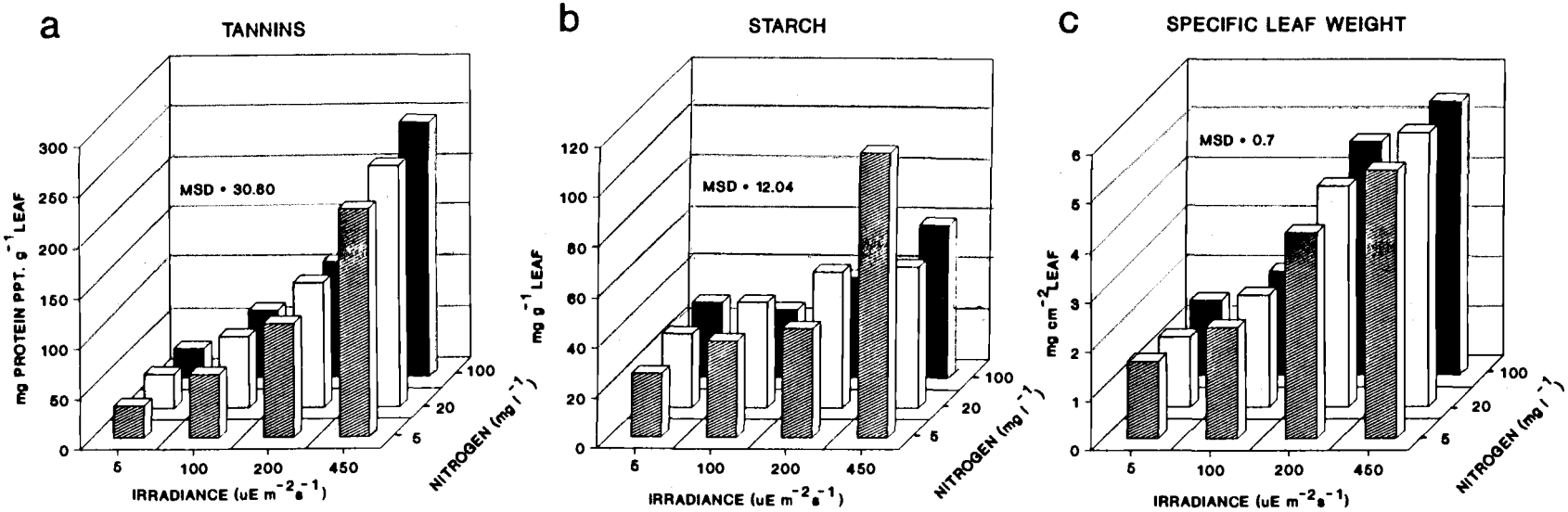

Figure I.4 a,b,c. Protein-precipitating capacity of condensed tannins, starch concentrations, and specific leaf weight of blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean.

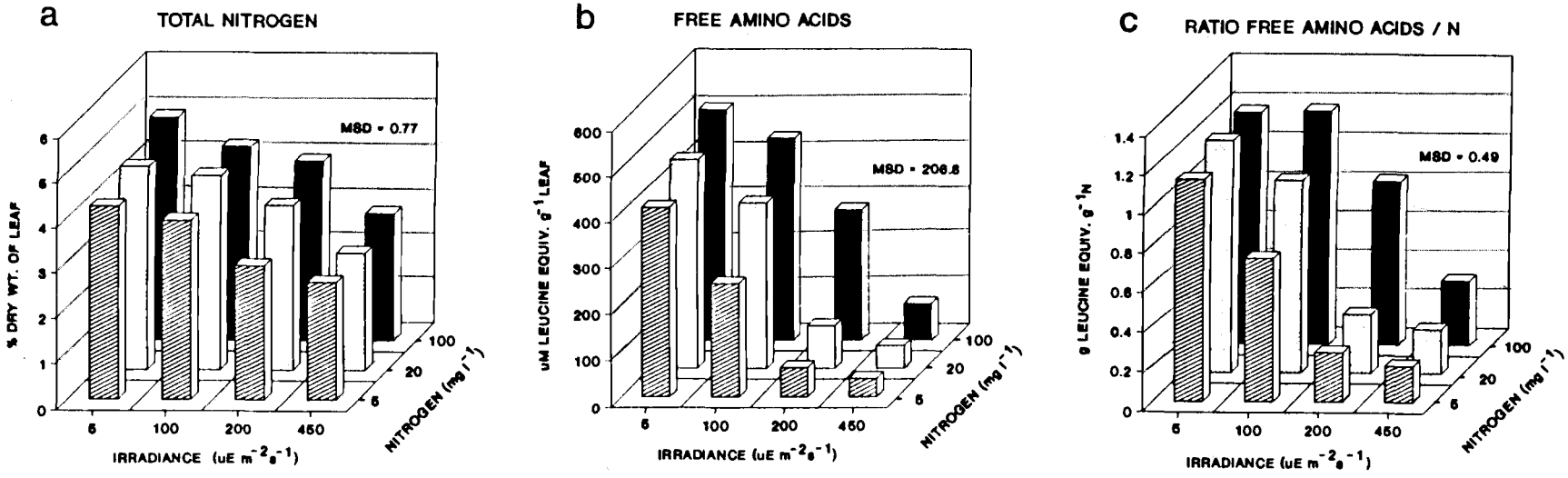

Figure I.5 a,b,c. Concentrations of total nitrogen, free amino acids, and the ratio of free amino acids / total nitrogen in blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean. The conversion of units from Fig. 5b to 5c involved a factor of 8.5348 uM leucine/ mg leucine equivalent.

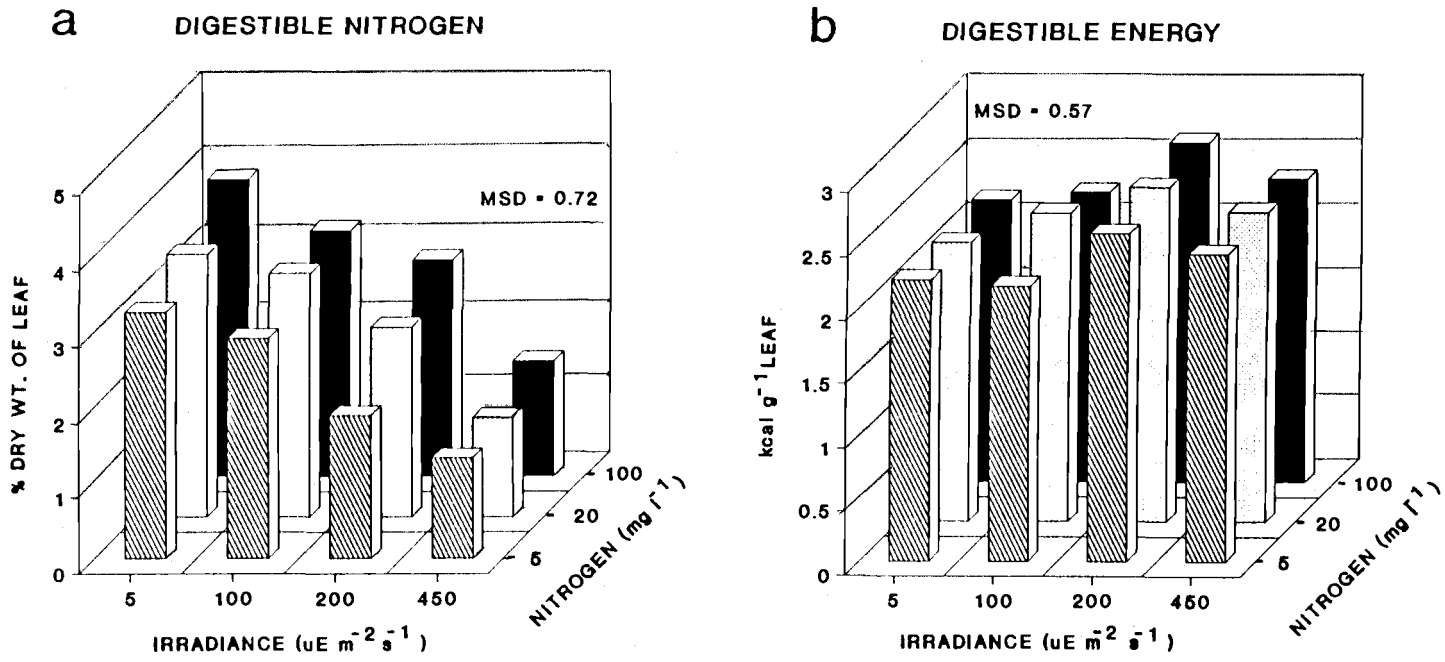

Figure I.6 a,b. Nutritional properties of blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply. Calculation of digestible nitrogen and digestible energy followed equations presented by (Robbins 1983, Robbins et al. 1987 a,b). Digestible energy calculation assumes a gross energy concentration in leaves of 4.5 kcal/g (Hanley and McKendrick 1985, Williams et al. 1987). Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean.

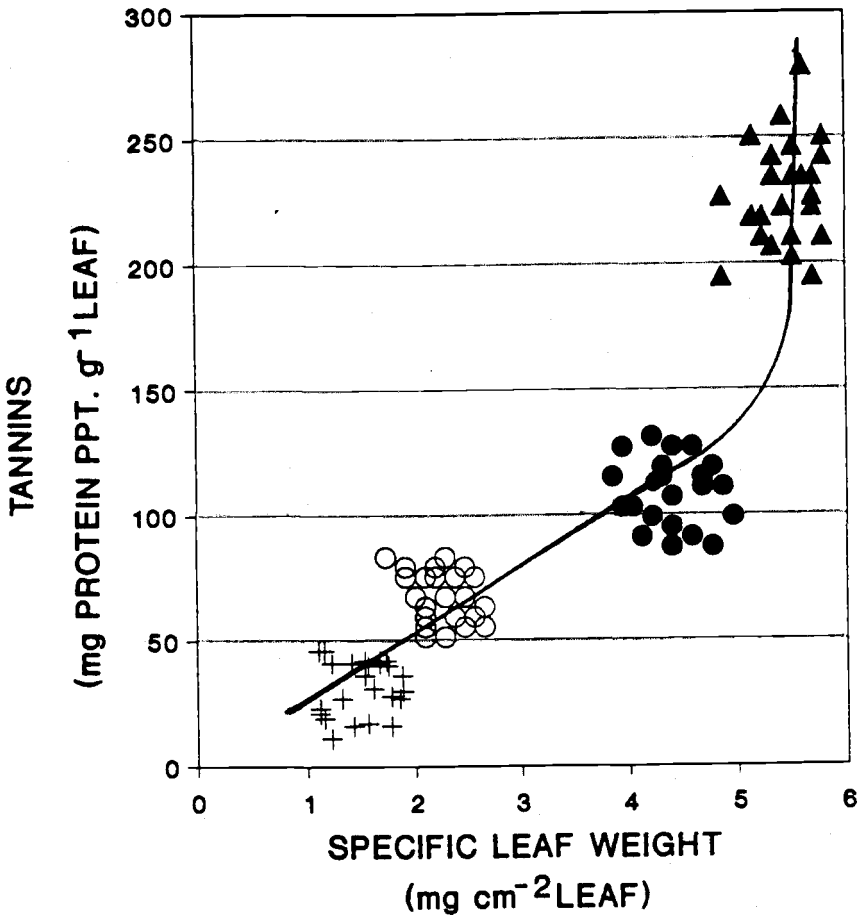

Figure. I.7. Leaf tannins in relation to specific leaf weight at four levels of irradiance. Tannins - $Y = 19.6e^{0.439x}$, in units of mg protein precipitated per g leaf; $R^2=.89$; n=96. Symbols correspond to irradiance treatments as follows (in uE $m^{-2}s^{-1}$):
+ = 5, ○ = 100, ● = 200, ▲ = 450.

Table I.1 Growth and physiological responses of blueberry (Vaccinium ovalifolium) in a growth room experiment to 12 combinations (4 X 3) of irradiance and nitrogen supply. By rows, values with the same letter do not differ, as determined by Scheffe's test at $p < .05$; n = 8 plants per treatment mean.

	IRRADIANCE ($uE\ m^{-2}\ s^{-1}$)											
	5			100			200			450		
RESPONSE VARIABLE	Nitrogen			Nitrogen			Nitrogen			Nitrogen		
	5	20	100	5	20	100	5	20	100	5	20	100
Net photosynthetic uptake ($mg\ CO_2\ h^{-1}$ / plant)	0.5 a	0.6 a	0.9 a	1.1 ab	1.8 ab	2.7 ab	3.3 bc	5.6 cd	7.5 d	7.9 d	9.3 d	10.3 d
Relative growth rate ($mg\ g^{-1}\ d^{-1}$)	2.0 a	-1.1 a	-0.02 a	5.9 ab	7.7 ab	-1.4 a	14.2 bc	20.7 c	10.2 abc	20.7 c	17.7 bc	15.6 bc
Root / shoot biomass ratio ($g\ g^{-1}$)	0.2 c	0.1 abc	0.2 bc	0.2 c	0.2 c	0.2 c	0.2 bc	0.1 abc	0.1 ab	0.1 abc	0.1 ab	0.1 a
Leaf mortality (# senescent leaves)	2.3 a	2.9 a	6.4 b	1.1 a	2.1 a	2.0 a	0.4 a	1.6 a	1.1 a	0.3 a	0.6 a	0.9 a
Total leaf area per plant (cm^2)	9.5 a	10.9 a	14.7 ab	20.2 ab	28.3 ab	41.2 bc	33.4 ab	68.4 c	89.8 cde	76.5 cd	99.4 d	107.3 de

Table I.2 Biochemical properties of blueberry (Vaccinium ovalifolium) in a growth room experiment with 12 combinations (4 X 3) of irradiance and nitrogen supply. By rows, values with the same letter do not differ, as determined by Scheffe's test at $p < .05$; n = 8 plants per treatment mean.

	IRRADIANCE (uE m^{-2} s^{-1})											
	5			100			200			450		
RESPONSE VARIABLE	Nitrogen			Nitrogen			Nitrogen			Nitrogen		
	5	20	100	5	20	100	5	20	100	5	20	100
Sugars (mg g^{-1} leaf)	40.2 ab	31.2 ab	49.6 abc	44.8 ab	27.8 a	49.3 abc	58.3 abc	113.4 de	135.2 e	72.5 bc	57.1 abc	81.5 cd
total nonstructural carbohydrates (mg g-1 leaf)	65.8 a	60.5 a	79.6 ab	83.6 ab	70.0 ab	76.3 ab	102.0 abc	167.9 de	175.4 de	186.6 e	113.6 bc	142.3 cd
Holocellulose (mg g^{-1} leaf)	213.7 ab	223.8 ab	201.6 ab	234.2 b	195.5 ab	205.1 ab	164.0 a	176.5 ab	161.4 a	188.5 ab	186.1 ab	200.1 ab
Hololignin (mg g^{-1} leaf)	258.0 d	253.0 d	264.1 d	231.2 cd	204.7 bc	235.6 cd	182.4 ab	153.6 a	153.9 a	187.5 ab	180.1 ab	178.3 ab
Total structural polymers (mg g^{-1} leaf)	471.6 e	476.8 e	465.7 de	465.4 de	400.2 bcd	440.6 cde	346.4 ab	330.1 a	315.3 a	376.0 abc	366.2 ab	378.4 abc
Leaf succulence (ratio fresh wt./dry wt.)	4.9 c	5.0 cd	5.7 e	4.7 c	4.7 c	5.5 de	3.3 b	3.4 b	3.2 b	2.4 a	2.0 a	1.9 a
NDF (% dry wt. leaf)	48.2 e	48.7 e	47.6 de	47.5 de	41.9 bcd	44.1 cde	33.6 ab	36.0 a	30.5 a	36.3 abc	37.2 ab	38.2 abc
NDS (% dry wt. leaf)	51.8 a	51.3 a	52.4 ab	52.5 ab	58.1 bcd	45.9 abc	66.4 de	64.0 de	69.5 e	63.7 cde	62.0 de	61.8 cde
% lignin + cutin in NDF (% dry wt. NDF)	26.1 a	26.4 a	27.1 a	28.8 abc	27.3 ab	27.2 ab	28.4 abc	28.5 abc	28.9 abc	30.8 bc	31.1 c	31.5 c

Table I.3. Regression equations describing the growth and nutritional quality of blueberry (Vaccinium ovalifolium) leaves under different regimes of irradiance and nitrogen supply; n=96.

Dependent Variable	Constant	Independent Variables: Specific Leaf Wt. a	Leaf Succulence b	TSP c	Tannins d	R^2	Prob> F	Curve Type
Growth rate (mg g^{-1}-d^{-1})	---	---	---	---	$27.09(1-e^{-4.09x})$	0.62*	.0001	Exponential Saturation
	1.17	3.54	-1.01	---	---	0.67	.0001	Linear
Tannins (mg protein ppt. g^{-1} leaf)	---	$19.6e^{0.439x}$	---	---	---	0.89*	.0001	Exponential
Digestible[e] Nitrogen (% d.w. leaf)	2.45	-0.35	0.26	---	---	0.91	.0001	Linear
Digestible[f] Energy (kcal/g leaf)	3.96	-0.034	---	-0.004	---	0.96	.0001	Linear

a mg cm^{-2} leaf
b ratio of leaf fresh wt./ dry wt.
c mg g^{-1} leaf of total structural polymers
d mg protein ppt. mg^{-1} leaf
e digestible nitrogen = digestible protein / 6.25
f digestible energy calculated as % energy digestibility * 4.5 kcal g^{-1} leaf
* Note: reported R^2 for nonlinear functions is an approximate, asymptotic R^2 (Draper and Smith 1981)

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CHAPTER II.

THE INFLUENCE OF IRRADIANCE AND NITROGEN SUPPLY ON THE NUTRITIONAL QUALITY OF BLUEBERRY FORAGE TO DEER :

II. A FIELD TEST

by

C. L. Rose

ABSTRACT

Resource limitations which alter the plant C/N ratio affect patterns of plant allocation to growth and chemical defense as well as foliar concentrations of nutrients important to the nutrition of ruminant herbivores. This field experiment tested the C/N ratio of blueberry (*Vaccinium ovalifolium* Sm.), an important understory forage to deer in southeastern Alaska, affected foliar concentrations of nutrients and digestion-inhibiting condensed tannins. Light was manipulated with shade cloth and nitrogen supply was manipulated with fertilizer and sucrose applied to mature shrubs in a forest clearing.

Results of this field study confirmed findings from an earlier growth room study. Light limitation which lowered the plant C/N ratio reduced plant shoot growth, net photosynthesis, and foliar concentrations of C-based compounds, including starch, sugars, % lignin + cutin in the cell wall, and tannins. By comparison, a low C/N ratio was also associated with increased concentrations of total structural polymers and nitrogenous compounds, including total nitrogen and free amino acids. Nitrogen supply had little effect on plant responses. Regression equations for digestible nitrogen and energy developed in the growth room study of Chapter I. accurately predicted field responses. Digestible nitrogen increased 600% under dense shade, but digestible energy varied little in response to light.

INTRODUCTION

Higher concentrations of nutrients and lower chemical defenses have been proposed to explain the strong preference by deer for forages growing beneath a forest canopy in southeastern Alaska compared to the same plant species in clear-cuts (Hanley et al. 1987, Van Horne et al. 1988). Intraspecific (phenotypic) variation in plant chemistry along gradients of resource availability in the environment can have significant effects on forage quality to herbivores (McKey 1979). In a previous growth room experiment (Chapter I.), changes in the nutritional quality of blueberry foliage (Vaccinium ovalifolium, Sm.), an important deer food, were evaluated in relation to controlled levels of irradiance and nitrogen supply. The C/N ratio of blueberry seedlings in the growth room responded markedly to irradiance but not to nitrogen supply. Foliage of shade-grown plants had over twice the concentration of digestible nitrogen, only slightly lower digestible energy, and 50-75% lower condensed tannins than forage grown at higher irradiance.

These findings confirmed predictions based upon the carbon/nutrient balance hypothesis of Bryant et al. (1983). This hypothesis states that changing resource availability (especially light and nitrogen) alters fluxes of carbon and nitrogen in plants. As the plant C/N ratio shifts, allocation patterns to growth and chemical defense change.

Changing allocation in turn alters nutrient concentrations, and leaf structural and biochemical properties which affect the digestibility and palatability of forages to deer.

The functional mechanism of the C/N ratio is related to the basic physiological principle that nutrient shortages (mainly N) are relatively more limiting to plant growth than to photosynthesis. Accordingly, growth limitation leads to the accumulation of surplus photosynthate which stimulates the production of carbon-based chemical defenses such as condensed tannins. Thus, nutrient limitations increase the plant C/N ratio and produce plant tissues low in nitrogenous compounds, but high in carbon-based compounds, including storage carbohydrates and tannins. By contrast, light limitations inhibit photosynthesis relatively more than either growth or nutrient absorption, which leads to the depletion of photosynthate. This reduces the plant C/N ratio and produces plant tissues with high concentrations of nitrogenous compounds, but low concentrations of carbohydrates and tannins. These principles have been confirmed in numerous studies, and on three species of willow (Salix dasyclados, S. aquatica, S. alaxensis) (Waring et al. 1985, Larsson et al. 1986, Bryant 1987).

The carbon/nutrient balance hypothesis focuses on the indirect effects of resource availability in the environment on the plant internal C/N ratio and allocation. Yet, environment also can affect allocation by directly

controlling the activity of different plant organs and metabolic pathways (Loomis 1932, 1953), as outlined in Figure I.1.(Chapter I.). In the present study, the carbon/nutrient balance hypothesis of Bryant et al. (1983) is interpreted in the broader physiological context of Loomis (1932, 1953) as the carbon/nitrogen balance concept. This broader interpretation includes the important distinction that allocation is determined not only by the indirect effects of a limiting supply of resources (especially light and nitrogen), but also by direct environmental effects on the plant, including hormone induction, gene expression and enzyme activation or inhibition.

Based upon research of understory biomass dynamics along a chronosequence of regenerating forests, Alaback (1980, 1982) speculated that irradiance was the key factor controlling understory growth in southeastern Alaska. Average daily irradiance or photon flux density in the understory of the coastal rainforests varies widely from < 10 uE $m^{-2}s^{-1}$ in closed-canopy regenerating forests, 10-50 uE $m^{-2}s^{-1}$ in dense canopy oldgrowth, 50-300 uE $m^{-2}s^{-1}$ in more open-canopy oldgrowth and low-volume timber stands, to a maximum of 400-700 uE $m^{-2}s^{-1}$ in open clearings under prevailing dense cloudcover (Tappeiner and Alaback 1989, Chapter III.). Ambient irradiance is reduced by persistent maritime cloudcover to less than a third that of cloudless conditions, thus light poses a potentially major limitation

to plant growth in this ecosystem.

Numerous factors also contribute to low availability of soil nutrients including a cool climate, porrly-drained anaerobic soils, nutrient leaching by heavy rainfall, and high organic matter content of soils (Sidle and Shaw 1983). Yet, nitrogen availability may improve somewhat after forest disturbance (Vitousek et al. 1982, Binkley 1984).

The present study was conducted to determine whether plant responses to varied light conditions and nitrogen supply in the field matched predictions based upon the growth chamber experiment of Chapter I.. Specifically, I tested the hypothesis that plant growth, nitrogen and tannin concentrations, and forage quality (digestible nitrogen and energy) were determined by the availability of light and nitrogen supply and could be accurately predicted by growth room equations based upon morphological and chemical properties of the leaves.

METHODS

Study area

The study area was located on Douglas Island at the northern end of the Alexander archipelago in southeastern Alaska (58°22′ N, 134°35′ W, Fig. II.1). The research site was a 6-year-old clearcut at 30 m in elevation and 5-10 % slope, situated on a glacio-marine deposit of the Gastineau Channel Formation composed of soft to compact sandy diamicton 1-3 m deep over bedrock (Miller 1975). Organic

soil 0.3-1.0 m deep overlying the mineral sediments was the zone of maximum plant rooting activity.

Previously forested by old-growth western hemlock and Sitka spruce, the site currently was vegetated by a dense moss carpet with an herb layer of bunchberry (Cornus canadensis L.), trailing bramble (Rubus pedatus Sm.), deerberry (Maianthemum dilatatum How.), and numerous fern species; and a shrub layer 1-2 meters tall of blueberry (Vaccinium alaskensis How., V. ovalifolium Sm., and V. parvifolium Sm.), rusty menziesia (Menziesia ferruginia Sm.), salmonberry (Rubus spectabilis Pursh.), and devil's club (Oplopanax horridum Sm.).

Experimental design and procedures

Experimental plants were naturally-established mature shrubs of early blueberry (Vaccinium ovalifolium, Sm.), which hybridizes with Alaska blueberry (V. alaskensis How.) and is often inseparable morphologically (Anderson 1959, Viereck 1972).

The experimental design was a 2-way factorial with four light levels and three levels of nitrogen availability for a total of 12 treatments. Eight zones approximately 10 m in radius and having uniform soil and vegetation were selected within the clearcut to serve as treatment replicates, and zones were used as a blocking factor in the analysis to discern possible effects of location within the clearcut on plant responses. To each of 12 randomnly-chosen shrubs in

each zone, one of the 12 light + nitrogen treatments was applied, ensuring that the shrubs were spaced widely apart to prevent interference among treatments. Only mature shrubs of similar size, diameter and form were included in the sample.

Light treatments were implemented during mid-May 1985, by suspending optically neutral cloth over the clump of shrubs so as to allow adequate air circulation. Light treatments were chosen to match a range of average daily irradiance levels found in the natural forests (Chapter III.), including (in uE m^{-2} s^{-1}): open clear-cuts (450), dense regenerating stands (5) and two intermediate values (100, 200).

Nitrogen treatments applied on the same date included a control (untreated soil), nitrogen amendment with 24 g/m^2 aqueous NH_4NO_3 sprayed around the base of each clump of shrubs. In another treatment, sucrose was added in granular form at the rate of 200 g/m^{-2} ground area to decrease nitrogen availability to plants by stimulating nutrient absorption by soil microbes (Turner and Olson 1976). The fertilizer and sucrose treatments were repeated again in mid-May, 1986. To determine whether nutrient treatments were effective in altering nutrient availability in the soil, three bags of ion exchange resins were buried 10 cm deep around the base of each sprout clump during July 1986, and left intact for 1 year until the plant foliage was harvested in July 1987. Table II.1 summarizes the

irradiance and nitrogen treatments applied to blueberry shrubs at the Douglas Island field site. Although deer were not excluded from the study area, deer browsing was not a problem due to the presence of feral dogs in the vicinity.

Photosynthesis, growth, and foliage collection

On July 15, 1987, twig samples were detached from the shrubs at mid-day, and transported in plastic containers under refrigeration to Oregon State University for gas exchange analysis. Gas exchange analysis on detached twigs has been shown to work satisfactorily for conifers (Lange et al. 1986). In the lab, twigs were placed in water and rates of light-saturated net photosynthesis were measured with a Licor 6000 portable flow-through gas exchange system and infrared gas analyzer (Licor, Inc.). Three mature leaves were placed into the gas-exchange cuvette equipped with temperature and humidity controls. Projected leaf areas were then determined on a leaf area meter (Licor, Inc.) to determine the net CO_2 exchange per unit leaf area.

Data on plant growth parameters measured at the time of harvest included the total number of leaves per plant, the fresh and dry weights of old twigs (2 years growth pre-treatment), and new twigs (2 years growth post-treatment), and the number of primary, secondary and tertiary twigs per main stem. Samples of mature leaves were then placed in air-tight plastic bags over dry-ice in a cooler and transported to an ultra-cold freezing facility (-80 °C) at

Oregon State University. Additional morphological data obtained on the frozen leaf samples including leaf succulence (fresh/ dry wt. ratio), specific leaf weight (mg/cm^2), and leaf size (cm^2). The data were also used to calculate total leaf mass and total leaf area per plant, and the current aboveground biomass production, excluding the biomass of main stems. Diameter growth of the main stem was assumed to be negligible because experimental shrubs were near the maximum height and diameter for the species.

Biochemical analyses

Sequential chemical analysis of the fresh, frozen leaf material followed the laboratory protocol outlined in Chapter I. and described in detail in Appendix A. Protein precipitating capacity of the foliage was measured with the BSA technique of Martin and Martin (1982) as modified by Robbins et al. (1987a). Protein precipitation by leaf extracts in blueberry was expressed in terms of amounts of condensed tannins because Van Horne et al. (1988) found that concentrations of condensed tannins were directly proportional to protein-precipitating activity of leaf extracts in this species.

Sugars were measured by colorimetric reaction with anthrone (Yemm and Willis, 1954) on fresh leaf tissue homogenized and extracted in 80% ethanol (Harborne 1984). For starch analysis, the plant material was dried and

ground in liquid nitrogen according to procedures described by Sandstrom and Loomis (1987). Starch was quantified by colorimetric reaction with anthrone, using a perchloric acid extract of plant tissue (Viles and Silverman 1949, McCready et al. 1950)

Holocellulose and hololignin were determined by modifying the standard methods of forage analysis (Goering and Van Soest 1970) to separate the leaf fiber into fractions digestible and nondigestible to ruminant herbivores such as deer. Holocellulose (the digestible fiber), composed of cellulose and acid-labile hemicellulose, was determined by weight loss of the tissue fiber component after hydrolysis in 72% sulfuric acid. Hololignin (the nondigestible fiber), composed of lignin, acid-resistant hemicellulose, and cutin, was determined by sample weight difference after ashing the cellulose-extracted residue in a muffle furnace (550 °C, 4 h).

Total Kjeldahl nitrogen was measured with a Technicon Autoanalyzer II on dried foliage ground to pass a 40-mesh screen, and digested in a Technicon block digestor using a sulfuric acid-Se/CuSO4 catalyst (Technicon Instruments Corp. 1975, 1976).

Total free amino acids were determined by the ninhydrin reaction (Moore and Stein 1954) using the same ethanol extract used for sugar analysis.

NDF (Neutral Detergent Fiber = hemicellulose, cellulose, lignin, and cutin), NDS (Neutral Detergent Solubles = 1 -

NDF), and the percentage lignin + cutin in the NDF were quantified by standard sequential detergent analysis techniques (Goering and Van Soest 1970), as modified by Mould and Robbins (1982). These variables then were used in equations supplied by Robbins (1983), Robbins and Moen (1975), and Robbins et al. (1987a, 1987b) to calculate in-vivo concentrations of digestible protein and energy digestibility as follows:

Digestible protein

$$Z = -3.87 + 0.9283 \text{ (crude protein)} - 11.82 \text{ (bsa)} \; ; \; R^2 = .99$$

where: Z = digestible protein; g/ 100 g forage
crude protein = % total N * 6.25; g/ 100 g forage
bsa = bsa protein precipitated; g/ 100 g forage

Digestible energy

$$Y_1 = [(0.9231e^{-0.0451x})(NDF)]; \; R^2 = .98$$
$$+ [-16.03 + (1.02 \; NDS) - 2.8 \; P]; \; R2 = .99$$

where: Y_1 = % dry matter digestibility; g/ 100 g forage
x = % lignin + cutin in the NDF
NDF = % of cell wall in tissue; g/ 100 g forage
NDS = 100 - % NDF; g/ 100 g forage
P = 11.82% * bsa = in vivo correction for bsa

$$Y_2 = -0.71 + 0.99 * Y_1; \; R^2 = .94$$

where: Y_2 = % energy digestibility; g/ 100 g forage

$$Y_3 = Y_2 * 4.65 \text{ kcal/ g forage; kal /g forage}$$

where: Y_3 = digestibile energy in kcal/ g forage

Values for digestible protein (Z) were then converted to units of digestible nitrogen by dividing by the factor 6.25 (Robbins 1983), to make the results easier to compare to the % total N values. Digestible energy (Y_3) was determined by multiplying the % energy digestibility (Y_2) by an

estimated average caloric value of 4.5 kcal/g leaf tissue (Hanley and McKendrick 1985, Williams et al. 1987).

Assessing the plant C/N ratio

The term C/N ratio functionally relates to the net flux of photosynthates (carbon) and nitrogenous substrates into a plant. Fluxes of carbon and nitrogen are difficult to measure in plants, therefore researchers have frequently used tissue concentrations of carbon and nitrogen to estimate the ratio. However, extrapolating from C and N concentrations in plant tissues to estimate the C/N ratio is questionable unless data on growth and other physiological responses are available for interpreting fluxes (Waring et al. 1985, Chapin et al. 1987). Furthermore, correct interpretation of the ratio is dependent upon the recognition that plants have inherent (genetically-fixed) allocation priorities (Waring 1985). For example, when plant growth is limited to a greater extent than photosynthesis, surplus carbon may accumulate as storage reserves or defense compounds in plant tissues even though the flux of carbon is reduced (Bryant et al. 1983, Waring et al. 1985, Larsson et al. 1986). This is true because the processes of growth have a higher allocation priority for carbon than either storage reserves or chemical defense. In this study, complementary data on growth, physiology and biochemistry were collected concurrently which permitted the interpretation of the

plant C/N ratio in terms of fluxes and resulting changes in allocation.

Statistical analysis

Statistical analyses were performed with SAS statistical software for personal computers (SAS Institute, Inc 1985). All variables having unequal variances were log-transformed and results reported for variables in the untransformed state. The data were analyzed as a 4 x 3 factorial. Two-way analysis of variance was conducted using the GLM procedure. In order to compare all treatment means and minimize the probability of Type II error despite significant interaction effects of light and nitrogen, the more conservative Scheffe's test was used. Quantitative relationships between selected variables were examined using linear and nonlinear regression analysis.

RESULTS

Growth and physiology

Most of the variation in plant responses was explained by irradiance. Nitrogen effects and irradiance x nitrogen interactions were generally significant in the ANOVA, but explained only a small amount of the variation in plant responses compared to the main effect of irradiance (Appendix B). However, using the more conservative Scheffe's test to compare treatment means (controls experiment-wise error rate), most significant plant responses were attributable to irradiance rather than to

nitrogen supply or to irradiance x nitrogen interactions.

Photosynthetic responses varied by factor of 2.5, and generally matched rates observed at the same irradiance regime in the growth room (Chapter I.). Negligible responses were observed with respect to nitrogen supply (Fig. II.2). However, photosynthetic rates were slightly less variable within treatments in field plants than in the growth room.

The production of new twig : old twigs did not respond significantly to either irradiance or nitrogen supply (Table II.2). However, the number of active secondary branches (secondary branches on main stem) was five-fold higher at high irradiance compared to dense shade (Table II.2). Increasing the supply of nitrogen at most irradiance treatments reduced the amount of branching, because twigs near the distal end of the main stem died.

Total leaf biomass increased by a factor of thirty from lowest to highest irradiance. Increased nitrogen supply significantly reduced the total leaf biomass at all but the lowest irradiance level (Table II.2). Total leaf area per plant increased more than 10-fold with increasing irradiance but decreased significantly with increasing nitrogen (Table II.2).

The current annual shoot growth, including leaves, and twigs, increased directly with irradiance directly with irradiance (Fig. II.3).

Leaf biochemistry and morphology

Leaf properties reflected a widely variable plant C/N ratio, attributed to changing irradiance but not to nitrogen treatments. As in the previous growth room study (Chapter I), leaves of plants grown under high irradiance accumulated C-based compounds and were depleted in nitrogenous compounds compared to shade leaves. Sun leaves of plants grown under high irradiance (sun leaves) had much higher concentrations of C-rich compounds including tannins, sugars, starch and % lignin + cutin in the cell wall, but lower concentrations of nitrogenous compounds, including total nitrogen, free amino acids and ratio of free amino acids to total nitrogen compared to shade leaves.

Regarding the C-based compounds, concentrations of condensed tannins (proportional to protein precipitation, see Methods), increased with irradiance in a pattern similar to the growth room, although the range of responses was 20% greater in the field plants (Fig. II.4a). Protein precipitation varied little among the two lower irradiance treatments, but doubled at successively higher irradiance treatments. Starch concentrations increased directly with irradiance as in the growth room experiment, although maximum starch concentrations were lower in shrubs grown in the field compared to the growth chamber (Fig. II.4b). Conversely, a more dramatic increase in sugars was noted in the field plants at higher irradiance compared to the

growth chamber seedlings (Table II.3). Total nonstructural carbohydrates increased directly to irradiance in a pattern similar to the growth room seedlings (Table II.3).

Holocellulose in field plants exhibited similar values in response to irradiance as the growth room seedlings except that mean values at the extremes of irradiance were significantly different (Table II.3). Hololignin also declined at increased irradiance (Table II.3). Consequently, structural polymers in field shrubs increased directly with irradiance, in a pattern similar to the growth room seedlings (Table II.3).

Total nitrogen, free amino acids, and the ratio of free amino acids to nitrogen increased dramatically under dense shade, and at a magnitude similar to the growth room seedlings (Fig. II.5a,b,c).

Specific leaf weights increased directly with irradiance and were unaffected by nitrogen. Specific leaf weight values in field plants closely resembled values in growth room seedlings except that leaves of field plants at minimum irradiance were 20% heavier (Fig. II.4c). Leaf succulence was inversely related to irradiance, but increased with added N in densely shaded foliage (Table II.3).

Forage nutritional quality

Forage nutritional quality was affected by irradiance but not by nitrogen supply (Appendix B). As with total

structural polymers, NDF (total cell wall fiber) was inversely related to irradiance and decreased by 25 % from lowest to highest irradiance (Table II.3), a slightly wider range than in growth room seedlings. NDS (total cell solubles) was directly related to irradiance (Table II.3).

The % lignin + cutin in the NDF (cell wall) of field plants increased directly with irradiance but was slightly higher in field plants than in the growth chamber seedlings (Table II.3).

As observed in the growth room, leaf digestible nitrogen in field plants was inversely related to irradiance (Fig. II.6a). Digestible energy was unresponsive to irradiance, as in the growth room seedlings (Fig. II.6b).

Predictive equations

Observed values for digestible nitrogen in field plants were very close to values predicted by equations developed in the growth room experiment (Fig. II.7a). Digestible energy values in the field averaged approximately 5-10% higher than values predicted by the growth room equations (Fig. II.7).

DISCUSSION

The physiological responses observed in blueberry are characteristic of facultative shade plants, which grow best at intermediate to high irradiance, but also grow with relative efficiency at low irradiance (Denslow 1980,

Hartshorn 1980, Whitmore 1984). The range of light-saturated net photosynthetic rates observed in the field plants under artificial shade confirmed results of the growth chamber experiment and emphasized the strong light limitation on physiological responses in this understory shrub. The total plant light compensation point (daily photon flux at which relative growth rate is 0) was inferred from the growth and photosynthesis data to be near 5 uE m^{-2} s^{-1}. However this value is likely to be affected by large reserves of carbon in rhizomes of field plants. Close agreement in net photosynthetic rates between the field and growth room also demonstrated that the technique of measuring gas exchange on detached twigs is valid for ericaceous shrubs as well as conifers (Turner and Olson 1976).

The lack of nitrogen limitation on photosynthesis in blueberry was surprising given the low values of mineralizable nitrogen in soils of a clear-cut reported by Sidle and Shaw (1983). The availabilty of extractable, mineralized nitrogen (mostly ammonium) in the Douglas Island control soils was an order of magnitude higher than values reported for soils in the subalpine mountain-fir zone of the Cascade mountains in Oregon (Waring et al. 1987), comparable to soils of pine and western hemlock in southern Wisconsin (Binkley et al. 1986), but only half that of soils in the Coast Range of western Oregon (K. Cromack, pers. comm.). Within a similar climatic zone,

soils from the Douglas Island clear-cut had 2-3 orders of magnitude higher values of extractable mineralized N than clear-cuts at low- and mid-elevation sites on Vancouver Island, British Columbia (Binkley 1984). Yet, soil N values on the Douglas Island clear-cut were similar to the values reported for high-elevation clear-cuts on Vancouver Island (Binkley 1984) .

Mycorrhizal plants are capable of deriving a substantial portion of their nitrogen as well as carbon supply from simple organic compounds in the soil (Abuzinadah and Read (1989). Much of the dissolved nutrient pool is in the form of simple organics rather than mineralized nitrogen in the acid histosols of the Alaskan coastal forests. Hence, to more accurately assess nutrient availability to plants in the Alaskan coastal forest ecosystem, new analytical methods are needed which include soluble organic forms of nitrogen. The fact that soil N treatments produced few responses in blueberry in both the growth room and field provides strong evidence that N was not an important limitation to plant growth in the field. Slow inherent growth rates undoubtedly contributed to V. ovalifolium's lack of response to nitrogen, a trait of many stress-tolerant plant species (Chapin 1980).

Sun leaves of blueberry under full light exposure in the field showed an intense reddening indicative of increased activity of the PAL enzyme which functions in tannin synthesis. The red color appeared during cold clear weather

in spring and intensified after hot, dry conditions in mid-late summer. The red coloration is related to insufficient electron transport and photo-oxidation damage to the photosynthetic apparatus. Acclimation to higher irradiance by the photosynthetic apparatus in shade-adapted plants is often limited by electron transport, which is further inhibited by temperature extremes (Baskin and Baskin 1978, Monson, 1982, Long et al. 1983, Powles 1984, Chazdon and Pearcy 1986). The appearance of photoinhibition symptoms in fully-illuminated field plants occurred at the same level of average daily irradiance (450 uE m^{-2} s^{-1}) at which maximum photosynthetic capacity of the leaves was identified in the growth room.

The lack of treatment response in the biomass of new twigs versus old twigs may have been a consequence of the low twig production in mature shrubs. Additionally, twig growth in blueberry has been found to be most responsive to N (Chester and McGraw 1983), and N did not limit plant growth in this study. The higher degree of secondary branching in sun plants was related not to branch production per se, but to increased survival of smaller branches at high irradiance. Reduced branch survival with increased N supply in shaded seedlings may have been a toxic effect caused by a lack of photosynthate for metabolizing and storing surplus N. At high irradiance, increased N supply may have exacerbated leaf stress by reducing the carbon available to repair membranes damaged

by photo-oxidation.

As in the growth room experiment, irradiance appeared to stimulate development of leaf biomass and total leaf area. Furthermore, increased nitrogen supply caused significant reductions in total leaf area and biomass at most irradiance levels. The greater relative response in plant growth at maximum irradiance in the field compared to the growth room could be explained by differences in light quality or to increased carbohydrate reserves in rhizomes of field plants.

Field plants had adequate nitrogen nutrition, judging from the poor response of growth and photosynthesis to either fertilization or sucrose application at any irradiance regime. Inverse trends in net photosynthetic rates versus tissue nitrogen concentrations indicated that blueberry plants grown under high irradiance had higher photosynthetic nitrogen-use efficiency, or PNUE (net photosynthetic rate per mg leaf N). By comparison, a direct relationship of PNUE to irradiance has been described for some understory species of the tropics (Waterman et al. 1984, Denslow et al. 1989).

The contrasting responses of PNUE to irradiance by understory plants in Alaska compared to the tropics may be explained on the basis of differing resource limitations (Seemann et al. 1987). Blueberry in the Alaskan coastal forests is limited strongly by light, but not by nitrogen. Improved lighting consequently increases photosynthesis and

shoot growth more than root growth and nitrogen assimilation. This produces a dilution in concentrations of nitrogen in blueberry leaves exposed to high irradiance.

In many tropical understory plants, nitrogen is far more limiting than light. Therefore with improved lighting, tropical plants allocate proportionally more photosynthate to root development and nitrogen assimilation. This produces an increase in concentrations of nitrogen in foliage of tropical understory plants despite increased photosynthesis and growth at higher irradiance (Waterman et al. 1984, Denslow et al. 1989). Not surprisingly then, N-fixing tropical plants which are light-limited exhibit trends in photosynthetic N-use efficiency and tissue N concentrations similar to blueberry in Alaska (Mole et al. 1988).

High concentrations of free amino acids in the most densely shaded blueberry foliage signified strong light limitations on the energy available for incorporating nitrogen into proteins (Margolis and Waring 1986). Concentrations of free amino acids in the most densely shaded foliage remained high even after two years, which indicated that physiological adjustment in plant nitrogen levels to shading was quite slow. However, the plants appear able to tolerate wide imbalances in the C/N ratio across an extreme range of light conditions.

High irradiance increased the production of C-based compounds in leaves of field plants, as was expected to

result from a higher C/N ratio. Sun leaves were enriched in tannins, sugars, starch, total nonstructural carbohydrates (sugars + starch), cell solubles (NDS),and % lignin + cutin in the cell wall. In addition, sun leaves were lower in succulence, holocellulose, hololignin, total structural polymers, and cell wall fiber (NDF) compared shade leaves. Important contrasts in plant biochemistry in the field compared to the growth room included : 1) 50% lower starch concentrations but 20% higher sugar concentrations, 2) a significant decrease in holocellulose concentrations across light treatments, 3) 25% lower leaf concentrations of total nitrogen at maximum irradiance, and 4) 20% higher concentrations of leaf tannins at maximum irradiance. Reasons for these differences are speculative, but probably relate to higher starch reserves, and/or differences in plant relative growth rates and the spectral quality of lighting in the field compared to the growth room.

Responses in forage quality were generally similar between the field and growth room, except that digestible nitrogen in blueberry foliage was 50% lower at maximum irradiance in the field compared to the growth room. Digestible nitrogen decreased as a function of lower concentrations of total N as well as reduced digestibility of nitrogen due to higher tannin concentrations. Faster growth rates in field plants likely contributed to the lower concentrations of total N at maximum irradiance in field plants compared to the growth room. Similarly,

stronger ultraviolet radiation in natural lighting may have directly induced PAL activity in tannin synthesis, which would have reduced nitrogen digestibilty in field plants. Yet, the success of growth room equations in predicting the nutritional quality of field plants indicated that leaf morphology accurately reflected acclimatory responses of plants to diverse and variable environments.

Rainforest plants in Africa (Acacia pennata DC, Barteria fistulosa Mast., Cynometra leonensis Hutch. & Dalz., Diospyros thomasii Hutch. & Dalz., and Trema guineensis Schum. & Thonn.) exhibit similar responses to blueberry in terms of leaf phenolics, digestibility, and nitrogen content across a wide range of light regimes (Waterman et al. 1984, Mole and Waterman 1988, Mole et al. 1988).

Additional research is needed to better understand the responses of understory plants to resource imbalances. Comparisons of plant responses to stress among different ecosystems would serve to elucidate the physiological mechanisms important to plant survival in stressful environments. Furthermore, a knowledge of understory responses would be useful to predicting the influence of management activities on forest wildlife.

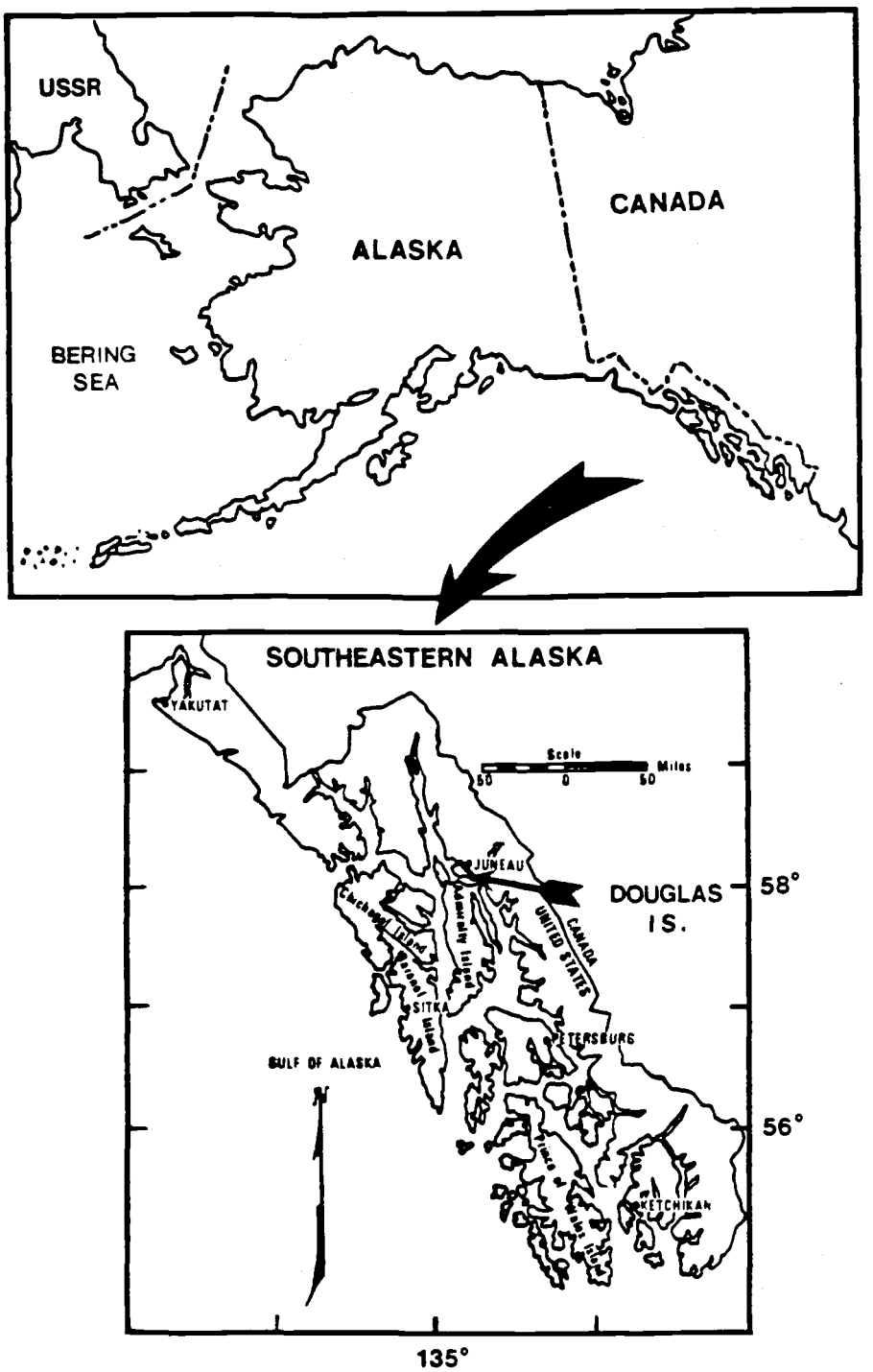

Figure II.1. Geographic setting of the study in southeastern Alaska and location of the field experiment on Douglas Island near Juneau, Alaska.

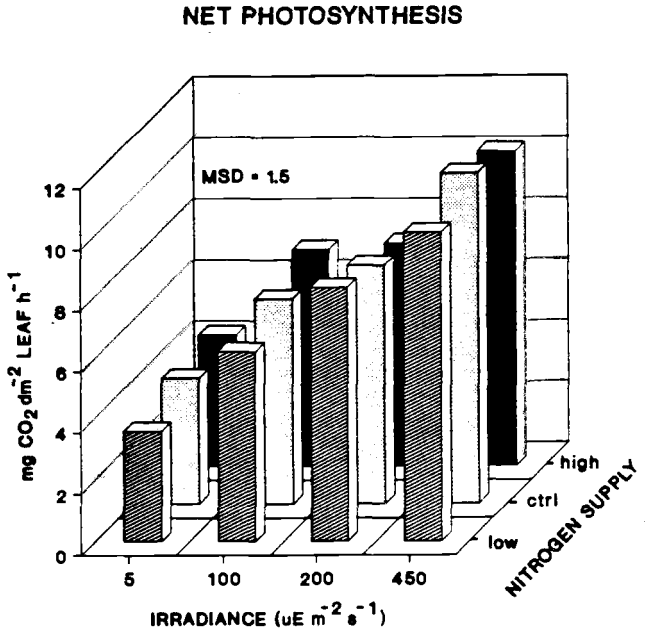

Figure II.2. Light-saturated rates of net photosynthesis in blueberry seedlings acclimated to 12 (4 * 3) combinations of irradiance and nitrogen supply in the field. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 3$ plants per treatment mean.

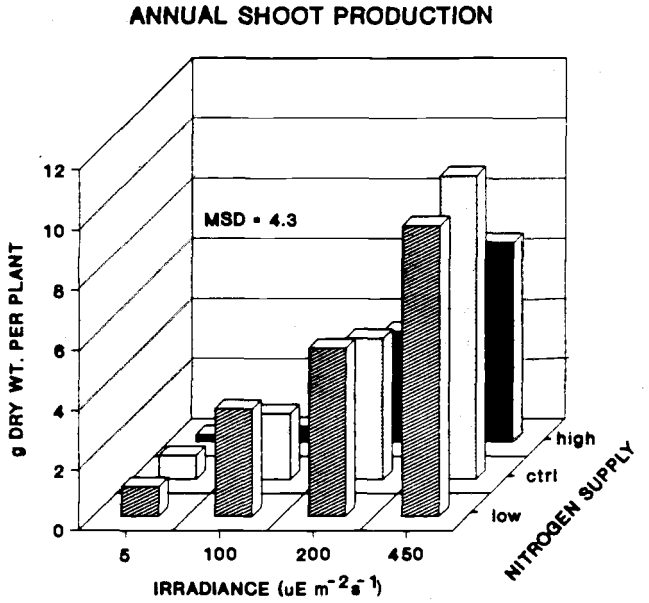

Figure II.3. The relationship of annual shoot production in blueberry leaves to 12 (4 * 3) combinations of irradiance and nitrogen supply in the field. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean.

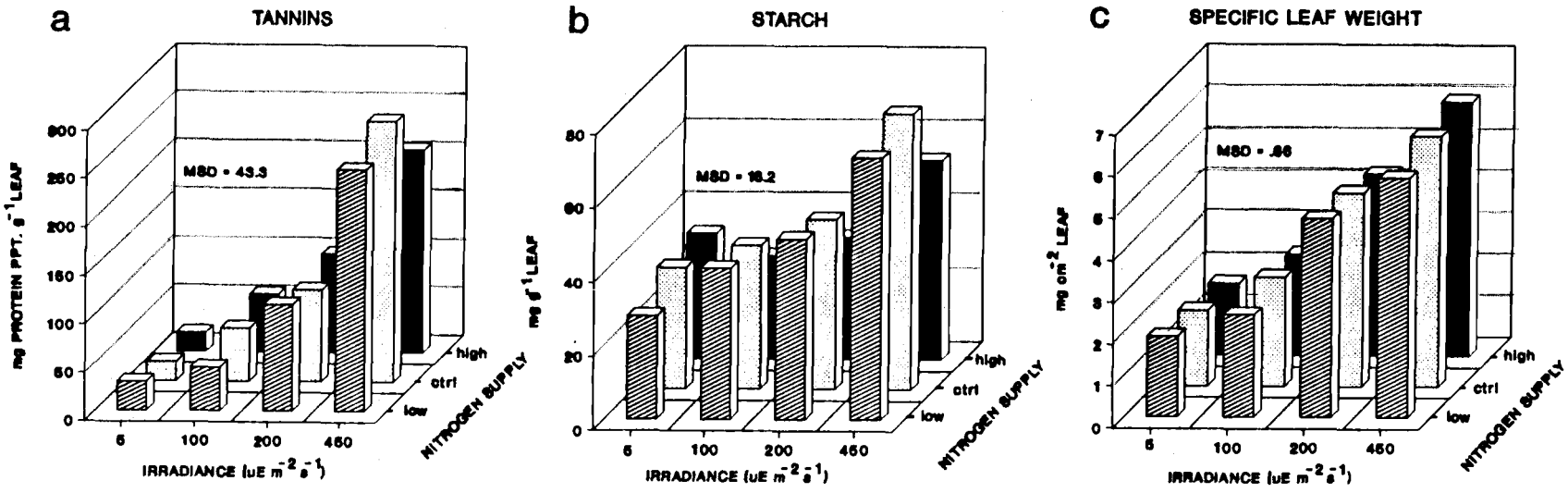

Figure II.4 a,b,c. Protein-precipitating capacity of condensed tannins, starch concentrations, and specific leaf weight of blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply in the field. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean.

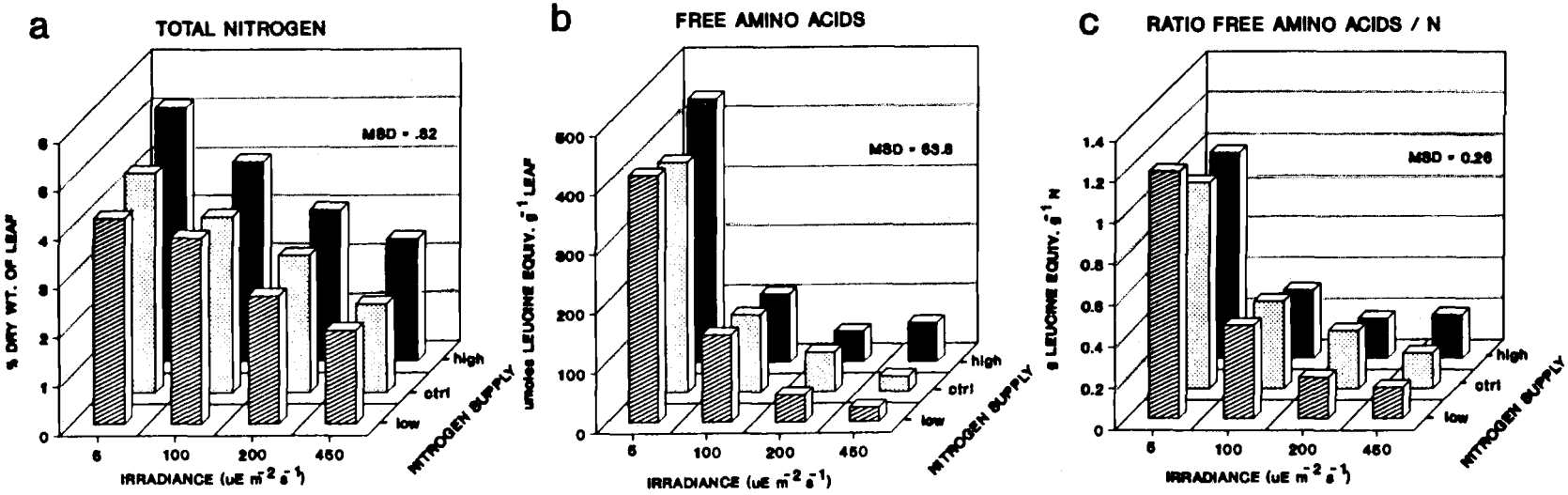

Figure II.5 a,b,c. Concentrations of total nitrogen, free amino acids, and the ratio of free amino acids / total nitrogen in blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply in the field. Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean. The conversion of units from Fig. 5b to 5c involved a factor of 8.5348 umoles leucine/ mg leucine equivalent.

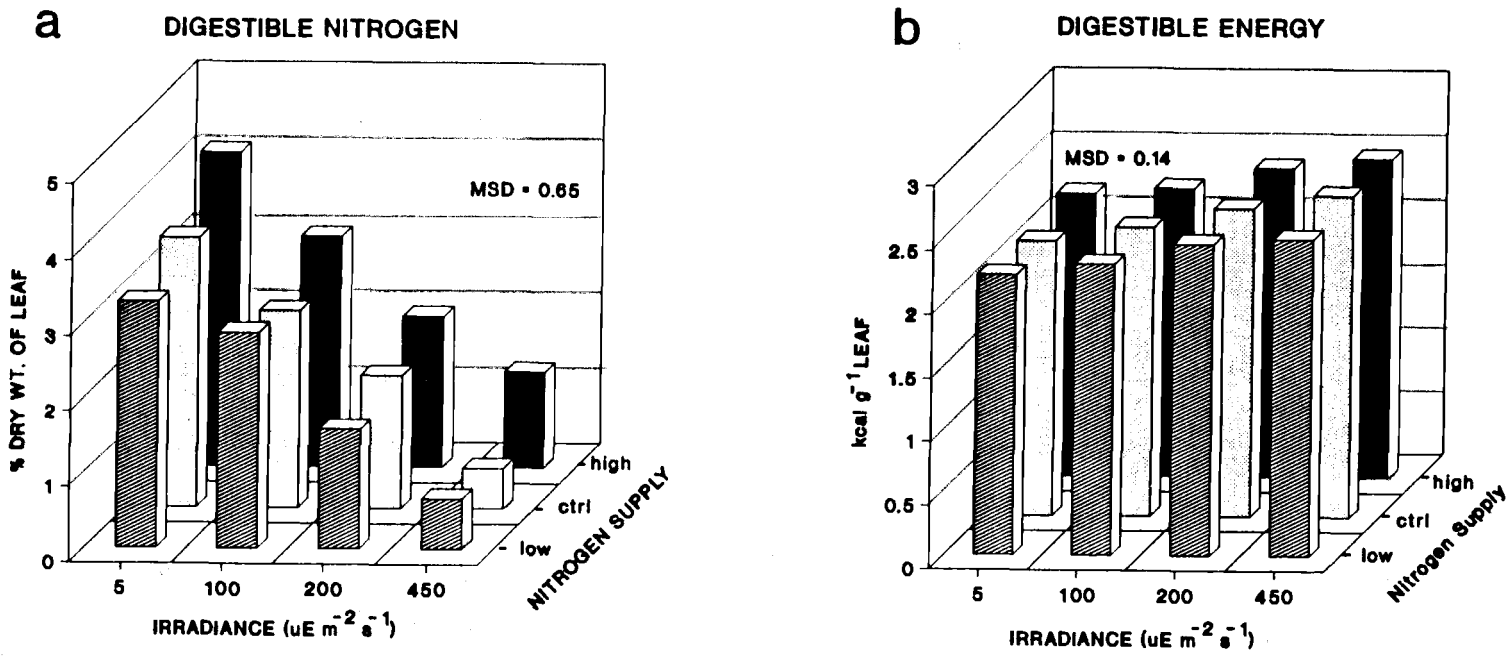

Figure II.6 a,b. Nutritional properties of blueberry leaves in relation to 12 (4 x 3) combinations of controlled irradiance and nitrogen supply in the field. Calculation of digestible nitrogen and digestible energy followed equations presented by (Robbins 1983, Robbins et al. 1987 a,b). Digestible energy calculation assumes a gross energy concentration in leaves of 4.5 kcal/g (Hanley and McKendrick 1985, Williams et al. 1987). Minimum significant differences (MSD) were determined by Scheffe's Test at $p < .05$; $n = 8$ plants per treatment mean.

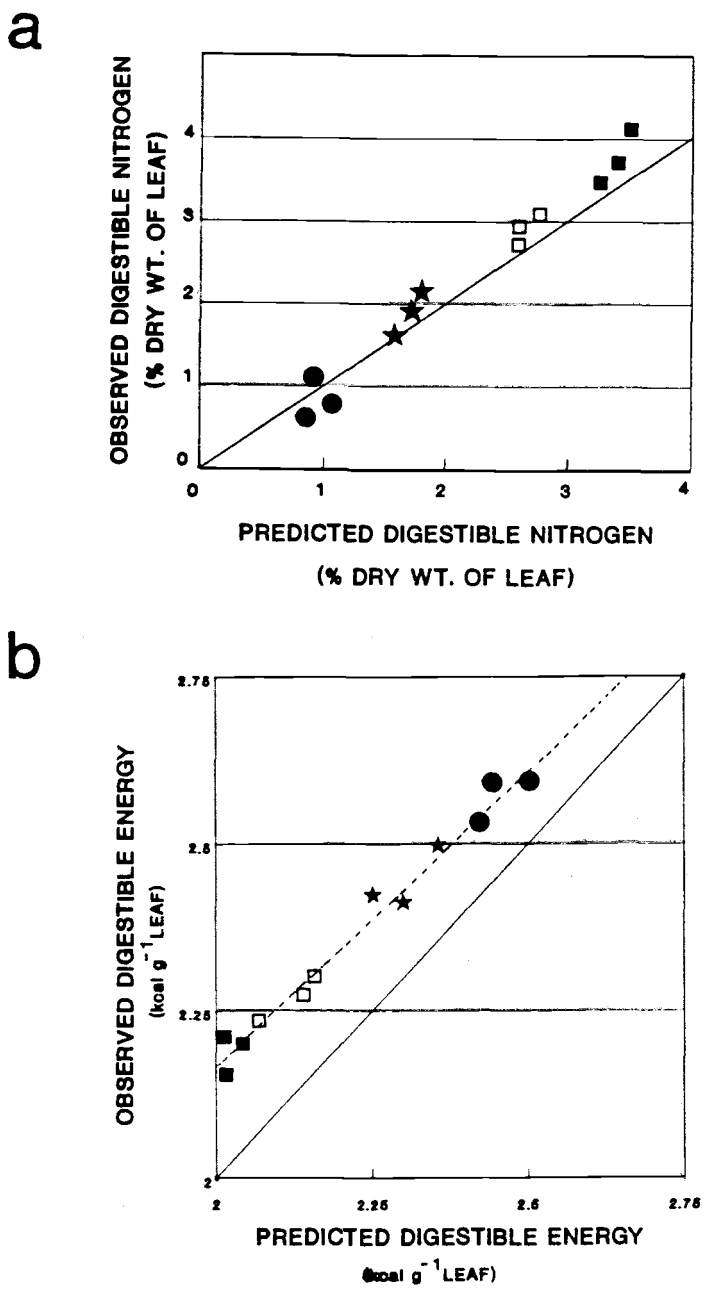

Figure II.7. Comparison of forage nutritional properties observed in the field to values predicted from the growth room experiment: (a) digestible nitrogen and (b) digestible energy. The solid line is that predicted by the regression in Chapter I. The dotted line in b is the actual regression line for the field data as follows:
$(Y = 3.98 - [0.036\ (B_1)] - [0.0036\ (B_2)]$; $R^2 = 0.89$;
B_1 = specific leaf weight (mg cm^{-2});
B_2 = total structural polymers (mg g^{-1} leaf).

■ = 5, □ = 100, ★ = 200, ● = 450 uE m^{-2} s^{-1} irradiance.

Table II.1. Average daily irradiance and soil nitrogen availability resulting from shade and fertilizer treatments applied to blueberry shrubs in the field during a period of two years. Light treatments consisted of ambient irradiance and three densities of shade cloth. Nitrogen treatments consisted of unaltered soil in the clearcut, sucrose addition to reduce nitrogen availability, and NH_4NO_3 fertilizer to increase N availability. Table values for extractable nitrogen from ion exchange resins quantify relative nitrogen availability among treatments during a 1-year period.

Average Daily[a] Irradiance ($uE\ m^{-2}\ s^{-1}$)	v.low	low	med	high
	5	100	200	450

Available N[b] ($mg\ g^{-1}$ dry wt. resins yr^{-1})	low	ctrl	high
NH_4	0.54 A	0.64 B	4.8 C
NO_3	0 A	0.01 B	1.0 C

[a] light regimes provided by shade cloth corresponded to conditions in the natural forests measured under prevailing cloudcover (Chapter III). Average daily irradiance ranged from a low comparable to closed-canopy even-aged forests, to a maximum comparable to open clearings, and two levels of intermediate shade. n = 8.

[b] low N = sucrose application rate = 200 g m^{-2} ground area.
control N = unaltered soil in clearcut.
high N = NH_4NO_3 application rate = 200 mg l^{-1} solution.
n = 6.

Table II.2 Estimated relative growth responses of blueberry shrubs to 12 (4 x 3) combinations of irradiance and nitrogen supply in a field experiment; n = 3.

	IRRADIANCE (uE m^{-2} s^{-1})											
	5			100			200			450		
	Nitrogen			Nitrogen			Nitrogen			Nitrogen		
	low	ctrl	high	low	ctrl	high	low	ctrl	high	low	ctrl	high
Ratio of new/old twig biomass (g g-1)	0.7 a	0.5 a	0.4 a	0.5 a	0.4 a	0.3 a	0.6 a	0.3 a	0.4 a	1.2 a	1.0 a	0.7 a
Number of 2°twigs	1.4 a	1.8 a	0.5 a	4.2 bc	3.1 ab	0.7 a	8.8 d	8.6 d	6.2 bc	9.6 d	9.2 d	5.9 c
Total leaf biomass (g $plant^{-1}$)	0.2 a	0.2 a	0.05 a	0.9 b	0.8 b	0.13 a	4.0 d	3.8 d	2.7 c	7.6 e	7.5 e	5.2 d
Total leaf area per plant (cm^2)	101 a	130 a	33 a	380 b	319 b	55 a	843 d	816 d	630 c	1331 e	1250 e	851 d

Table II.3. Morphological and biochemical responses of blueberry leaves to 12 (4 x 3) combinations of irradiance and nitrogen in a field experiment; n = 8.

	IRRADIANCE (uE m^{-2} s^{-1})											
	5			100			200			450		
	Nitrogen			Nitrogen			Nitrogen			Nitrogen		
	low	ctrl	high	low	ctrl	high	low	ctrl	high	low	ctrl	high
Sugars (mg g-1 leaf)	50 a	63 ab	62 ab	61 a	68 b	75 bc	83 c	82 c	88 c	92 c	94 c	110 d
Nonstructural carbohydrates (mg g-1 leaf)	79 a	96 a	97 a	102 a	107 a	103 a	132 b	128 ab	121 ab	163 c	169 c	164 c
Holocellulose (mg g-1 leaf)	203 b	213 b	218 b	220 b	199 b	221 b	187 ab	176 ab	162 a	159 a	177 ab	161 ab
Hololignin (mg g-1 leaf)	260 bc	284 c	232 b	221 b	252 bc	231 b	192 ab	211 ab	215 ab	192 a	178 a	195 ab
Structural polymers (mg g-1 leaf)	463 bc	297 c	450 bc	441 b	451 bc	455 bc	379 a	387 a	377 a	351 a	355 a	356 a
Leaf succulence (fresh / dry wt.)	5.3 d	5.5 de	6.0 e	4.1 c	4.5 c	4.9 cd	3.3 b	3.6 bc	3.4 b	2.4 a	2.3 a	2.6 a
NDF (% dry wt. leaf)	47 c	49 c	46 c	45 bc	44 bc	45 bc	39 ab	40 abc	39 ab	37 a	35 a	36 a
NDS (% dry wt. leaf)	53 a	51 a	54 a	55 ab	56 ab	55 ab	61 bc	60 abc	61 bc	63 c	65 c	64 c
% lignin + cutin in NDF	27.9 a	27.1 a	28.5 a	27.5 a	28.9 ab	28.1 a	29.0 ab	30.1 abc	29.9 abc	31.7 c	31.5 bc	30.8 b

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CHAPTER III.

NUTRITIONAL VARIABILITY IN BLUEBERRY FORAGE AMONG FOREST ENVIRONMENTS IN SOUTHEASTERN ALASKA : RESPONSE TO IRRADIANCE AND NITROGEN SUPPLY

by

C. L. Rose

ABSTRACT

This study confirmed the applicability of the plant C/N ratio to interpreting the nutritional quality of blueberry foliage (*Vaccinium ovalifolium* Sm.), and extended findings of previous growth room and field studies to natural conditions in southeastern Alaska.

Foliage characteristics of blueberry responded strongly to irradiance, but not to nitrogen. Light penetration through the canopy was only .2 - 1% of ambient irradiance in even-aged forests and 2 - 6% of ambient in old-growth stands. Concentrations of extractable soil ammonium did not differ among soils from different ages of forests, but most soils had adequate nitrogen for plant growth.

The standing biomass and rates of biomass production in blueberry were highest in the clearcut, moderate in old growth, and negligible in even-aged forests. Blueberry foliage in clear-cuts had four times higher concentrations of condensed tannins, but 30% lower total N compared to plants growing under a forest canopy. Blueberry foliage in forests of all ages had adequate digestible energy to meet the dietary needs of deer, but in clear-cuts digestible nitrogen was 30%-50% lower than the minimum dietary requirements. Opportunities exist to manage the availability and quality of forage to deer by manipulating the amount of light reaching the understory in regenerating forests of southeastern Alaska.

INTRODUCTION

Populations of Sitka black-tailed deer (Odocoileus hemionus sitkensis Sm.) in southeastern Alaska are influenced by the abundance and nutritional quality of understory forage (Klein 1965). Recent clear-cutting has converted extensive areas of pristine old-growth forests into open clear-cuts and dense, naturally-regenerated conifer stands. Such large-scale disturbance to the native forests may also alter the availability and nutritional quality of understory forage to deer (Wallmo and Schoen 1980, Schoen 1981, Kirchhoff et al. 1983).

Regenerating forests pose two major nutritional problems to deer in southeastern Alaska. The first relates to forage quality. Recent studies in southeastern Alaska suggest that forage growing in open habitats may be lower in nutrients and higher in chemical defenses such as condensed tannins (Hanley et al. 1987, Van Horne et al. 1988, Chapter II.). Tannins reduce both the digestibility and palatability of forages to ruminant herbivores such as deer (Feeny 1970, Ribereau-Gayon 1972, Mould and Robbins 1981, 1982, Robbins et al. 1987 a,b). This I reasoned might explain the fact that Alaskan deer prefer forage growing beneath a forest canopy despite more abundant forage in open clear-cuts (Wallmo and Schoen 1980, Rose 1982, Hanley et al. 1987).

The second problem concerns the annual levels of forage

production. The standing biomass and biomass production of understory herbs and shrubs peaks during the first 10-15 years following clear-cutting. Understory biomass then rapidly declines as the conifer canopy closes. Understory plants often do not re-establish until the overstory canopy begins to open at 150-300 years. Consequently, habitats managed on a 100-year timber harvest rotation cycle may remain virtually barren of understory forages throughout most of the timber harvest rotation (Alaback 1980, 1982).

Studies on the nutritional ecology of Alaskan deer have shown that the quality and quantity of understory forage may limit population densities of deer in Alaska (Klein 1965, Hanley and McKendrick 1985, Hanley et al. 1987 Spalinger et al. 1988). Yet, relatively little is known about the physiological responses of understory plant species to changing light and nitrogen availability in regenerating forests in coastal Alaska, nor the consequences to forage resources for deer.

Species composition in the understory changes little during stand development in the Alaskan rainforests. Thus, acclimation, or plastic responses of plants to the changing forest environment should be a key factor influencing forage resources in the understory. Plasticity in plant growth, defense, and forage nutritional quality has been interpreted in relation to the relative availability of carbon and nitrogen resources to a plant (Bryant et al. 1983). The amount of digestible energy and nitrogen that

deer can actually obtain from their diet are critical nutritional factors to deer (Hanley and McKendrick 1985).

Based upon studies of vegetation dynamics in successional forests of Sitka spruce-western hemlock forests in southeastern Alaska, Alaback (1980, 1982) concluded that light was a major factor limiting understory productivity in the coastal rainforest. Nitrogen supply to plants may also be low due to poor soil drainage, cool temperatures, and organic matter accumulation (Sidle and Shaw 1983). Nitrogen availability may improve though, after disturbances such as clear-cutting (Vitousek et al. 1982, Binkley 1984a).

In previous growth room and field experiments, the level of irradiance but not nitrogen supply was shown to strongly affect the plant C/N ratio as evident by changes in the growth, production of chemical defenses (condensed tannins) and nutritional quality of blueberry foliage. The present study examined whether these findings were valid for oldgrowth and a variety of ages of regenerating forests in southeastern Alaska.

METHODS

Study area

Five study areas were selected from locations throughout the Alexander archipelago in southeastern Alaska (58°22′ N, 134°35′ W, Fig. III.1). Attempts were made at each study area to match an old-growth stand with a nearby

clear-cut and a dense regenerating conifer stand (Table III.1). All areas were situated on typical upland forests of Western hemlock (Tsuga heterophylla Raf.) and Sitka spruce (Picea sitchensis Bong.), on flat to gently sloping terrain.

Experimental design and procedures

The experimental design was a simple analysis of variance with stand type (clear-cut, even-aged regeneration, and oldgrowth) as the single categorical factor, and site (different study locations) as a blocking factor. Experimental plants belonged to the taxon Vaccinium ovalifolium (Sm.), which hybridizes with V. alaskensis (How.) (Anderson 1959, Viereck and Little 1972). All stands were sampled during the peak growing season (July 15-30) during the period 1984-1986. Study sites were characterized as to the forest light regime and nutrient availability. The light regime was determined as the average daily irradiance using ozalid paper (Emmingham and Waring 1973, Friend 1961). The ozalid paper was calibrated to readings of daily photon flux density (PAR) with a Kipp solarimeter placed in an open area near Juneau during mid-July 1985. Calibration curves for the ozalid paper were obtained both for cloudless conditions and for uniform overcast conditions. Without exception, conditions were overcast during the periods when light regimes were measured at the study sites. The ozalid paper was sealed in

transparent glass petri dishes and mounted on the top of poles elevated above the canopy of the surrounding shrubs to prevent shading by foliage. The dishes were left in place for 24 h, and then collected and placed in a dark container until they were developed. The degree of exposure was then estimated for the ozalid paper, and entered into regression equations relating the number of exposed pages to irradiance.

The extractable levels of soil N were assessed with buried bags of ion exchange resins (Binkley and Matson 1983, Binkley 1984b). Six resin bags were placed approximately 10 cm deep in the soil organic horizon at random locations in each study site. The bags were collected after a period of 1 year, extracted in NaCl, and analyzed for ammonium and nitrate on a Technicon Autoanalyzer (Technicon Inc. 1975).

Growth/biomass assessment and foliage collection

The standing biomass and biomass production of blueberry shrubs was calculated from data on stem numbers and diameters of mature blueberry shrubs within a 25 m^2 circular plot. Allometric biomass equations for V. ovalifolium were provided by Alaback (1980). Samples of mature leaves were collected from three random locations in clearcuts and even-aged stands, and at six locations in oldgrowth to account for the more variable lighting in the older stands. Each foliage sample consisted of composite

collections of mature leaves from several shrubs in a patch. In sampling stands of uniform canopy cover such as clear-cuts and dense even-aged forests, attempts were made to collect leaves from microsites representative of the stand as a whole. In clear-cuts for example, leaves were selected only from exposed terminal branches that were not subject to shading. And in dense-regenerating forests, plants were sampled only beneath a dense, unbroken overstory canopy. However, in oldgrowth-stands, sampling was conducted systematically at 5m-intervals along random transects to include canopy variability.

Leaf samples were placed in plastic bags, stored on ice, and promptly frozen. Samples were then transferred to an ultra-cold freezer (-60° C) for storage.

Leaf morphology and biochemical analysis

Morphological data obtained on the frozen leaf samples included specific leaf weight and leaf succulence. Leaf area was measured on a Licor Leaf Area Meter (LiCor, Inc), after fresh weights were determined. Leaf tissue was then dried at 90°C for 30 min., then at 70° C for 48h and re-weighed. Biochemical analyses for free amino acids, total Kjeldahl nitrogen, NDF, and % lignin + cutin in NDF followed procedures described in detail in Chapters I. and II. and/or in Appendix A.

Protein precipitating capacity of the foliage was measured with the BSA technique of Martin and Martin (1982)

as modified by Robbins et al. (1987a). Protein precipitation by leaf extracts in blueberry was expressed in terms of amounts of condensed tannins because Van Horne et al. (1988) found that concentrations of condensed tannins were directly proportional to protein-precipitating activity of leaf extracts in this species.

Total Kjeldahl nitrogen was measured with a Technicon Autoanalyzer II on dried foliage ground to pass a 40-mesh screen, and digested in a Technicon block digestor using a sulfuric acid-Se/CuSO4 catalyst (Technicon Instruments Corp. 1975, 1976).

Total free amino acids were determined by the ninhydrin reaction (Moore and Stein 1954) using the same ethanol extract used for sugar analysis.

NDF (Neutral Detergent Fiber = hemicellulose, cellulose, lignin, and cutin), NDS (Neutral Detergent Solubles = 1 - NDF), and the percentage lignin + cutin in the NDF were quantified by standard sequential detergent analysis techniques (Goering and Van Soest 1970), as modified by Mould and Robbins (1982). These variables then were used in equations supplied by Robbins (1983), Robbins and Moen (1975), and Robbins et al. (1987a, 1987b) to calculate in-vivo concentrations of digestible protein and energy digestibility as follows:

Digestible protein

$Z = -3.87 + 0.9283$ (crude protein) $- 11.82$ (bsa) ; $R^2=.99$

where: Z = digestible protein; g/ 100 g forage

crude protein = % total N * 6.25; g/ 100 g forage
bsa = bsa protein precipitated; g/ 100 g forage

Digestible energy

$$Y_1 = [(0.9231e^{-0.0451x})(NDF)]; R^2 = .98 + [-16.03 + (1.02\ NDS) - 2.8\ P]; R^2 = .99$$

where: Y_1 = % dry matter digestibility; g/ 100 g forage
x = % lignin + cutin in the NDF
NDF = % of cell wall in tissue; g/ 100 g forage
NDS = 100 - % NDF; g/ 100 g forage
P = 11.82% * bsa = in vivo correction for bsa

$$Y_2 = -0.71 + 0.99 * Y_1; R^2 = .94$$

where: Y_2 = % energy digestibility; g/ 100 g forage

$$Y_3 = Y_2 * 4.65 \text{ kcal/ g forage; kal /g forage}$$

where: Y_3 = digestibile energy in kcal/ g forage

Values for digestible protein (Z) were then converted to units of digestible nitrogen by dividing by the factor 6.25 (Robbins 1983), to make the results easier to compare to the % total N values. Digestible energy (Y_3) was determined by multiplying the % energy digestibility (Y_2) by an estimated average caloric value of 4.5 kcal/g leaf tissue (Hanley and McKendrick 1985, Williams et al. 1987).

RESULTS

Light and nitrogen

The average daily irradiance was much higher in open clear-cuts than in either even-aged stands or oldgrowth (Figure III.2). Mean values of average daily irradiance did not differ significantly between oldgrowth and even-aged stands, probably due to the small sample size. Variability in irradiance was higher in oldgrowth though, compared to

clear-cuts and even-aged forests.

Extractable nitrogen varied widely among stands, but did not differ significantly by stand type, that is, among oldgrowth, even-aged, and clear-cut stands (Fig. III.3). Similar to the control soils in the field experiment of Chapter II, the extractable soil N in all stands was predominantly in the form of ammonium, and nitrate levels were negligible.

Biomass

The standing biomass (stem count), as well as biomass production of blueberry shrubs varied as follows : open clear-cuts > oldgrowth > even-aged regeneration (Table III.3).

Foliage morphology, biochemistry, and nutritional quality

The leaves of plants growing in open clear-cuts had more than 2.5 times higher specific leaf weight (mg dry wt. / cm^2 leaf area) than leaves of plants beneath a forest canopy (Fig. III.2a). Leaf succulence (fresh/dry wt. ratio) followed the opposite trend (Fig III.2b). That is, leaves exposed to the higher irradiance regime of clear-cuts had more dry matter accumulation per unit leaf area and less moisture than in shaded environments.

With regard to biochemical changes, leaves collected from clear-cuts had significantly higher concentrations of condensed tannins compared to leaves in either even-aged regenerating stands or oldgrowth (Fig III.2c). The cell

wall or NDF fraction in leaf tissue was lower in plants from clear-cuts than from even-aged stands or oldgrowth (Table III.3). These NDF values were similar to data reported by Rose (1982) for clearcut and forest microsites in the southern coastal region, and by Hanley and McKendrick (1983) for forests in the northern region. The soluble component of plant cells (NDS) calculated as 1 - NDF, was highest in leaves from clear-cuts (63.2 %), but lower in oldgrowth (53.7%) and even-aged stands (52.1%). The weight % of lignin + cutin in NDF was highest in plant foliage from clear-cuts compared to oldgrowth and even-aged stands (Table III.3).

Leaves in the clear-cut had about 25% lower concentrations of total nitrogen than in the forests, but free amino acids and the ratio of free amino acids to total nitrogen did not vary by stand type (Fig III.3 a,b,c). Concentrations of digestible nitrogen declined by nearly two-thirds at higher irradiance in the clear-cut compared to the forest stands as a result of both the lower total N concentrations and lower N digestibility (higher tannin accumulation) (Fig. III.4a). Digestible energy remained constant across forest types (Fig. III.4b).

DISCUSSION

Acclimation, or plastic changes in plant growth, chemical defense and nutritional composition along gradients of resource availability are well documented

(McKey 1979, Gartlan et al. 1980, Crawley 1983, Bryant 1987, Waring and Pitman 1985, Waring et al. 1985, Larsson et al. 1986). Wide fluctuation in the standing crop and biomass production of blueberry among different forest environments in Alaska has been previously described in detail by Alaback (1980, 1982). Blueberry growth responses can be attributed to large changes in light levels but not to nitrogen availability among different ages of forests. Although values of average daily irradiance were 2 - 20 higher in oldgrowth compared to even-aged stands, all the forest light values were low compared to the clear-cut.

Even so, shrub biomass and production was considerably higher in oldgrowth compared to the even-aged stands. The more abundant understory in oldgrowth compared to even-aged stands may also be related to differences in stand history, light spectral quality or sunfleck activity. Sunflecks are important to the energy balance and photosynthetic performance of mant subcanopy pecies (Chazdon and Pearcy 1986, Chazdon 1988, Pfitsch and Pearcy 1989).

Extractable soil N varied widely within stands, ranging from low values comparable to Vancouver Island B.C. (Binkley 1984b) and the Oregon Cascades (Waring et al. 1987) to higher values reported for pine forests in Wisconsin (Binkley et al. 1986) and the Oregon Coast Range (K. Cromack pers. comm.). The high variability in values of extractable soil N, combined with the lack of plant response to nitrogen treatments strongly suggested that N

did not limit shrub growth under any irradiance regime in the natural forests.

Nitrogen concentrations found in blueberry leaves in the natural forests were similar to values reported by Klein (1965) in southeastern Alaska. Total N concentrations in blueberry leaves under varying levels of irradiance in the natural forests followed similar trends to the artificially shaded plants in the growth room and field experiments. These data strongly suggested that the observed concentrations of foliar nitrogen were a function of the dilution of tissue N by growth. However, leaf N concentrations from shaded leaves in the even-aged and oldgrowth forests were approximately 30% lower than leaves subjected to similar, but stable irradiance regimes in the growth room and field experiments. Assuming that tissue N levels reflected differential rates of plant growth, then it is apparent that artificial and natural shading did not have comparable effects on plant growth and carbon assimilation. This also could be related to sunfleck activity in the natural forests.

The weak response of free amino acids to irradiance in the natural stands contrasted sharply to the strong light response seen in the growth room and field experiments. One possible explanation is that free amino acids reflected only temporary imbalances in the plant C/N ratio under extreme shade stress. Over time periods longer than the artificial shading experiments in the field (2 years),

blueberry apparently adjusted nitrogen assimilation to maintain more constant levels of free amino acids and total N in foliage.

The high degree of light limitation found with blueberry in the Alaskan rainforest is not uncommon for subcanopy species (Boardman et al. 1972). Physiological adjustments in understory plants to varying light quality and quantity have well-documented effects upon the primary productivity and ecological differentiation of plant species in many forests (Denslow 1980, Brokaw 1985 a,b, Popma and Bongers 1988). The strong relationship of tannins to irradiance also has been observed with plants in other forest ecosystems (Bryant and Kuropat 1980, Bryant et al. 1983, Muller et al. 1987, Mole and Waterman 1988).

Shaded leaves offered a better nutritional package to deer by virtue of higher concentrations of digestible nitrogen and lower tannins compared to sun leaves. If the digestible nitrogen concentrations in different forest types are compared to the minimum daily requirements for different levels of metabolism in deer, major nutritional problems are apparent. Levels of digestible nitrogen in blueberry foliage from clear-cuts are inadequate to meet the nutritional needs for maintenance metabolism in an adult doe, and especially deficient to support the nutrition of a doe with a fawn (Hanley and McKendrick 1985). Because deer in coastal Alaska periodically suffer high mortality during severe winters, high reproductive

rates are necessary to replenish the populations. Under major nutritional limitations of low nitrogen, reproductive success could be expected to decline. Low birth rates attributed to poor nitrogen nutrition might explain the slow population recovery of deer in some regions of southeastern Alaska that have been extensively clear-cut.

Digestible energy would be unlikely to limit deer nutrition where adequate forage biomass was present, because energy concentrations were stable and exceeded the nutritional requirements in all forest types. However, this conclusion is based upon energy calculations which assume a constant gross energy content in leaves of 4.5 kcal g^{-1} leaf. While this assumption is adequate for most herbaceous plants and agricultural species, much more variation is observed in woody plants, evergreens, and ericaceous plants which have higher amounts of secondary metabolism (Golley 1961, Hadley and Bliss 1964). Vaccinium and other ericaceous plants can vary by as much as 30% in gross energy and potentially by as much as 40-50% in digestible energy. Under high irradiance regimes, the potential nutritional advantage of the higher gross energy content of foliage is negated by the reduced palatability of the high-tannin forage to deer. In fact, a study of forage preference with V. ovalifolium collected from the Douglas Island site indicated that deer ate twice as much forage collected in an oldgrowth forest compared to an adjacent clear-cut (Hanley and McKendrick 1985). Similarly,

colobus monkeys in Africa specifically select low-phenolic leaves of certain tree species (McKey et al. 1978). And ptarmigan in Alaska select forage that is high in readily-digestible energy (soluble carbohydrates), but low in phenolics (Bryant and Kuropat 1980).

In young clear-cuts, forest management poses the most serious nutritional problems to deer in terms of reduced foliar levels of digestible nitrogen and forage palatability. In older even-aged forests, the important problem relating to forest management is the low production of understory forage. Consequently, in managed forests deer face the dilemma of abundant but low-quality forage in clear-cuts, and of very limited quantities of high-quality forage in dense conifer stands. Habitat management in regenerating forests should strive to improve the nutritional quality of understory forage in young clear-cuts, and to sustain adequate biomass and productivity of understory vegetation in older regenerating forests.

These habitat objectives are attainable by modifying management prescriptions to alter light availability in the understory of regenerating forests. For example, conventional methods of clear-cutting could be changed to retain partial overstory shade in cutover units. Selective cutting would preserve timber values, produce a light environment favorable to the production and quality of understory vegetation and be more compatible with many forest concerns and uses other than wildlife, including

protection of soil and water quality, fisheries and recreation.

Thousands of acres of dense regenerating conifer stands can be thinned precommercially to prevent closure of the overstory canopy and maintain a productive understory. For once the understory is lost, recovery can be expected to be slow (Alaback 1980, 1982, Tappeiner and Alaback 1989). In older commercial-aged stands with little or no herbaceous vegetation, thinning programs are needed which include selective tree removal and the planting of native seed and/or rhizomes to restore the understory to former levels of diversity and productivity.

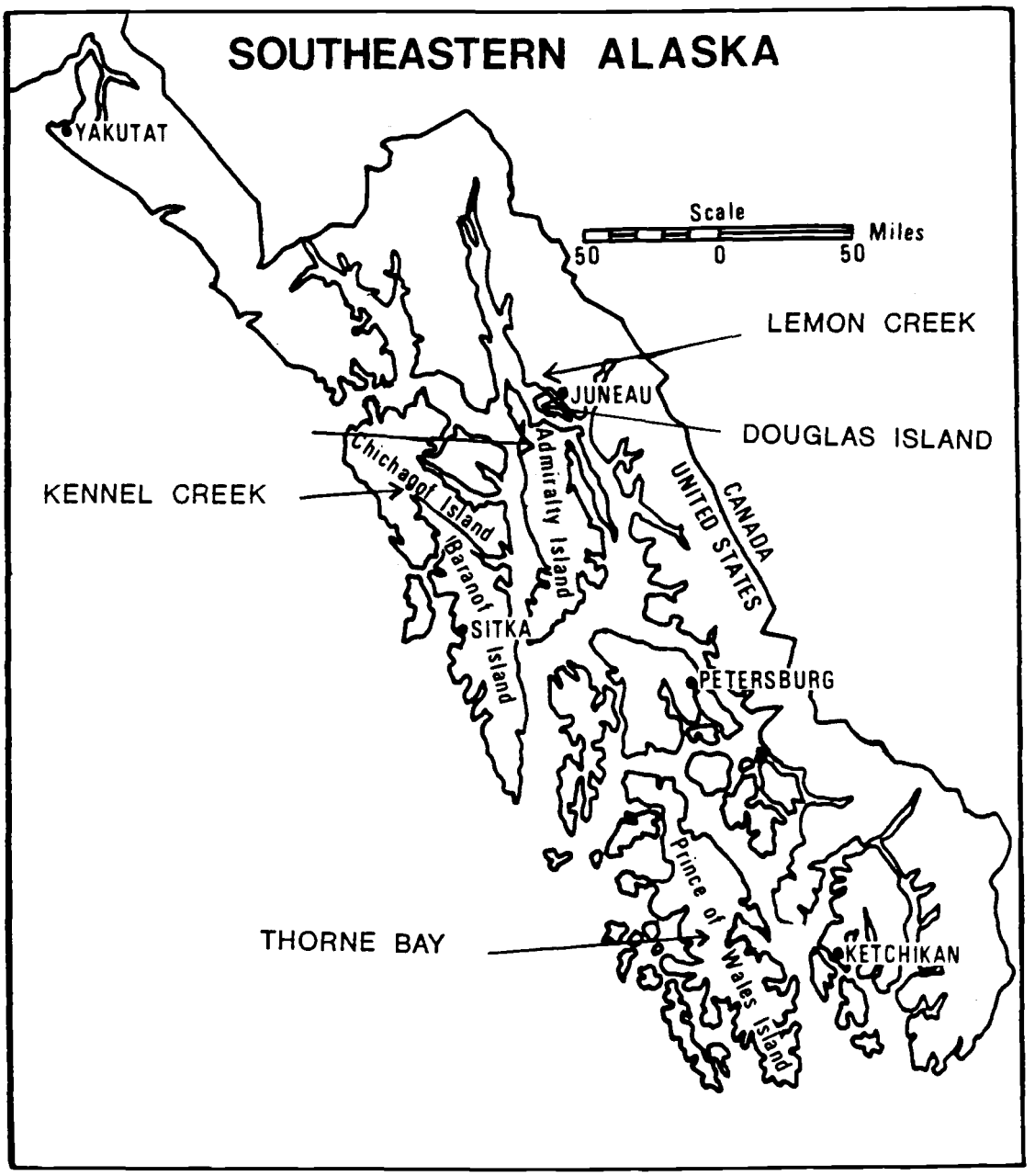

Figure III.1. Locations of the five study sites in southeastern Alaska.

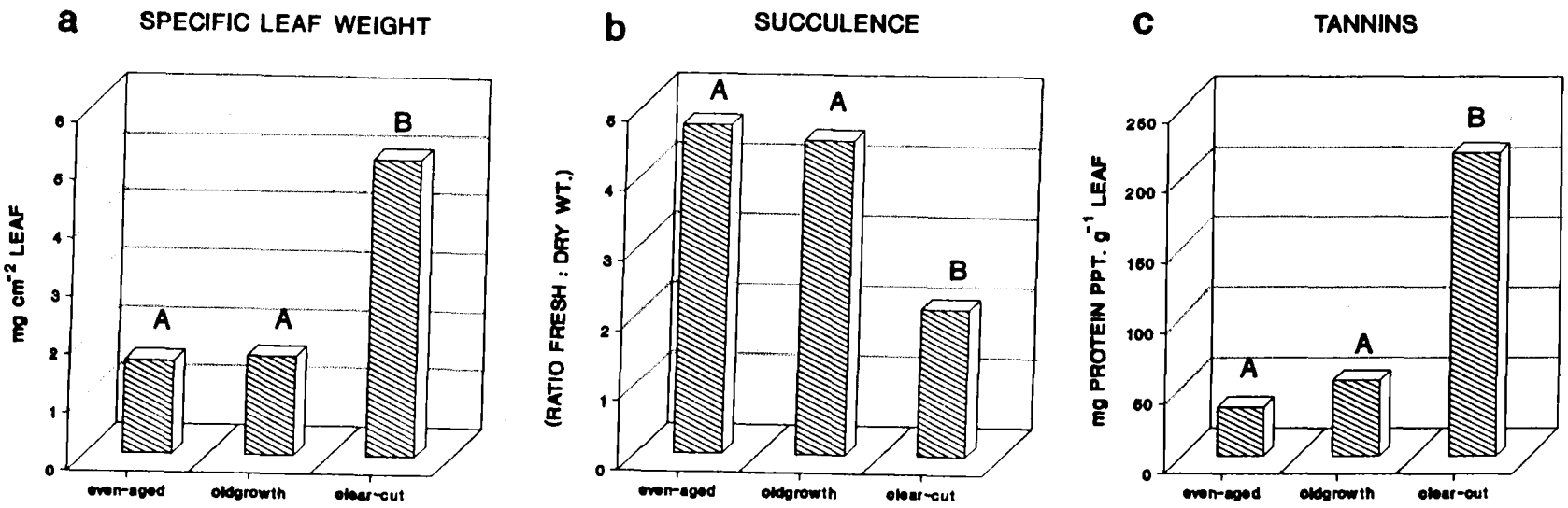

Figure III.2 a,b,c. Leaf responses including a) specific leaf weight, b) leaf succulence and c) tannin concentrations among forests of different ages and canopy structure. Values with the same letter do not differ as determined by Scheffe's Test at $p < .05$; $n = 3$ samples per stand x 5 stands, except in oldgrowth where $n = 6$ samples per stand x 3 stands.

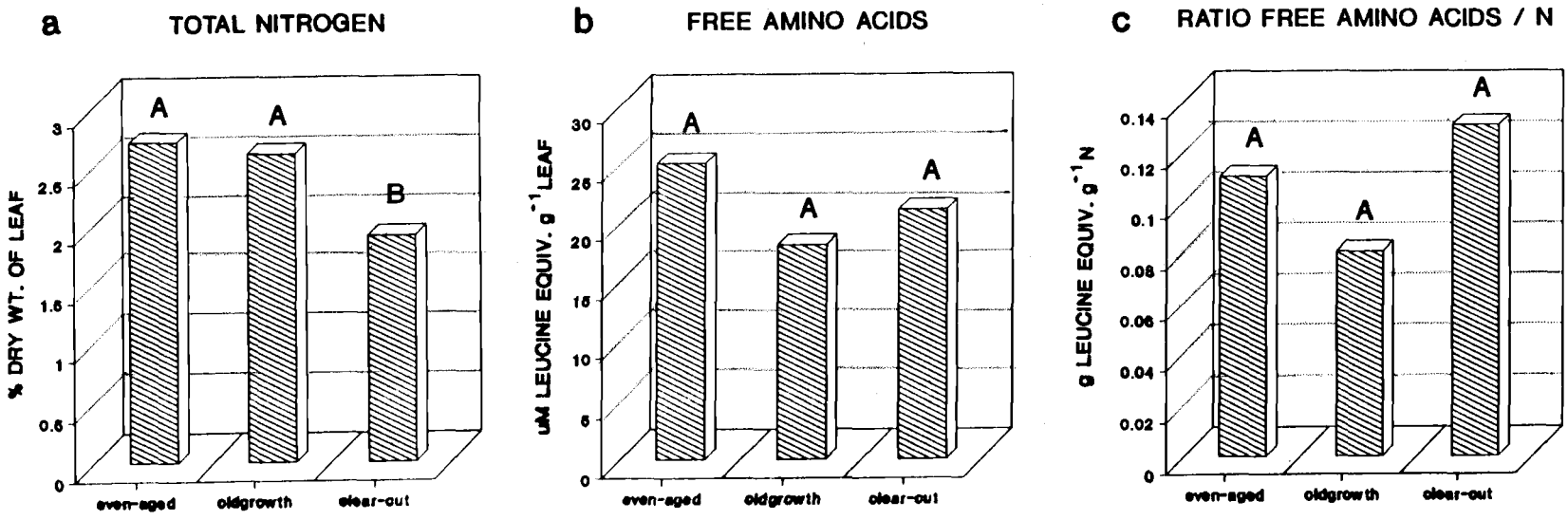

Figure III.3 a,b,c. Variation in concentrations of a) total N, b) free amino acids and c) ratio of free amino acids / N among forests of different ages and canopy structure. Values with the same letter do not differ as determined by Scheffe's Test at $p < .05$; n = 3 samples per stand x 5 stands, except in oldgrowth where n = 6 samples per stand x 3 stands.

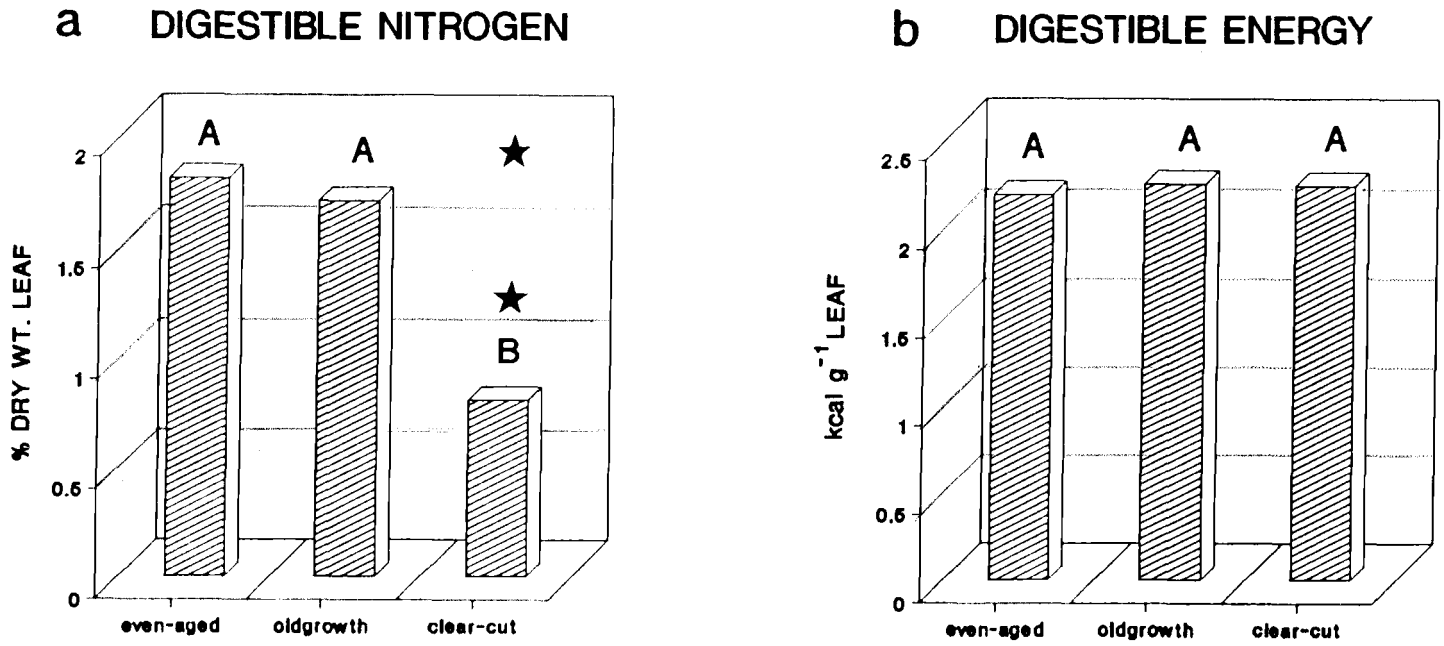

Figure III.4 a,b. Leaf concentrations of a) digestible nitrogen and b) digestible energy among forests of different ages and canopy structure. Calculation of digestible nitrogen and energy followed equations presented by Robbins (1983) and Robbins et al. (1987 a,b). Digestible energy calculation assumes a gross energy concentration in leaves of 4.5 kcal/g (Hanley and McKendrick 1985, Williams et al. 1987). Values with the same letter do not differ as determined by Scheffe's Test at $p < .05$; $n = 3$ samples per stand x 5 stands, except in oldgrowth where $n = 6$ samples per stand x 3 stands. The stars in 4a. denote the dietary requirement of digestible nitrogen for an adult doe without (lower value) and with one of more fawns (upper value).

Table III.1 Study site locations and stand types sampled in southeastern Alaska.

Site	Location	Stand Types Sampled		
		even-aged[a]	clear-cut[a]	oldgrowth[b]
Eagle Crest	Douglas Island	x	x	x
Hawk Inlet	Admiralty Island	x	x	
Kennel Creek	Chichagof Island	x	x	
Lemon Creek	Juneau Mainland	x	x	x
Thorne Bay	Prince of Wales Island	x	x	x

[a] n = 3 leaf samples per site
[b] n = 6 leaf samples per site

Table III.2. Values of average daily irradiance and nitrogen availability across a variety of forest ages and canopy cover. Irradiance was measured with ozalid paper calibrated to a solarimeter (PAR). Extractable soil nitrogen (NH_4^+) was determined with ion-exchange resins placed in soils for a period of one year. Values include means, with standard errors in parentheses.

Site	Even-aged		Clear-cut		Oldgrowth	
	I[a]	N[b]	I[a]	N[b]	I[a]	N[b]
Eagle Crest	4 (6)	.233 (.037)	450 (36)	.600 (.123)	11 (10)	.438 (.017)
Hawk Inlet	4 (4)	.064 (.017)	370 (26)	nd	nd	.085 (.009)
Kennel Creek	1 (4)	.048 (.014)	460 (36)	.681 (.278)	nd	nd
Lemon Creek	5 (2)	1.93 (1.23)	430 (12)	.733 (.166)	23 (12)	1.29 (.174)
Thorne Bay	3 (6)	nd	480 (15)	nd	9 (7)	nd

[a] Average daily irradiance = uE m^{-2} s^{-1}; n = 3.
[b] Extractable soil NH_4 = mg g^{-1} resin $year^{-1}$; n = 6.
nd = not determined.

Table III.3 Plant responses to varying irradiance regimes in forests of different ages and structure. Across rows, values with the same letter do not differ as determined by Scheffe's Test at $p < 0.05$.

Variable	--- STAND TYPES --- Even-aged[a]	Clear-cut[a]	Oldgrowth[b]
Biomass production ($g\ m^{-2}$)	12 A	975 C	273 B
Stem density (stems 25 m^{-2} ground)	2 A	57 C	16 B
Neutral Detergent Fiber (% dry wt. of leaf)	47.9 B	36.8 A	46.3 B
% lignin + cutin in NDF	27.9 A	31.8 B	26.8 A

[a] n = 3 samples per stand * 5 stands
[b] n = 6 samples per stand * 3 stands

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APPENDICES

APPENDIX A.

SEQUENTIAL CHEMICAL ANALYSES AND LABORATORY PROTOCOL

METHODS SUMMARY

The methods of sequential analysis used in this study quanitified total free amino acids and sugars on an 80% ethanol extract of fresh leaf tissue. Starch was determined on dried and ground leaf tissue extracted with acetone and 80% ethanol and then treated with 35% perchloric acid to solubilize starch. Holocellulose and hololignin were determined by treating the residual fiber after starch extraction with concentrated sulfuric acid followed by ashing in a muffle furnace. Free amino acids, sugars, and starch were measured colorimetrically whereas holocellulose and hololignin were determined by weight difference. The following section describes the basic procedures and chemistry involved in the different analytical methods.

Free amino acids

There is great variation in the relative proportions of different free amino acids between plant species, plant parts, and with physiological status of the plant (Synge 1955), thus this method is most accurately applied to quantify total free amino groups, rather than free amino acids per se.

Fresh plant tissue was weighed into a test tube, and homogenized with a Polytron in 80% ethanol. 80% ethanol provides a good overall solvent for most amino acids (Stuart 1935, Harborne 1984), and does not extract starch as do the more dilute alcohol solutions (De Angelis 1982).

Insoluble polyvinlypyrrolidone (PVPP) was added to the extract to reduce interference problems with phenolics (Sanderson and Perera 1966). The ethanol solution was then evaporated to precipitate proteins, pigments and lipids, and produce an aqueous solution of amino acids (Stuart 1935, Loomis and Shull 1937, Draper 1976, Harborne 1984).

Free amino acids were quantified colorimetrically with the ninhydrin reagent described by Moore and Stein (1954). Ninhydrin, (1,2,3 triketohydrindene hydrate) is a commercially available reagent which reacts with intact amine groups to produce a red-purple color product with maximum absorption at 570 nm. The color is reproducible to ± 0.5% and is stable for about 60 min, then a decline of about 1 % per hour occurs (Troll and Cannon 1953).

Soluble sugars

Free sugars were extracted along with free amino acids in the fresh plant tissue using an 80% ethanol solution (Harborne 1984). The advantage of determining sugars on the ethanol extract of fresh rather than dried tissue is avoidance of the Maillard reaction in which reducing sugars react with proteins to form complexes that can resist ordinary separatory and hydrolytic operations (Pirie 1956).

Following evaporation of the ethanol extract, the aqueous sugar solution was filtered to remove precipitates (Harborne 1984). Neutral lead acetate also was used to remove any remaining tannins, pigments, lipids, or proteins

that could interfere with carbohydrate determinations. Excess lead was removed with sodium oxalate (Stuart 1935, Loomis and Shull 1937, Horowitz 1959).

Sugars were determined colorimetically using the anthrone reaction (Morse 1947, Morris 1948). Anthrone is a commercially available reagent which reacts with hexose sugars to produce a green color product with maximum absorbance at 650 nm (Yemm and Willis 1954).

Starch

Plant tissue was homogenized in liquid N in order to fracture the plant cell wall and attain maximum extraction of starch (Loomis and Shull 1937, Sandstrom and Loomis 1987). Before starch extraction, interfering sugars, pigments, proteins and lipids were by extraction in acetone followed by treatment in boiling 80% ethanol (McCready 1970). Starch was then solubilized in 35% perchloric acid by soaking the tissue directly in the acid and filtering the extract.

Starch was quantified colorimetrically with the anthrone reagent as described in Viles and Silverman (1949), and McCready et al. (1950). Perchloric acid was included in the standards because the acid influences color development of the carbohydrate-anthrone reaction (Hansen and Moller 1975).

Holocellulose

Holocellulose (cellulose and acid-labile hemicellulose)

was measured by weight difference of the perchloric acid-insoluble residue following hydrolysis in concentrated sulfuric acid. Cellulose was first gelatinized in 72% cold sulfuric acid followed by rinsing in boiling water to acheive hydrolysis (Loomis and Shull 1937).

Hololignin

Hololignin (lignin + acid-stable hemicellulose + cutin) was determined by weight difference of the holocellulose-extracted residue following ashing in a muffle furnace at 550° C for 4 h (Goering and Van Soest 1970). The residue remaining after cellulose extraction was combusted in a muffle furnace to remove all remaining organic materials. An estimate of lignin was obtained by subtracting the weight of the ash residue from the weight of the sample prior to combustion.

SEQUENTIAL CHEMICAL ANALYSES AND LABORATORY PROTOCOL

I. Free Amino Acids and Soluble Sugars

Solutions

Free Amino Acids

1) Sodium acetate buffer (4N) - Add 32.8 g anhydrous sodium acetate to 35 ml distilled de-ionized water (ddH_2O) heated to just below the boiling point on a stir plate, and let dissolve. When cool, add 26-28 ml glacial acetic acid to bring the pH of the buffer solution to 5.5-5.6. Pour the buffer into a 100 ml volumetric flask and bring to volume with ddH_2O.

2) Potassium cyanide - Add 0.065 g potassium cyanide to 100 ml water. This is an anti-bacterial agent.

3) Methylcellosolve - (ethylene glycol monomethyl ether). HPLC-grade reagent is required. Peroxide contamination is a problem with this reagent, so keep bottle tightly closed in a refrigerator. Caution - this reagent is a toxic terratogen. Use only in a fume hood.

4) Ninhydrin - Add 3.75 g ninhydrin reagent to 30 ml methylcellosolve in a 100-ml volumetric flask on a magnetic stir plate. Mix slowly until dissolved, then bring to volume with 100 ml of methylcellosolve. Store in refrigerator and avoid exposure to light. Reagent is good for approximately one month.

5) 80% Ethanol - Dilute 840 ml 95% ethanol to 1 l with ddH_2O.

6) 60% Ethanol - Dilute 75 ml 80% ethanol to 100-ml volume with ddH_2O.

7) Moore-Stein reagent - In fume hood mix 100 ml of solution # 1 (buffer) with 9.3 ml of solution # 2 (KCN), and 123 ml of solution # 3 (methylcellosolve). This reagent is good for approximately one month.

8) Leucine standards - Stock solution (200 ppm) consists of 200 mg of D-leucine in 1 l of ddH_2O. Add leucine to 1 l volumetric flask half-filled with ddH_2O, stir until dissolved, then bring to volume with ddH_2O.

Standards - 0 ppm = 100 ml ddH_2O
5 ppm = 2.5 ml stock + 97.5 ml ddH_2O
10 ppm = 5.0 ml stock + 95.0 ml ddH_2O
20 ppm = 10.0 ml stock + 90.0 ml ddH_2O
40 ppm = 20.0 ml stock + 80.0 ml ddH_2O
60 ppm = 30.0 ml stock + 70.o ml ddH_2O

Soluble Sugars

9) Anthrone - Slowly add 500 ml of concentrated sulfuric acid to 200 ml of ddH_2O in a heavy glass bottle on a stir plate. Place un-capped bottle in an ice bath and allow to cool. This makes 655 ml of 72% sulfuric acid. Add 1.146 g of Anthrone powder to the acid solution and allow to mix for 2 or more h on a stir plate away from bright light.

10) Sugar standards - Stock solution (360 ppm) consists of 360 mg of D-glucose in 1 l of ddH_2O. Mix as described for leucine standards.

Standards - 0 ppm = 100 ml ddH_2O

18 ppm = 5.0 ml stock + 95.0 ml ddH_2O

36 ppm = 10.0 ml stock + 90.0 ml ddH_2O

90 ppm = 25.0 ml stock + 75.0 ml ddH_2O

180.0 ppm = 50.0 ml stock + 50.0 ml ddH_2O

Procedure

Sample preparation and processing

1) Pat dry fresh leaf tissue.

2) Weigh 0.5-1.0 g of fresh leaf tissue into a plastic centrifuge tube.

3) Place leaf powder in a 30-ml plastic centrifuge tube, add 15 ml of 80 % ethanol and homogenize with a Polytron for several seconds. Rinse homogenizer shaft with 15-25 ml of 80% ethanol solution into the centrifuge tube.

4) Immediately add insoluble polyvinylpyrrolidone (PVPP) equal to the weight of the leaf sample and stir with a glass rod.

5) Centrifuge samples at 10 G for 10 min., then carefully pour the supernatant into a 250-ml beaker leaving the insoluble residue at the bottom of the tube. Repeat the centrifugation/decantation steps two more times with 20-30 ml of ethanol, and bring to a final volume of 100 ml with the 80% ethanol solution.

6) Place beakers under a fume hood and allow ethanol to evaporate down to 5-15 ml.

7) Pour the remaining solution through a folded Whatman #1, 12.5 cm filter paper in a long-stem glass funnel and

collect the filtrate in a 100-ml volumetric flask. Bring to volume with ddH_2O. Discard the filter paper residue.
8) Store the free amino acid extracts in a refrigerator and read within two days for maximum accuracy, or freeze the extract in plastic vials at -40° for longer-term storage.
9) To the sample solution remaining in the volumetric flask, add 100 ul of saturated lead acetate and shake well. After 20 min. add 500 ul of saturated sodium oxalate, place in a refrigerator, and wait several hours or overnight for a white precipitate to settle out. This extract is used for sugar analysis. Analyze the samples within a couple of days for maximum accuracy, or freeze the extract in plastic vials at -40° for longer-term storage.

Free Amino Acid Color Reaction And Reading

10) Immerse a rack of 15-ml test tubes in icewater to a depth of about 10 cm.
11) Pipette 2 ml of each amino acid extract into a test tube, and do the same for the prepared standards. Duplicate aliquots are recommended. If the color of the reacted solution is too dark (greater than 1.0 absorbance units), use less sample solution and bring to a volume of 2 ml with ddH_2O. Correct for this dilution as described in the calculation section.
12) Add 1.0 ml of Moore-Stein reagent to each test tube.
13) Add 0.5 ml of the ninhydrin solution to each test tube, cover tube loosely with a bottle cap, and mix briefly on a

vortex.

14) Place rack in a container of vigorously boiling water 10 cm deep, cover, and heat for 20 min. exactly. The rate of heat supply should be sufficient to bring the bath quickly back to boiling. Remove rack from boiling water bath and place immediately in icewater to a depth of 10 cm. Allow tubes to cool approximately 10 min.

15) Add 5 ml of 60% ethanol to each tube and mix on a vortex.

16) Read samples for absorption at 570 nm on a spectrophotometer using leucine standards from 0 - 60 ppm. Read samples immediately as the color begins to fade after 1 h. Vortex each tube before reading, as vertical color gradients form rapidly.

Soluble Sugar Color Reaction And Reading

17) Immerse a rack of 15-ml test tubes in icewater to a depth of about 10 cm.

18) Pipette 0.5 ml of the sugar extract in a test tube and do the same for the prepared standards. Duplicate aliquots are recommended. If the color of the reacted solution is too dark, use less sample solution and bring to a volume of 0.5 ml with ddH_2O. Correct for this dilution as described in the calculation section.

19) Add 5 ml of the anthrone solution and mix briefly on a vortex. If solution turns cloudy white use less of the sample solution (use 0.25 ml diluted to .5 ml with ddH_2O).

Correct for the dilution as described in the calculation section.

20) Place the rack in a bath of vigorously boiling water for 12 min. exactly, then set rack in icewater, and let samples cool.

21) Read samples for absorption at 625 nm on a spectrophotometer using glucose standards from 0-180 ppm.

II. Starch, Holo-cellulose and -lignin

Solutions

Starch

1) Acetone - reagent grade.

2) 35% Perchloric Acid - Slowly add 500 ml of concentrated perchloric acid to 500 ml ddH_2O in a heavy glass bottle on a stir plate. Place uncapped bottle in an ice bath and allow to cool.

3) Anthrone - Slowly add 500 ml of concentrated sulfuric acid to 200 ml of ddH_2O in a heavy glass bottle on a magnetic stir plate. Place bottle in an ice bath and allow to cool. This makes 655 ml of 72% sulfuric acid. Add 1.146 g of Anthrone powder to the acid solution and allow to mix for 2 or more hours on a magnetic stir plate away from bright light.

4) Starch standards - Stock solution (360 ppm) consists of 360 mg of D-glucose in 1 l of ddH_2O. Mix as described for leucine standards.

Standards - 0 ppm = 35 ml acid + 65 ml ddH_2O

18 ppm = 5 ml stock + 35 ml acid + 60 ml ddH_2O

36 ppm = 10 ml stock + 35 ml acid + 55 ml ddH_2O

90 ppm = 25 ml stock + 35 ml acid + 40 ml ddH_2O

180 ppm = 50 ml stock + 35 ml acid + 15 ml ddH_2O

Holocellulose

5) 72% Sulfuric acid - Slowly add 500 ml concentrated sulfuric acid to 200 ml of ddH_2O in a stock bottle on a stir plate, and place in icewater to cool. This makes 655 ml of 72% sulfuric acid.

Procedure

Sample preparation and processing

1) Dry leaf tissue in an oven at 80° C for one-half hour, then at 70° C for at least 48 h.

2) Macerate tissue with liquid N in a dry mortar.

3) Weigh 0.50 g of leaf powder onto a piece of Whatman #1 filter paper 9.0 cm in diameter. Fold the filter paper to form a tube with one narrow end and one wide end. Insert the narrow end into the wide end to seal the plant tissue inside.

4) Place the sample folded in filter paper into a 25-ml test tube with 10 ml of 100% acetone and allow to sit a couple of hours or overnight. Pour off the acetone, repeat this step until the extract is colorless.

5) Place the sample still folded in filter paper into a 100-ml test tube, cover with 80% ethanol, and place in a water bath to boil gently. Cover the tube with a cap to

reduce evaporation. After one to two hours, pour the extract off and repeat the extraction until the extract is colorless. Three extractions can be completed over a 4-6 h period.

6) Wash the residue from the filter paper with three, 5-ml rinses of 35% perchloric acid into a 125 ml Erlenmeyer flask. Use two more rinses of 5 ml to remove the sample from the sides of the flask, for a total of 35 ml of acid. Cover the flask with foil and agitate flasks overnight on an orbital shaker.

7) Filter solutions in the flask through Gooch crucibles (30-50 ml, coarse) into a 100 ml volumetric and bring to volume with ddH_2O. This extract is used for starch analysis.

8) Rinse the crucible and residue well with hot water using a light vacuum on a filter flask, and finish with an acetone wash and to dry the sample.

9) In a fume hood, add 10 ml of 72% sulfuric acid to the starch-extracted residue in Gooch crucibles and stir with a glass rod to form a smooth paste. Then add an additional 20 ml of acid and stir. Set crucibles in a pyrex pan for 24 h under a fume hood. A temperature less than 12° C is preferred to prevent the carbonization of cellulose forming unfilterable colloids (Loomis and Schull 1937).

10) Place Gooch crucible on a filter flask and apply a strong vacuum to remove the acid. Rinsing with very hot water in a squirt bottle improves the filtering speed.

11) Rinse exterior of crucible and residue with hot water.

12) Place crucible in a 70° C oven for 24 h and weigh.

13) Determine weight loss from the acid digestion. This is the estimate of holocellulose.

14) Cover the crucible containing the residue from #13 above with a ceramic lid and place in a muffle furnace at 550° C for 3 h, then cool to 100° C and place in a drying oven at 70° C for several hours and weigh. Determine weight loss during ashing. This is the estimate of hololignin.

Starch Color Reaction and Reading

15) Add 0.5 ml of the starch extract to 15-ml test tubes in a rack immersed to a depth of 10 cm in icewater.

16) Add 5 ml of Anthrone solution to each tube, cover test tubes loosely with a cap, and mix briefly on a vortex. If the solution turns cloudy white use less of the sample solution (use 0.25 ml sample extract diluted to 0.5 ml with ddH_2O). Correct for this dilution as described in the calculation section.

17) Place the rack of test tubes into a large container of vigorously boiling water 10 cm deep, for 12 min., then set the rack in icewater, and let samples cool.

18) Read the samples for absorption at 625 nm on a spectrophotometer using glucose standards from 0-180 ppm.

III. Calculations

For colorimetric analyses, calculate a regression equation for ppm of standard solution based upon absorbance

readings on the spectrophotometer. Then use the regression equation to calculate sample values in ppm, and convert this value to concentrations of chemical fractions using the following equations:

Free Amino Acids :

A x B/C x (D x E x F) = u moles leucine g^{-1} dry wt. of foliage

where:

A = absorbance reading in ppm

B = sample volume of .1 l

C = sample fresh wt in g

D = extract dilution ratio. For 1.0 ml of sample, the ratio is 2. For 2 ml of sample, D = 1

E = fresh wt./dry wt. ratio

F = factor for converting mg leucine to u moles leucine per g dry wt. of foliage (8.5348)

Soluble Sugars :

A x B/C x (D x E) = mg sugar g^{-1} dry wt. of foliage

where:

A = absorbance reading in ppm

B = sample volume of .1 l

C = sample fresh wt in g

D = extract dilution ratio. For .25 ml of sample, the ratio is 2.For 0.50 ml sample, D = 1

E = fresh wt./dry wt. ratio.

Starch :

A x (B/C) x D x E = mg starch per g dry wt. of foliage

where:

A = absorbance reading in ppm

B = sample volume of .1 l

C = sample dry wt. in g

D = extract dilution ratio. For .25 ml of sample, the ratio is 2. For 0.5 ml sample, D = 1

E = conversion factor of .9 to account for hydration in the conversion from starch to sugar

Cellulose :

[(A - B)/C] = mg cellulose per g dry wt. of foliage

where :

A = dry wt. (mg) of fiber component before acid digestion

B = dry wt. (mg) of fiber component after acid digestion

C = original dry wt. (g) of sample

Lignin :

[(A - B)/C] = mg lignin per g dry wt. of foliage

where :

A = dry wt. (mg) of fiber component before ashing

B = dry wt. (mg) of fiber component after ashing

C = original dry wt. (g) of sample

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APPENDIX B. ANOVA statistics for plant response variables under different regimes of irradiance and nitrogen supply, n = 96.

Response Variables	Total Explained Variation (R^2)	Partial Explained Variation		
		Light	N	Light*N
Growth and Physiology				
Net photosynthesis [a]	.61 **	.55 **	ns	ns
Growth efficiency [a]	.21 *	ns	ns	ns
Net photosynthetic uptake	.78 **	.57 **	.13**	.08 *
Relative Growth rate	.83 **	.70 *	.07 **	ns
Root/shoot ratio	.60 **	.45 *	ns	ns
Leaf mortality	.68 **	.42 *	.12 **	.15 **
Leaf Biochemistry and Morphology				
Protein Precipitation	.84 **	.83 **	ns	ns
Sugar	.84 **	.56 **	.11 **	.17 **
Starch	.71 **	.46 **	.06 **	.20 **
Nonstruct. carbohydrates	.86 **	.63 **	.02 *	.21 **
Holocellulose	.40 **	.30 **	ns	ns
Hololignin	.85 **	.79 **	ns	ns
Structural polymers	.80 **	.74 **	ns	ns
Total N conc. (TN)	.86 **	.74 **	.08 **	ns
Free amino acids (FAA)	.81 **	.68 **	.09 **	.03 *
FAA / TN	.75 **	.62 **	.08 **	.05 *
Specific leaf wt.	.98 **	.97 **	ns	ns
Succulence	.96 **	.91 **	.01 **	.04 **
NDF [b]	.80 **	.74 **	ns	ns
NDS [c]	.81 **	.73 **	ns	ns
% Lignin/NDF	.58 **	.54 **	ns	ns
Digestible protein [d]	.90 **	.82 **	.06 **	.02 *
Digestible energy [d]	.72 **	.63 **	.03 *	.06 *

* Pr (F) 0.001 to 0.05
** Pr (F) < 0.001

a n = 32
b Neutral detergent fiber = total cell wall
c Neutral detergent solubles = cell solubles, or 1 - NDF
d calculated from Robbins (1983), Robbins et al. (1987a, 1987b).